REVIEW ARTICLE

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BIOLOGICAL MACROMOLECULES

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Summary

This article describes the principal types of naturally occurring macromolecules under the classification of polysaccharides, proteins, glycoproteins, proteoglycans and nucleic acids. One of the specialised opening features is a composite tabulation of the full structures of the principal monosaccharides, amino acids, and purine and pyrimidine bases which go to make up these macromolecules. Comparisons are drawn between the complexities of the biological structures and the simplicities of man's attempts to simulate them, and readers' attentions are drawn to the vast range of uses and functions of biological macromolecules which arise from the large number of specific primary structures possible. Several examples of the foregoing macromolecular types are given to illustrate the subject, these examples ranging from connective tissue support polymers to enzymes, from plant structural polysaccharides to microbial polymers etc. Considerable emphasis is given to the uses to which the biological macromolecules have been put in isolated and multiple processes and systems.

As their name implies, biological macromolecules are naturally occurring, high molecular weight materials. However, they are not necessarily simple polymers of a single monomer unit. They may contain repeating units but these frequently have

a complex structure. In a brief article of this nature it is not possible to give a comprehensive coverage of the subject; we therefore introduce, in outline, many aspects of biological macromolecules and quote a number of examples in greater detail to illustrate them. Selected references are given to enable the reader to obtain further information on many points.

Classification

There are three principal classes of natural macromolecules–proteins[1] (polymers of L–amino acids, see Table 1), nucleic acids[2] (polymers of purine or pyrimidine bases, see Table 2, phosphate and D–ribose or 2–deoxy–D–ribose, Figure 1) and polysaccharides[3] (polymers of monosaccharides, see Table 3). Covalent combinations of protein and carbohydrates give glycoproteins[4] (protein chains with a small number of pendant oligosaccharide chains, Figure 2a) and proteoglycans[5] (protein chains with many pendant polysaccharide chains, Figure 2b).

Polysaccharides, proteins, nucleic acids and their derivatives are of almost universal occurrence in living organisms, where they perform a variety of functions, many of which are not fully understood. The analysis of a macromolecule usually follows a sequence of identification based on biological activity, isolation and purification. The macromolecule is broken down to its component units which are separated chromatographically (for example as in Figure 3) to give the overall component analysis[4, 6, 7]. This is followed by determination of the sequence of component units to elucidate the primary structure, by physicochemical assessment of the secondary structure and finally by determination of the overall three dimensional tertiary and quaternary structure, using physicochemical and chemical methods. Such identification is very important, but the necessary equipment is expensive. Thus centres for analysis, such as the University of Birmingham Macromolecular Analysis Centre which carries out polysaccharide and protein component analysis, primary amino acid sequencing and peptide synthesis, are playing an important role and can be predicted to become greater contributors to the elucidation of macromolecular structure.

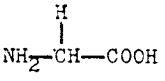
Biosynthesis and Man's Attempts

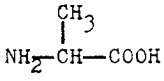
The control of polymerization reactions to yield synthetic polymers with the required properties is, when compared to represent nature, relatively crude. Man-made polymers are attempts to produce materials which mimic naturally occurring macromolecules but which are synthesized from a restricted number of monomers with limited chemical diversity. Most have a simple repetitive structure, unlike the majority of biological macromolecules, and the properties of the polymer produced are controlled by temperature, concentration and reaction time, etc.

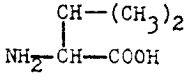
The synthesis of biological macromolecules is controlled by templates. These coding molecules are macromolecules which are present during biosynthesis and pro-

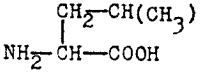
TABLE 1. STRUCTURES OF *a*–AMINO–ACIDS[a]

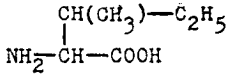
Neutral amino-acids (one amino-group and one carboxy-group)

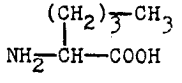

Glycine (g)

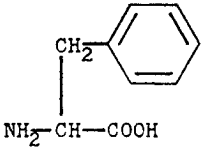

Alanine (g)

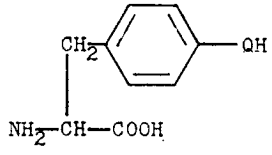

Valine (g,e)

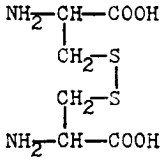

Leucine (g,e)

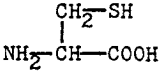

Isoleucine (g,e)

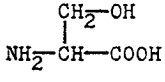

Norleucine (l)

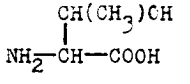

Phenylalanine (g,e)

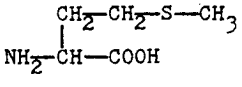

Tyrosine (g)

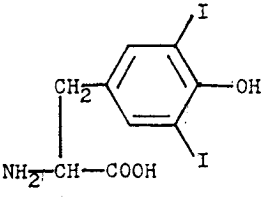

Cystine (g)


Cysteine (g)


Serine (g)


Threonine (g,e)


Methionine (g,e)


Di-iodotyrosine (or iodogorgic acid) (l)

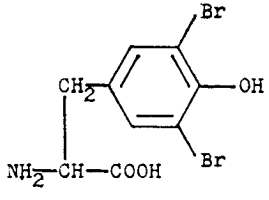
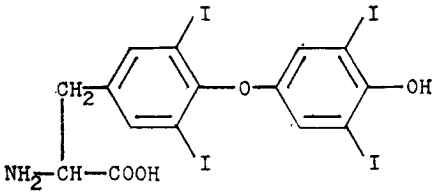
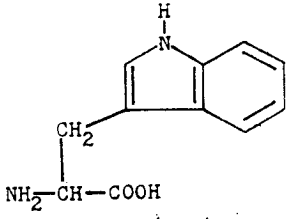
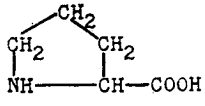
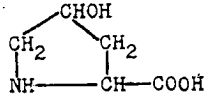

Dibromotyrosine (l)

TABLE 1 (cont.)

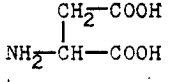

Thyroxine (l)

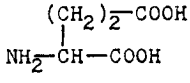

Tryptophan (g,e)

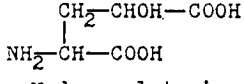

Proline (g)


Hydroxyproline (l)

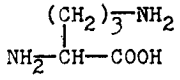
Acidic amino-acids (one amino-group and two carboxy-group)

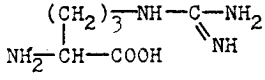

Aspartic acid (g)

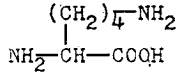

Glutamic acid (g)

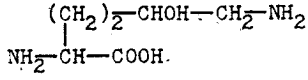

γ-Hydroxyglutamic acid (l)[b]

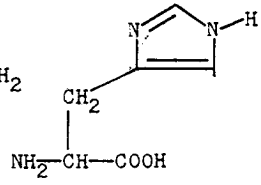
Basic amino-acids (two amino-groups and one carboxy-group)

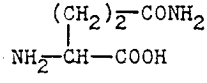

Ornithine[c]


Arginine (g)


Lysine (g,e)


δ-Hydroxylysine


Histidine (g)


Glutamine (g)

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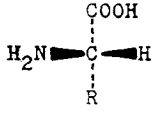
[a] General stereochemistry for L-amino-acids 

[b] Occurrences in proteins uncertain

[c] Ornithine is probably not present in proteins, but is formed by the hydrolysis of arginine

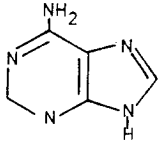
(e) essential in man

(g) general occurrence

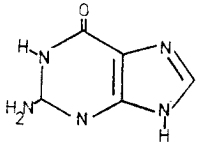
(l) less common occurrence

TABLE 2. STRUCTURES OF PURINE AND PYRIMIDINE BASES

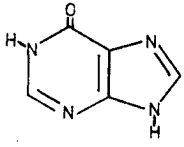
Purine bases



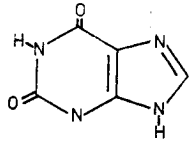
adenine
(DNA,RNA)



guanine
(DNA,RNA)

