J.Sci.Soc.Thailand, 21(1995) 293-303

SCREENING OF GLYCOHYDROLASE ENZYMES IN THAI PLANT SEEDS FOR POTENTIAL USE IN OLIGOSACCHARIDE SYNTHESIS

RUDEE SURARIT[1,2], M.R. JISNUSON SVASTI[2,3], CHANTRAGAN SRISOMSAP[2], WIPA SUGINTA[3], SAUVARAT KHUNYOSHYENG[2], SIRINUN NILWARANGKOON[3], PINTUSORN HARNSAKUL[2], AND EM-ON BENJAVONGKULCHAI[4]

[1] *Dept. of Physiology and Biochemistry, Faculty of Dentistry, Mahidol University, Yothi St., Bangkok 10400;*

[2] *Laboratory of Biochemistry, Chulabhorn Research Institute, Vibhavadee Rangsit Rd., Bangkok 10210;*

[3] *Dept. of Biochemistry, Faculty of Science, Mahidol University, Rama VI Rd., Bangkok 10400;*

[4] *Dept. of Biochemistry, Faculty of Dentistry, Chulalongkorn University, Henri Dunant St., Bangkok 10330, Thailand.*

(Received September 21, 1995)

ABSTRACT

Thai plant seeds from 50 species in 17 families have been screened for glycohydrolase activity. The enzymes α-D-mannosidase, β-D-glucosidase, β-D-fucosidase, α-D-galactosidase, β-D-galactosidase, and β-D-N-acetyl-glucosaminidase were found in significant amounts (>0.1 μmole/ min/ g seed), and 38 species contained one or more of these enzymes. Reversal of hydrolytic action was tested by prolonged incubation with 50% (w/w) monosaccharide at pH 5, at elevated temperature. Significant levels of oligosaccharide synthesis was observed with α-D-mannosidase from Albizzia procera *Benth., β-D-glucosidase from* Dalbergia cochinchinensis *Pierre, and β-D-galactosidase from* Hibiscus sabdariffa *L. var.* altissima.

INTRODUCTION

Glycohydrolases (EC 3.2) catalyze the hydrolysis of glycosidic linkages formed between the hemiacetal hydroxyl group of a cyclic aldose or ketose and the hydroxyl group of a compound, which may be a constituent of an alkyl or aryl compound or a polyol. These enzymes hydrolyze simple glycosides, oligosaccharides and polysaccharides, as well as complex carbohydrates, such as glycoproteins and glycolipids into their individual building blocks, and occur widely in plants, fungi, animals, and bacteria[1,2]. The oligosaccharides which they hydrolyze play many important roles in biological processes, including various key metabolic events, growth related responses, defense mechanisms and cellular regulation[3,4], and also serve as sources of nutritious food for humans and animals.

Oligosaccharides may be extracted from biological sources, such as human milk, the colostrum of various animals, the faeces of unweaned infants[5], but the quantities obtained are small and prices are very high. Moreover, chemical synthesis[6-7] of specific oligosaccharides

is cumbersome, due to the many reactions under strong conditions that are required and the difficulty of obtaining regiospecific products. Enzymatic synthesis[5,8] is an attractive choice since it employs milder conditions and potentially has high specificity. Glycosyltransferase enzymes (EC 3.4) have been used for the oligosaccharide synthesis but often these enzymes are found in low amounts and require expensive substrates. Recently, glycohydrolases have become subjects of interest, not only for their applications in elucidating the structure of oligosaccharides[3,9,10], but also for their potential use in the synthesis of oligosaccharides by reversal of their hydrolytic actions[11,12]. Moreover, equilibrium controlled synthesis or kinetically controlled synthesis has been successfully performed with commercially available enzymes such as glucanase[13], mannosidases[14-16], glucoamylase[17] and galactosidase[12]. We initiated our studies in the field of oligosaccharide synthesis by searching for new glycohydrolases from naturally available plants in Thailand. This paper reports the glycohydrolase activities in the seeds of some Thai plants and indicates that some of these enzymes have potential use for oligosaccharide synthesis.

MATERIALS AND METHODS

Plant seeds

Plant seeds were kindly provided by the Field Crops Research Institute, Department of Agriculture, Ministry of Agriculture, Thailand, the Gene Bank, National Research Council of Thailand and the ASEAN-Canada Forest Tree Seed Center, Muaklek, Saraburi, Thailand. The actual selection of most plants was based primarily on their availability and abundance.