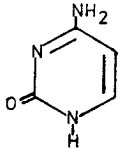


hypoxanthine
(RNA)

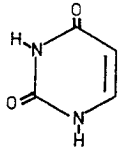


xanthine
(RNA)

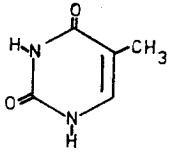
Pyrimidine bases



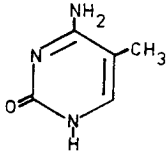
cytosine
(DNA,RNA)



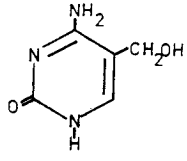
uracil
(RNA)



thymine
(DNA,RNA)



5-methyl cytosine
(DNA,RNA)



5-hydroxymethyl cytosine
(DNA,RNA)

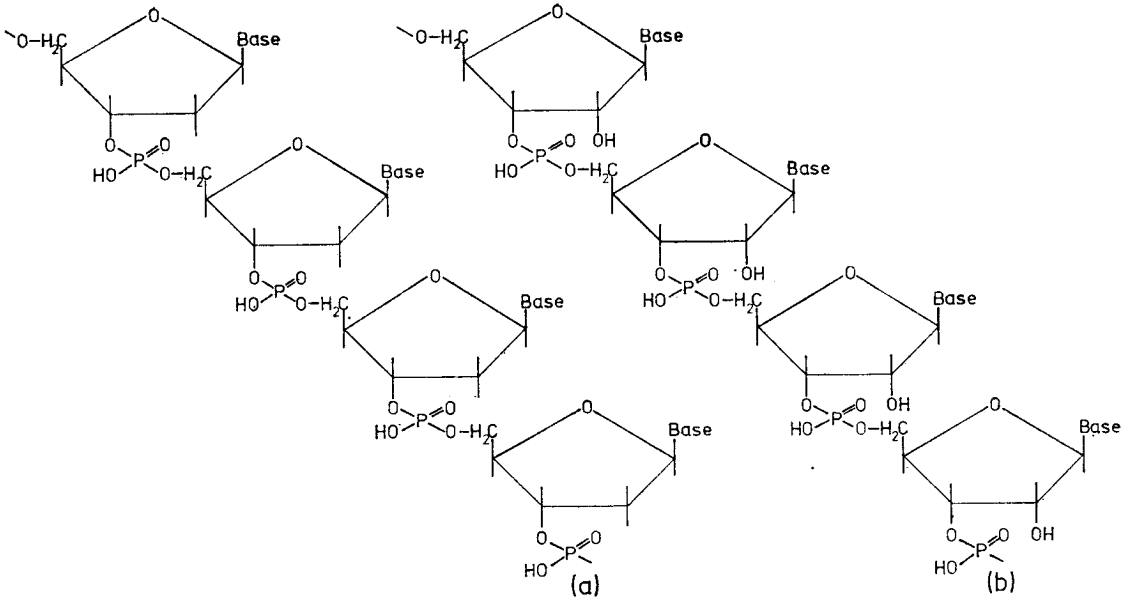


Fig. 1. Structure of (a) DNA and (b) RNA chains

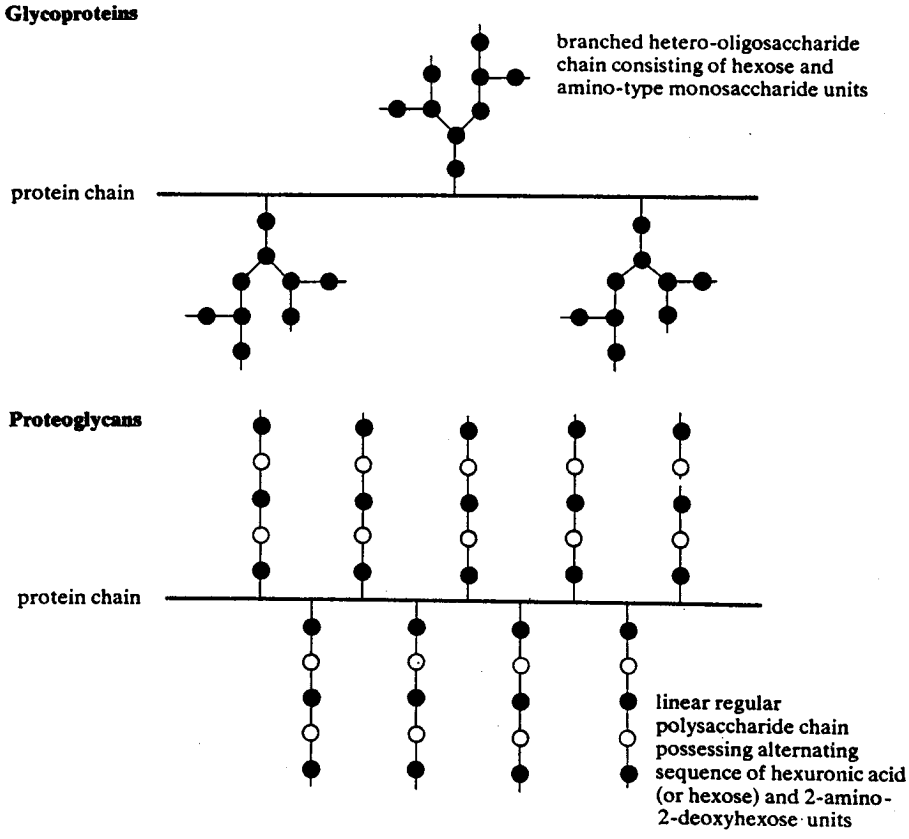


Fig. 2. General representation of (a) glycoproteins and (b) proteoglycans

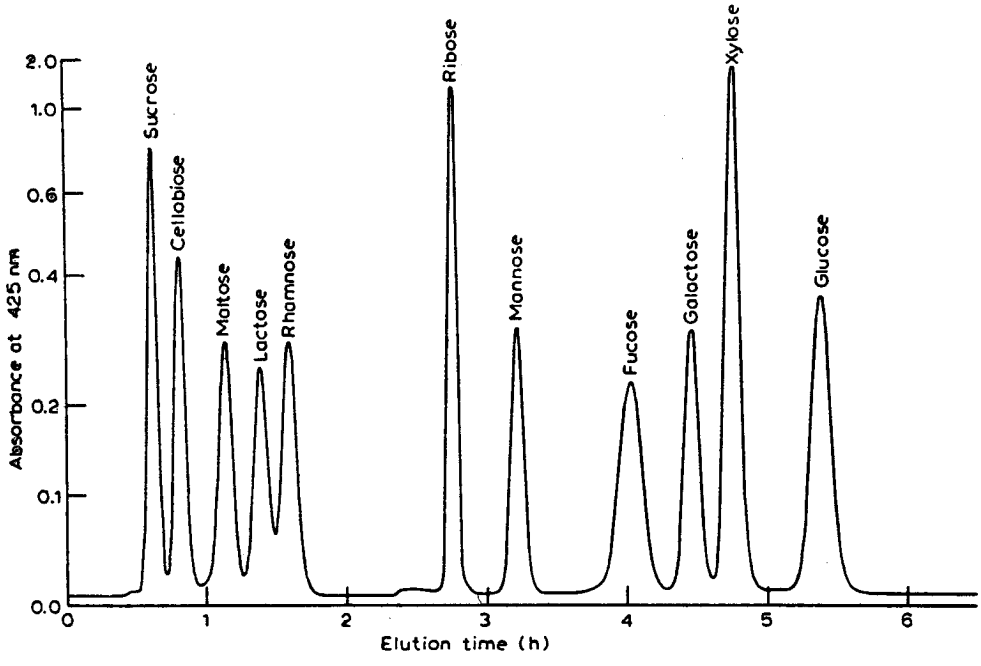
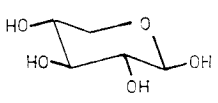


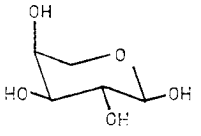
Fig. 3. Chromatography of neutral carbohydrates as derived from polysaccharides, glycoproteins etc., on Jeol LC-R-3 ion-exchange resin (borate form) using borate solutions as eluant

TABLE 3. STRUCTURES OF NATURALLY OCCURRING MONOSACCHARIDES[a]

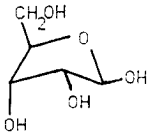
Pentoses



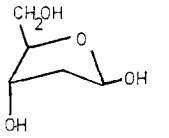
D-Xylopyranose (g)



L-Arabinopyranose (g)

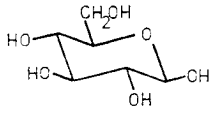


D-Ribofuranose (g)

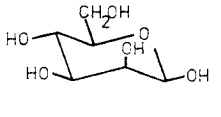


2-Deoxy-D-ribo-furanose (g)

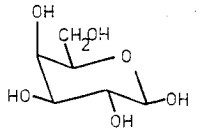
Hexoses



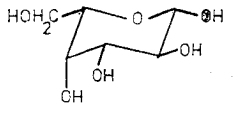
D-Glucopyranose (g)



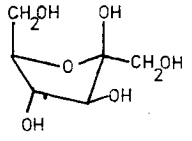
D-Mannopyranose (g)



D-Galactopyranose (g)

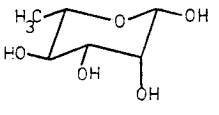


L-Galactopyranose (1)

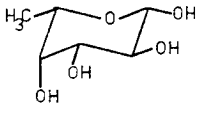


D-Fructofuranose (g)

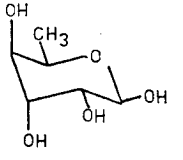
Deoxyhexoses



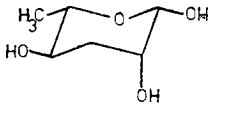
L-Rhamnopyranose (1)



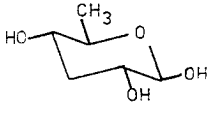
L-Fucopyranose (g)



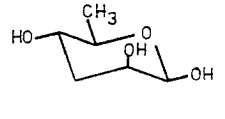
6-Deoxy-D-talose (1)



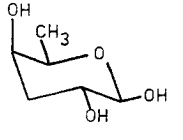
3,6-Dideoxy-L-manno-pyranose (Ascarylose) (1)



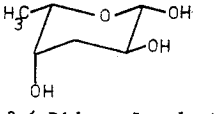
3,6-Dideoxy-D-gluco-pyranose (Paratose) (1)



3,6-Dideoxy-D-manno-pyranose (Tyvelose) (1)

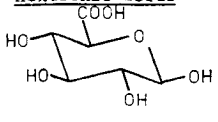


3,6-Dideoxy-D-galacto-pyranose (Abequose) (1)

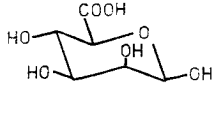


3,6-Dideoxy-L-galacto pyranose (Colitose) (1)

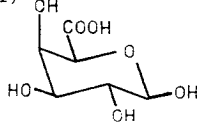
Hexuronic acids



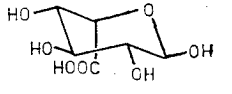
D-Glucopyranuronic acid (g)



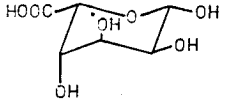
D-Mannopyranuronic acid (g)



D-Galactopyranuronic acid (g)



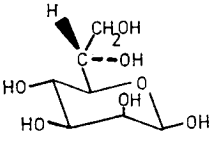
L-Idopyranuronic acid (1)



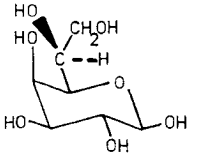
L-Gulopyranuronic acid (1)

TABLE 3 (cont.)

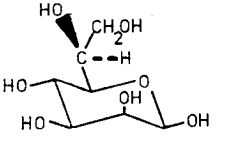
Heptoses



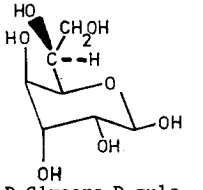
L-Glycero-D-manno-heptopyranose (1)



D-Glycero-D-galacto-heptopyranose (1)

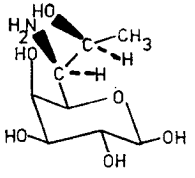


D-Glycero-D-manno-heptopyranose (1)

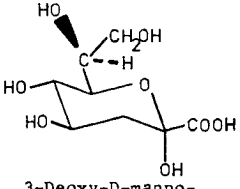


D-Glycero-D-gulo-heptopyranose (1)

Octoses

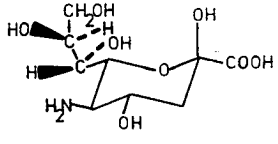


6-Amino-6,8-dideoxy-D-erythro-D-galacto-octopyranose (Lincosamine) (1)

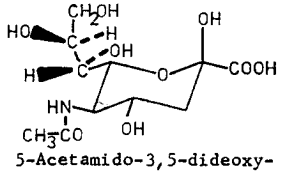


3-Deoxy-D-manno-octopyranulosonic acid (KDO) (1)

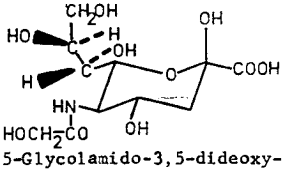
Nonoses



5-Amino-3,5-dideoxy-D-glycero-D-galacto-2-nonopyranulosonic acid (Neuraminic acid) (1)

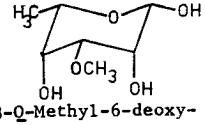


5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonopyranulosonic acid (NANA) (g)

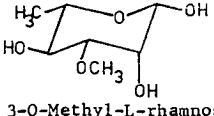


5-Glycolamido-3,5-dideoxy-D-glycero-D-galacto-2-nonopyranulosonic acid (NGNA) (1)

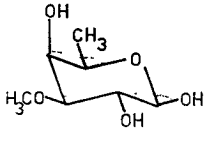
Ether derivatives



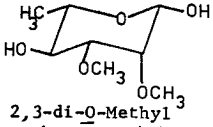
3-O-Methyl-6-deoxy-L-talose (Acovenose) (1)



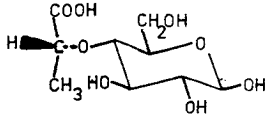
3-O-Methyl-L-rhamnose (Acofriose) (1)



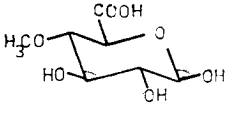
3-O-Methyl-D-fucose (Digitalose) (1)



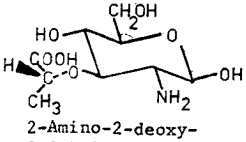
2,3-di-O-Methyl L-rhamnose (1)



4-O-D-Lactyl-D-glucopyranose (1)



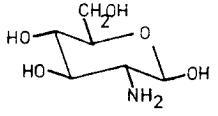
4-O-Methyl-D-glucopyranuronic acid (1)



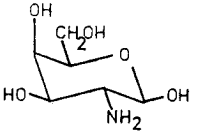
2-Amino-2-deoxy-3-O-D-lactyl-D-glucopyranose (Muramic acid) (1)

TABLE 3 (cont.)

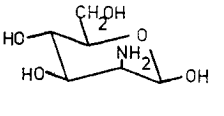
Hexosamines



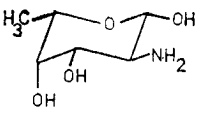
2-Amino-2-deoxy-D-glucopyranose (Glucosamine) (g)



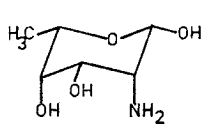
2-Amino-2-deoxy-D-galactopyranose (Galactosamine) (g)



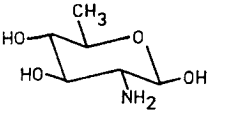
2-Amino-2-deoxy-D-mannopyranose (Mannosamine) (1)



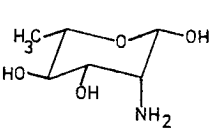
2-Amino-2-deoxy-L-fucopyranose (Fucosamine) (1)



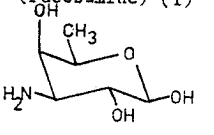
2-Amino-2,6-dideoxy-L-talopyranose (Pneumosamine) (1)



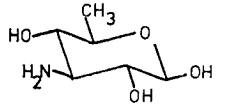
2-Amino-2,6-dideoxy-D-glucopyranose (Quinovosamine) (1)



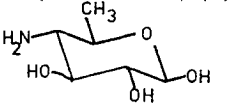
2-Amino-2,6-dideoxy L-mannopyranose (Rhamnosamine) (1)



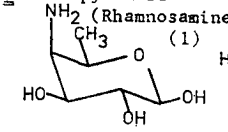
3-Amino-3,6-dideoxy-D-galactopyranose (1)



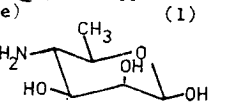
3-Amino-3,6-dideoxy-D-glucopyranose (1)



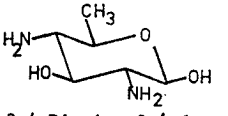
4-Amino-4,6-dideoxy-D-glucopyranose (Viosamine) (1)



4-Amino-4,6-dideoxy-D-galactopyranose (Thomasamine) (1)

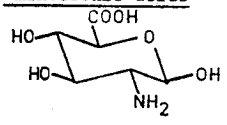


4-Amino-4,6-dideoxy-D-mannopyranose (Perosamine) (1)

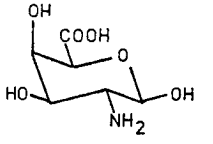


2,4-Diamino-2,4,6-trideoxy-D-glucopyranose (Bacillosamine) (1)

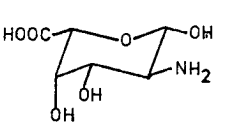
Aminouronic acids



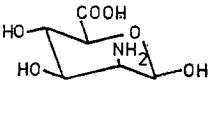
2-Amino-2-deoxy-D-glucopyranuronic acid (1).



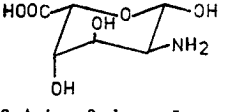
2-Amino-2-deoxy-D-galactopyranuronic acid (1)



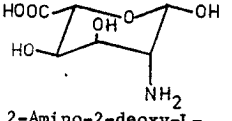
2-Amino-2-deoxy-L-galactopyranuronic acid (1)



2-Amino-2-deoxy-D-mannopyranuronic acid (1)



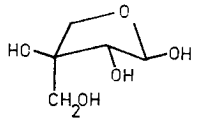
2-Amino-2-deoxy-L-gulopyranuronic acid (1)



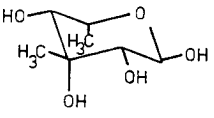
2-Amino-2-deoxy-L-altropyranuronic acid (1)

TABLE 3 (cont.)

Branched-chain monosaccharides



Apiose (l)



Vinelose (l)

[a]Each shown in one anomeric form only
(g) general occurrence
(l) less common occurrence

vide the exacting control of the synthesis which man has found so difficult to follow. The processes of biosynthesis of proteins, nucleic acids and polysaccharides are essentially very similar with inherent coding systems dictating the primary sequence produced. An illustration of the complexities of biosynthesis and the variety of specific catalysts required is given in Figure 4 which shows the biosynthesis of monosaccharides from D–glucose, and their modification and activation necessary for the biosynthesis of chondroitin 4–sulphate proteoglycan, a proteoglycan important for the maintenance of tissue structure in man. The protein chain is assembled on ribosomes from the component amino acids. The information concerning the sequence of amino acids is transferred from the DNA in the cell nucleus to the ribosomes by a single-stranded messenger ribonucleic acid (mRNA) (see also under nucleic acids). D–Xylose, D–galactose and D–glucuronic acid are attached in turn to the (glycosylated) L–serine residues by glycosyltransferases to give the 'linkage region' of the polysaccharide chain. To this linkage region the repeating unit is added by the sequential action of two glycosyltransferases and sulphatotransferase to give the polysaccharide chain.

Man has attempted for many years to synthesise natually occurring macromolecules with various degrees of success[8-10]. Once the sequence of amino acids, for example within an enzyme molecule, is known synthesis of the enzyme becomes a possibility. The technique of solid-phase synthesis[11] in which the peptide chain being synthesised is attached to an insoluble support to facilitate the removal of product at various stages of the reaction, (a process which is becoming routinely automated) has enabled a number of enzymes to be synthesised. Over 10 years ago assembly of 19 peptide fragments gave ribonuclease A with 80% specific activity[12]. Many syntheses have been thwarted by problems of purity at various stages of the reaction with the result that low activities are recorded. It is now well established[13] that the sequence of amino acids in a protein molecule carries with it all the information required for the polymer to assume the correct three dimensional stereochemical structure responsible for the natural molecule's high activity and so if the correct sequence is obtained with a high degree of purity high activities may be expected. Current work in this field centres on the production of enzyme analogues which have lower molecular weight and only copy the enzyme's binding and active sites.

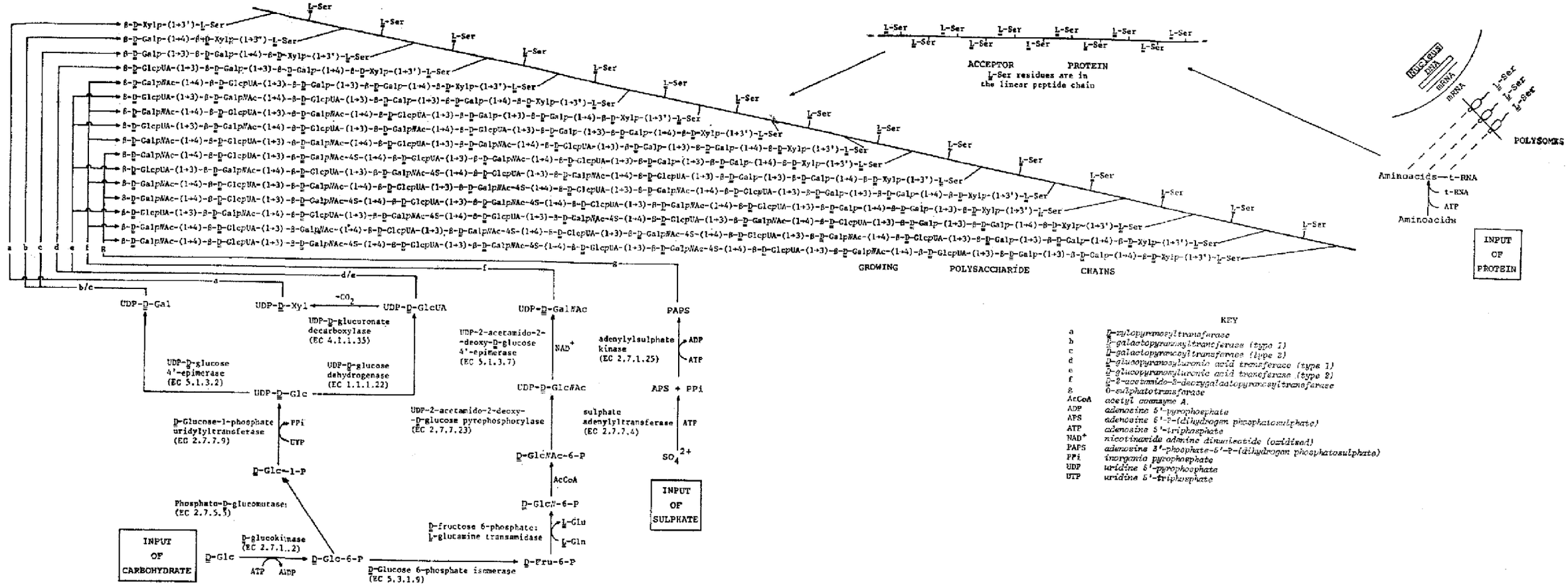


Fig. 4. Representative collation of the stages of the biosynthetic pathway to chondroitin sulphate proteoglycan

These compounds are used as models in the study of enzyme action by variation of the amino acid sequence at critical parts of the molecule.

The synthesis of polysaccharides (and the carbohydrate part of glycoproteins and proteoglycans) is even more elusive, because it is necessary to determine and then reproduce not only the sequence of monosaccharide units, but also, for each monosaccharide, the correct ring size, the correct selection of the hydroxy-groups for linkage to adjacent units and the correct selection of the stereochemistry at carbon–1. Such parameters give rise to sixteen possibilities for the way in which a single neutral hexose unit, such as D–galactose, may be linked within a carbohydrate chain.

In nucleic acid synthesis the major limitation of a purely chemical approach is the length of the defined sequence segments which may be prepared pure and in satisfactory yield. This problem is largely overcome when chemical synthesis is used in conjunction with enzymatic synthesis. This dual approach has been remarkably successful in synthesis of genes and their control regions.

The major contribution to gene synthesis has been that of Khorana and his colleagues[14], who synthesized the gene for the precursor of *Escherichia coli* $tRNA^{Tyr}$. This involved the specific joining of 26 chemically synthesized segments to form 4 duplexes which were enzymically joined. Subsequently the promoter and the terminator regions were sequenced and synthesized. The total gene so synthesized could be accurately transcribed to give a precursor which could be processed into $tRNA^{Tyr}$. Since this synthetic total gene was for a suppressor tRNA, it was possible, by cloning, to establish that it was biologically active because expression *in vivo* could be demonstrated by suppression of amber mutations.

The greatest difficulties for the prospect of man's synthesis of biological macromolecules arise therefore in the initial determination of sequences and primary structure (these are still unknown for the vast majority of macromolecules). The difficulty is even more extended for molecules which contain carbohydrate chains as part of the structure and where several molecules each in their own three dimensional form, need to be combined by non-covalent bonds to yield the overall, active molecules—the subunit phenomenon. Added to this is the fact that certain changes in the sequence do not necessarily upset the biological function. For example, two molecules produced by two species but with the same biological activity will differ in some parts of the sequence of monomer units along the chain. An example is bovine insulin which has the sequence L–Ala^{8}–L–Ser^{9}–L–Val^{10} and which is active in man although human insulin has the sequence L–Thr^{8}–L–Ser^{9}–L–Ile^{10}. As a result of biosynthetic and metabolic mechanisms the molecules from one of the species will all possess the same sequence.

Some further appreciation of the vast number of the possibilities and problems in natural macromolecules can be derived mathematically. For a macromolecule composed of a sequence of y monomer units, where any one of x monomer types can occupy each of the monomer positions along the sequence, the number of sequences possible (P) is derived from:—

$$P = x^y$$

Thus, in the case of proteins, where there are about 20 common amino acids, for a molecule 100 units long, which is by no means a large protein, there are $2^{100} \times 10^{100}$ ($= 1.27 \times 10^{130}$) sequence possibilities. This, together with the fact that it has not been demonstrated that there is only one possible tertiary structure for each of these sequences, explains the wide variety of biological activities possible in natural polymers. It is therefore obvious that the actual synthetic production of such macromolecules is beyond man and production has to be by isolation from nature with man frequently augmenting the biosynthetic process.

Uses and Functions

Man-made polymers are designed to satisfy a particular and pre-determined purpose. Biological macromolecules are extracted from systems in which they have specific functions (some of which we do not yet understand) in the maintenance of natural processes and are used extensively and to a far greater extent than man-made polymers. Industrial applications of the specialist properties of biological macromolecules are usually based on their natural function although they may not be recognised as macromolecules by the user. As more becomes known about biological functions, new applications can be expected to be developed. Table 4 indicates the vast diversity of biological functions of biological macromolecules and highlights a number of their industrial uses.

Proteins, Glycoproteins and Proteoglycans

Proteins act extensively as enzymes, which catalyse the chemical reactions which take place in the living cell, including hydrolysis, oxidation/reduction, group transfer, biosynthesis, etc. Control mechanisms of the processes of life are frequently mediated by proteins or glycoproteins, e.g. the glycoprotein and protein hormones such as follicle stimulating hormone and growth hormone respectively, the muscle contractant molecules actin and myosin, and metabolism modifiers such as insulin. Structural proteins such as keratin, collagen and elastin make up horn, wool, nails, tissue, etc. and are responsible for the rigidity and/or elasticity of such materials. Proteoglycans are major components of mammalian tissues and are responsible for maintaining tissue shape, stability, and transport phenomena. Many proteins have a specific chemical job to do as opposed to exerting a physicochemical effect, e.g. oxygen and iron transport by haemoglobin and transferrin respectively; similarly in the form of antibodies glycoproteins are produced as an immunological defense response to invasion of the body by foreign macromolecules.

Proteins in the commercial world are primarily used as foods. Whereas no single food item contains only protein, items such as fish, egg, meat, soya bean, sunflower seed, peas, beans and milk all contain significant proportions of protein among their non-water components. The growing threat to resources posed by the

rate of population growth on the available resources and increasing demand by industry is the principal concern, on a tonnage basis, of commercial aspects of protein production and use. Novel, edible protein sources for foods and foodstuffs include single cell sources, bacteria, yeasts, micro-fungi and algae, and extensive processing has now been set up to use vegetable protein sources such as soya bean. Processing of such protein sources has now reached the stage of production of successful simulation of protein from other sources, including meat. Since enzymes are catalysts, they are required in a lesser amount, but they are nevertheless produced in large quantities for conversion of large quantity materials, e.g. starch to D-glucose by glucoamylase. Small scale production of proteins is very active e.g. hormones for therapeutic use, antibodies for vaccination, and antibodies and antigens for clinical chemical assays particularly in the form of radioimmunoassays.

TABLE 4. FUNCTIONS AND USES OF BIOLOGICAL MACROMOLECULES

Macromolecule	Biological function	Industrial and medical uses
Protein	Amino acid source	Antibiotics
	Antibodies	Antibodies
	Enzymes	Antigens
	Hormones	Enzymes
	Nutrient storage	Food–body protein production
	Structural material	Hormones
	Transport	
Glycoproteins	Antibodies	Antibodies
	Blood group active substances	Enzymes
	Circulatory antifreeze agents	Hormones
	Connective tissue	
	Enzymes	
	Hormones	
	Immunoglobulin	
	Protective agents	
Proteoglycans	Connective tissue	Blood anticoagulants
	Filtration media	
	Lubricants	
	Shock resistors	
Nucleic acids	Storage and replication of genetic information	Antiviral agents
		Chemotherapeutic agents
		Genetic engineering
Polysaccharides	Carbohydrate sources	Construction materials
	Energy stores	Emulsifiers
	Structural materials	Film-forming agents
		Food–energy source
		Food bulking
		Gelling agents
		Pharmaceutical adsorbents
		Plasma extenders
		Stabilisers
		Vaccines

Water-insoluble enzymes

Enzymes artificially rendered water-insoluble have been developed comparatively recently, and yet the overall principle of attachment of an enzyme to an insoluble matrix is simple and simulates the natural mode of action and environment of enzymes embedded in biological membranes. The insolubilisation of enzymes with retention of activity has already made an impact on the chemistry of biological molecules and systems. The advantages of insoluble enzymes over their soluble counterparts are numerous, deriving principally from the fact that they can effectively perform the same catalytic action as soluble enzymes, yet without contaminating the substrate solution. The stability of an enzyme is often increased on insolubilization, and, as they can also be re-used without loss of activity, insoluble enzymes, once prepared, are more economical than soluble enzymes.

The enzymatic isomerization of D–glucose to D–fructose has attracted significant attention from scientific as well as industrial quarters[15,16]. In recent years, high D–fructose sugars ('isosyrups') produced by enzymatic isomerization of high dextrose-equivalent starch-derived syrups (Figure 5) have been introduced successfully on a high tonnage basis. A decisive factor for the ability of isosyrups to compete with cane sugar (sucrose) is that of enzyme costs. Whereas the enzyme (glucoamylase) costs for the conversion of starch to D–glucose (the first part of the conversion process) are relatively small, the cost of the D–glucose isomerase for the conversion of D–glucose to D–fructose (the second part of the conversion process) is high. The development of immobilized D–glucose isomerase makes multiple use of the enzyme possible and thus makes economy of the isomerization process more attractive[15]. Current research is being addressed to simplification/reduction of the need for cofactors in the *D*–glucose feed solution which, unless removed, contaminate the product.

It can be predicted that the development of supports which possess magnetic character[17-20] will be of great significance to the industrial use of enzymes. Such supports allow the immobilized enzyme to be removed from the substrate solution without the use of filtration or centrifugation. This allows the enzyme to be recovered from solutions containing insoluble by-products or contaminants (such as undegraded insoluble starch in the liquefaction of starch).

Many research laboratories have devised both special supports[21,22] to carry the enzyme and highly specific methods of immobilization in order to obtain active immobilized enzymes with additional specific properties particular to the immediate use. The repeated reusability and high operational stability of such systems has led to a decrease in the cost of routine analyses such as the analysis of D–glucose using D–glucose oxidase. Systems have been developed to incorporate immobilized enzymes: onto nylon reaction tubes[23] which are coupled to automated analysis equipment to give automated enzyme analysers for continuous determination of, for example, D–glucose in serum or urine using immobilized D–glucose oxidase; into ion-selective electrodes[24] to give enzyme electrodes for the detection of specific compounds such as L–amino acids using L–amino acid oxidase in conjunction with a cation-selective electrode to measure the ammonium ions produced; and more recently into nylon pipette tips[25] attached to pipettes to give immobilized enzyme pipettes used for a rapid, cheap,

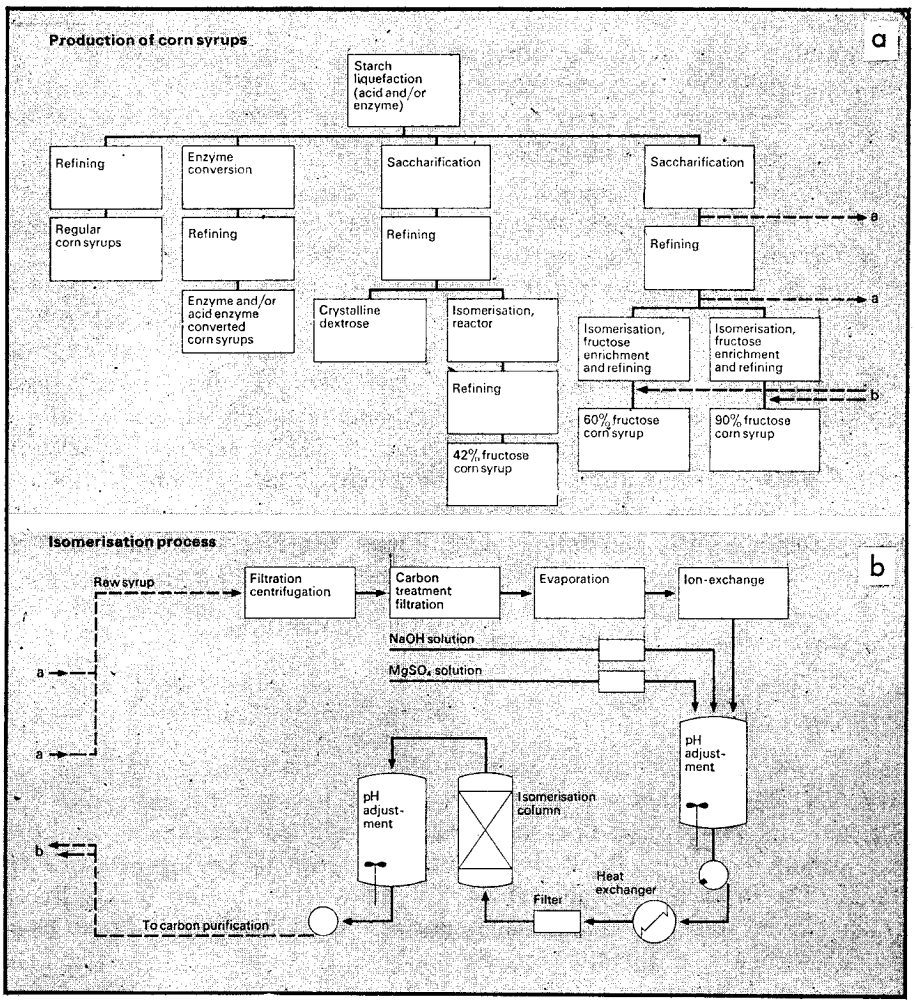


Fig. 5. (a) Key points in the processes for the production of corn syrups, particularly high fructose syrups. (b) shows detailed system arrangement for the isomerization stage

routine method for the determination of blood urea using immobilized urease. The use of immobilized enzymes for biological analysis has recently been reviewed[26].

For large scale industrial production, financial and practical constraints dictate that less sophisticated techniques than those outlined above be employed for enzyme immobilization. One such system devised for the isomerization of D-glucose to D-fructose (see above) is the cross-linking of microbial cells with glutaraldehyde[27] to give an insoluble mass in which, although the cells are now dead, some D-glucose isomerase remains active with the cross-linked cells providing the support matrix. This system is particularly attractive to industry due to the fact that the cost of producing a purified enzyme prior to crosslinking has been eliminated.

Another newer novel approach to the field of immobilized enzymes is the immobilization of microbial cells with retention of life[28] so that they can reproduce and thereby act as an automatically self-renewing form of immobilized enzyme. Apart from the obvious advantage this type of system has for industrial use, the potential of such systems has yet to be fully realised. One of the original ideas behind the development of immobilized enzymes was the study of how enzymes react in their natural environment, immobilized enzymes often being a closer representation of natural systems than soluble enzymes. The immobilization of whole cells means that enzymes can be readily studied in situations which resemble their natural environment. With the development of more immobilized cell systems it may become possible to apply this technology to the investigation of enzyme deficiency disorders by studying comparatively the action of particular enzymes in affected cells and in normal cells. A development of this could lead to more accurate diagnosis and ultimate treatment (by implantation of immobilized whole normal cells into the affected areas) of enzyme deficiency disorders.

Antibodies and Antigens

The production of antibodies and antigens is a widespread adjunct to clinical chemistry, not only for the production of vaccines, but also for radio- and enzyme-immunoassay kits which are essentially reagents for diagnoses and monitoring of therapy. Immunoassay, in which the unknown amount of homogeneous antigen to be determined is allowed to compete with a known amount of radio-, fluorescent marker-, or enzyme-labelled homogeneous antigen for binding antibody is now used extensively. Although antibodies can normally only be raised to macromolecular antigens, by chemical coupling of a small molecule to a carrier macromolecule and using the product as antigen, it is possible to raise antibodies to the original small molecule. Thus the concept of immunoassay is applicable to the sensitive assay of large and small molecules. For example, measurement of thyroxine (T4) in serum, long recognised as the most useful and, until now, the most time consuming and error-prone human-thyroid function test, can now be done simply, accurately and rapidly using a solid-phase radioimmunoassay procedure[29].

The immunological reactions that occur between antibodies and antigens are also being used to purify such macromolecules—by immunoadsorption[30]. The antibody (or antigen) is attached to a column matrix, and the solution containing the

antigen (or antibody) to be purified is loaded. The specific macromolecule becomes adsorbed (for subsequent elution) whilst impurities pass right through. This technique is permitting important and special antibodies, hitherto inaccessible by conventional purification techniques, to be purified. By this technique it has been possible to separate and purify whole antibody molecules and even to separate structurally similar, but immunologically different, chains which form part of the immunoglobulin macromolecule (see Figure 6) allowing the whole field of body defence mechanisms to be studied afresh. Such current research is attracting attention and finance from leading pharmaceutical companies.

Nucleic Acids

Deoxyribonucleic acid (DNA) is the molecule of heredity and ribonucleic acid (RNA) in the molecule involved in the transcription of genetic information. DNA and RNA are polymers containing sugar, base and phosphate (Figure 1). The sugars (2–deoxy–D–ribose and D–ribose in the case of DNA and RNA respectively) together with the phosphate perform a structural role whereas the purine and pyrimidine bases (Table 2) carry genetic information. The function of a DNA molecule is to carry the genetic information of a cell in such a way that this information can be passed uncorrupted from one generation to the next. For this to occur the DNA must be a stable molecule which can be exactly duplicated so that the two daughter cells arising at mitosis may each receive identical copies. The biological information is stored in the base sequences of the DNA. Mechanisms exist to correct for any errors which may occur in the duplicating process, or any damage arising through the action of chemicals or radiation. The information present in the DNA is made available to the cell by an ordered mechanism which does not affect the integrity of the DNA; conversely, the act of duplication of the DNA does not drastically interfere with the expression of the information contained therein.

In the process of transcription the genetic information is transferred from the DNA to the complementary mRNA. Finally in the process of protein synthesis, the genetic information is translated from the four-letter language of the mRNA into the twenty-letter language of the proteins by dictating which transfer RNA, with its specific amino acid, shall operate next on a growing protein chain.

The commercial aspects of DNA and RNA are very limited. The production of both is largely for the purpose of laboratory research. Deoxyribonucleic acid is produced for conversion to deoxynucleosides among which there are certain antiviral agents and it is in this field and that of cancer therapy (5–fluoro–deoxyuridine is currently used[31]) that derivatives of the nucleic acids hold greatest potential.

Polysaccharides

Polysaccharides are natural macromolecules of almost universal occurrence in living organisms where they perform a variety of functions. Examples are the mul-

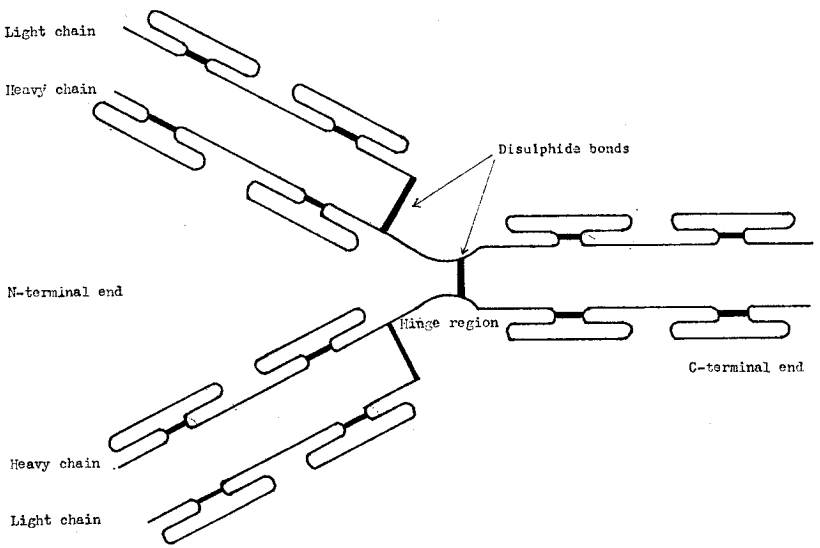


Fig. 6. Schematic representation of an immunoglobulin G macromolecule

tipurpose multiperformance material—wood (consisting largely of cellulose which is a polymer of D–glucose, and lignin) and the foodstuff—starch (mixed polymers of D–glucose). It is readily recognised that polysaccharides may act as skeletal substances in the cell walls of higher land plants and seaweeds, that they provide reserve food supplies in plants, micro-organisms, and animals, and that they may function as protective substances, in plants in the form of exudate gums sealing off sites of injury, and in micro-organisms as encapsulating shields. They also function as thickening agents in the joint fluids of animals and as protectants preventing tissues from desiccation.

Polysaccharides may also perform more specific roles, for example by being responsible for the type-specificity of the *Pneumomocci* pneumonia-generating organisms. Other macromolecules, which are not composed entirely of sugar units, contain blocks of monosaccharide residues as part of the molecular structure. The blood group substances, for example, are a group of glycoproteins in which the arrangements of sugar residues in the carbohydrate chains contribute towards the specificities of the different types, the differences between the different types being the result of a change on a single carbohydrate unit. For example removal of an α–linked 2–acetamido–2–deoxy–D–galactopyranosyl group from type A carbohydrate or of an α–linked D–galactopyranosyl group from type B carbohydrate by specific enzyme action results in both types being converted to type O carbohydrate. A composite structure has been proposed[32] to indicate which monosaccharide residues confer which specificity to the blood group substances (Figure 7).

Polysaccharides of economic importance are mainly derived from the plant kingdom. Cellulose, which is by far the most abundant of all polysaccharides, finds direct use in fibres from cotton, jute, flax, hemp and ramie, and as wood pulp it is used both for paper-making and as the starting material for formation of derivatives used in the manufacture of rayons and other fibres, films and sheetings, and certain plastics. Starch is, of course, the main carbohydrate polymer for human consumption notably in the form of cereal grains and root crops such as potatoes. Starch is also the main carbohydrate starting material for fermentation processes. Gums and mucilages from land plants and seaweeds find many uses in stabilizing emulsions, and as thickening and gelling agents.

Xanthan Gum

Xanthan gum is a high molecular weight carbohydrate (Figure 8) produced by bacteria which is important in both the food and non-food industries[33-36]. The wide range of industrial applications arise from its capacity, in common with other polysaccharides particularly high grade plant gums, for altering the rheological properties of aqueous solutions either by gelling or through the alteration of its flow characteristics, and current world wide production is about 5.5 Kt per annum. The advantage of microbial production and isolation compared to production by and from growing plants is that relatively cheap substrates can be used in a production unaffected by maritime pollution, weather or local disturbances (which do affect the plant polysaccharides), and which is far quicker.

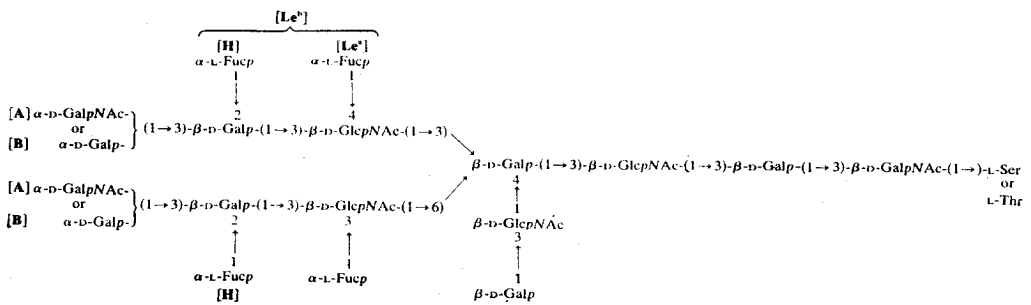


Fig. 7. Composite structure of the carbohydrate chain of blood group glycoproteins, indicating the residues which confer blood group A, B, H(O), Le^a and Le^b specificity

Xanthan is used extensively by the petroleum industry in both drilling and recovery procedures. In mud drilling xanthan is used to enable the clays etc. to remain in colloidal form through its emulsifying properties whilst providing a solution which has a high viscosity at low stress which decreases with increasing stress and which lubricates the drill bit and provides a counterbalance to the upward pressure of the oil. Mud drilling accounts for an annual consumption of about 2 Kt and a much larger proportion is used (potentially up to 4.5 Kt annually) is used to enhance the actual oil recovery. The polysaccharide solution improves the water flooding techniques by increasing the efficiency of contact with, and displacement of, oil through increasing viscosity and reducing permeability. The insensitivity of xanthan solutions to change viscosity with temperature or salt concentrations commonly found in oil wells gives it a major advantage over petroleum based polymers which, at present, are favoured for this purpose due to their lower cost.

Other non-food uses of xanthan include the gelling of explosives to aid desensitisation, glue expansion, suspension and stabilisation of herbicides, pesticides, fertilisers and fungicides, and production of glazes, paint, polishes, and pastes. The quantity of xanthan required is usually less than that of the hydrocolloid which it has replaced.

The major food use of xanthan is as a stabiliser but its advantageous mouth feel and flavour release properties add to its attraction. It is included in salad dressings, sauces, beverages, processed cheese and in the generation of textured food. It is also used in tinned, frozen and instant foods (including desserts, toppings and whips) in conjunction with locust bean gum to yield a stable gel. Since it is not metabolised, its use in low calorie foods is envisaged and it may even become a standard ingredient of bread. Its great range of compatability indicates that the gum has potential use in many pharmaceutical formulations.

In the production of xanthan by fermentation, *Xanthomonas campestris* is cultured in a well-aerated medium containing commercial D-glucose, a suitable nitrogen source, phosphate and trace elements. At the conclusion of the fermentation process, xanthan gum is recovered by precipitation with propan–2–ol, washing, drying and milling. The principal problem is the high cost of the propan–2–ol and current research is directed at selective isolation by specialist reagents[37], electrolysis and electrolytic flotation which at the same time overcome the extreme problems of handling the very viscous fermentation products.

Microbial Polysaccharides

Certain polysaccharides which are elaborated by microorganisms may be produced on a large scale[34-36]. The best known of these are the dextrans from strains of *Leuconostoc* species which are used as blood plasma extenders. The macromolecules of microbes are important in infection and biological warfare. Heparin, of animal origin, and the polysaccharide component of a proteoglycan (see above), is used extensively in medical chemistry as a blood-anticoagulant.

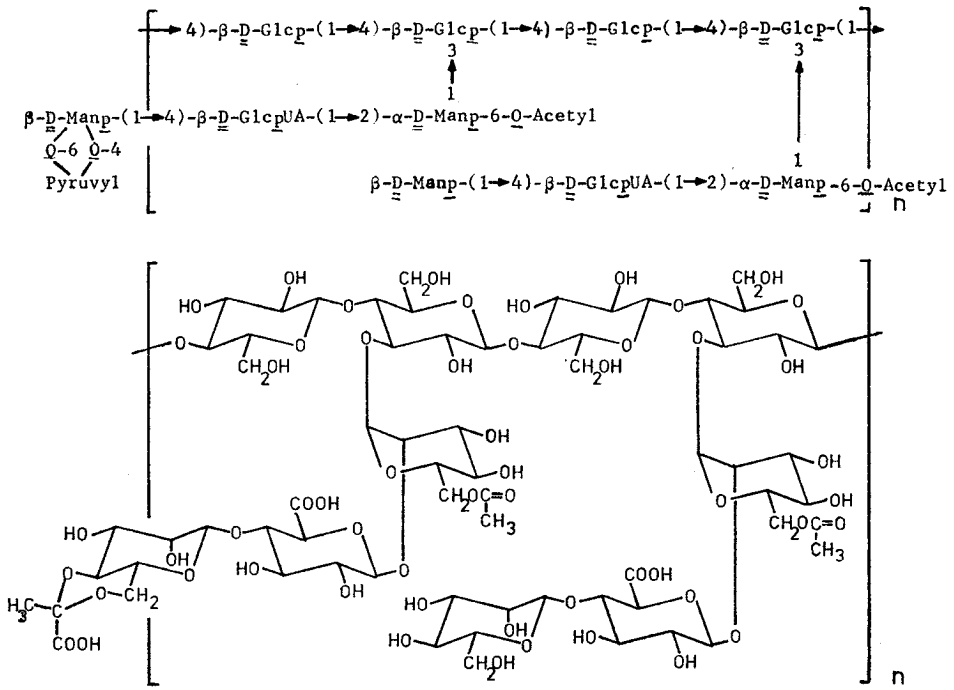


Fig. 8. Primary carbohydrate sequence for xanthan gum. The polysaccharide is shown in the acidic form. The pyruvic acid is present in non-stoichiometric proportions

Recently purified polysaccharides of bacterial origins have been prepared for use as vaccines against meningococcal[38] and pneumococcal[39] infections and, due to the ability of certain such polysaccharides to cross react with other antisera, they may also provide immunity against other infections[40, 41]. The advantages of using purified polysaccharide vaccines is the low risk of adverse reactions compared to vaccines containing whole cells (either living or dead) and such vaccines can give immunity to infection caused by antibiotic-resistant bacteria.

Conclusions

It is clear that with all the possible different sequences etc. in polysaccharide, protein and nucleic acid structures, and all the chemical modifications possible, there is still much to be discovered. The future for the field of chemistry of naturally occurring macromolecules is exciting since it can contribute further and extensively to an understanding of the chemical processes of life and to aiding society in an expanding way.

There is a great need for more emphasis to be put on the application of biological macromolecules in industry. Several recent reviews have been published[42, 43] outlining the present state of the art and calling for more innovation and application. There is much more work to be done on extraction and charactization of macromolecules from hitherto undeveloped sources such as jungles, swamps and desert areas of little explored regions of the earth. New materials will be found and new sources of already known materials will be developed. For industries to expand or be opened up in developing countries there is a need to have energy sources and supplies of materials which are not dependent on this world's dwindling oil supplies. Microbial technology could hold this key, particularly for the developing countries which have access to unexploited reserves of biological macromolecules and can set up completely new systems rather than having to convert from existing oil based methods.

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บทคัดย่อ

บทความนี้บรรยายถึงโมเลกุลขนาดใหญ่ที่สำคัญซึ่งเกิดขึ้นในธรรมชาติ คือ โปลีแซคคาไรด์ โปรตีน ไกลโคโปรตีน โปรติโอไกลแคนส์ และกรดนิวคลีอิก โดยได้ให้โครงสร้างของหน่วยย่อยที่เป็นองค์ประกอบของสารเหล่านี้ อันได้แก่ โมโนแซคคาไรด์ กรดอะมิโน เพียวรีน และพีริมิดีนเบส ได้เปรียบเทียบระหว่างความสลับซับซ้อนของโครงสร้างของสารที่มีในธรรมชาติ กับความพยายามอย่างง่าย ๆ ของมนุษย์ที่จะเลียนแบบสร้างมันขึ้นมา และได้ชี้ให้เห็นถึงประโยชน์และหน้าที่อันกว้างขวางของโครงสร้างจำเพาะหลายชนิดที่จะพบในธรรมชาติหรือสร้างขึ้นได้ โดยยกตัวอย่างจากโปลีเมอร์ในเนื้อเยื่อเกี่ยวพันจนถึงเอนไซม์ จากโปลีแซคคาไรด์ที่เป็นสารโครงสร้างในพืชจนถึงจุลชีวัน ได้เน้นถึงประโยชน์ที่จะได้รับจากการนำเอาโมเลกุลชีววิทยาขนาดใหญ่มาใช้ในกระบวนการและระบบต่าง ๆ ทั้งชนิดแยกและชนิดรวม