Extraction of enzymes

Seeds were surface-sterilized by treatment with sodium hypochlorite, imbibed overnight in double distilled water and homogenized in 2 volumes of 0.05 M sodium acetate buffer, pH 5.0 containing 1 mM phenylmethylsulfonylfluoride (PMSF) and 5% (w/v) polyvinyl-polypyrrolidone (PVPP). The homogenate was filtered through cheesecloth and centrifuged at 12,000 g for 30 min. The supernatant was stirred with 25% (w/v) Dowex 2-X8 for 1 h, and centrifuged again at 12,000 g for another 30 min to yield the crude extract.

Activity screening

The enzyme activities were screened using synthetic p-nitrophenyl (p-NP-) glycoside substrates[2], which were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.. The standard assay mixture contained 50 μl of suitably diluted enzyme, 100 μl of 0.01 M p-NP-glycoside, 850 μl of 0.1 M sodium acetate buffer, pH 5.0. The reaction was incubated at 30° C for 10 min and stopped by addition of 2 M Na_2CO_3. p-Nitrophenol released was quantitated spectrophotometrically at 400 nm. One unit of enzyme is defined as the amount of enzyme releasing 1 μmol p-nitrophenol per min at 30° C.

Partial purification

While crude extracts were used directly for determination of hydrolytic activity, they were generally too dilute in terms of enzyme concentration for synthesis studies. Thus

crude extracts were usually partially purified by 35%-65% ammonium sulfate fractionation at 4 °C. In the case of β-galactosidase from *Hibiscus sabdariffa* L. var. *altissima* was freed from α-mannosidase contamination by passing the dialyzed ammonium sulfate fraction through a DEAE-cellulose column, equilibrated with 10 mM potassium phosphate buffer, pH 7.0, and using the unbound fraction.

Test of oligosaccharide synthesis

Oligosaccharide synthesis was studied using reverse reactions of the enzymes. Each enzyme (2-10 U/ml final concentration) was incubated with suitable monosaccharides at high concentration (30-80% (w/w)) in sodium acetate buffer pH 5, at elevated temperature (50°- 60° C) for 1-14 days. Products were analyzed by h.p.l.c. using Waters 625 LC instrument equipped with refractive index detector (Waters 410), typically employing an Aminex HPX-87C column at 85° C.

RESULTS

Screening of glycohydrolase enzymes in Thai plant seeds

The hydrolytic activity of nine glycosidase enzymes, namely α-D-mannosidase, β-D-glucosidase, α-D-glucosidase, β-D-fucosidase, β-D-galactosidase, α-D-galactosidase, α-D-arabinosidase, β-D-N-acetyl-glucosaminidase and α-L-fucosidase were tested in the crude extracts from the seeds of fifty Thai plant species from 17 families. The results (Table 1) showed that six major enzymes were found in seed extracts at levels greater than 0.1 μmole/min/ g seed. A total of 38 species contained one or more of these enzymes at these levels. α-D-mannosidase was the most frequently found enzyme, present in most species of bean (e.g. *Glycine max* and *Vigna radiata* L. Wilzcek) and also in other species, such as *Albizzia procera* Benth. *Hibiscus* spp. and *Corcorus* spp., generally contained several glycosidases, including α-D-mannosidase, β-D-galactosidase, β-D-N-acetyl-glucosaminidase, α-D-galactosidase and β-D-fucosidase. Most interestingly, very high levels of β-D-glucosidase and β-D-fucosidase were found in *Dalbergia cochinchinensis* Pierre, more than 10-fold higher than the level of glycohydrolases found in other plant seeds. Lower levels of the latter two enzymes were also present in *Dalbergia nigrescens* Kurz.. *Gliricidia sepium* Steud. was found to contain relatively high activity of β-D-fucosidase, while the β-D-glucosidase activity was found to be not significant.

Test for oligosaccharide synthesis

Incubation of ammonium sulfate fractions, containing α-D-mannosidase from *Albizzia procera* Benth. with 50% (w/w) D-mannose at 50° C for 7 days gave good yields of mannose disaccharides and trisaccharides detectable by h.p.l.c. (Figure 1). A lower level of synthesis was observed with partially purified β-D-galactosidase from *Hibiscus sabdariffa* L. var. *altissima*, when incubated with D-galactose (Figure 2). Incubation of *Dalbergia cochinchinensis* Pierre crude extract containing β-D-glucosidase and β-D-fucosidase with 50% (w/w) of D-glucose gave intermediate levels of synthesis, with both disaccharides and trisaccharides being observed (Figure 3). Synthesis yield may be estimated from the combined area of the

TABLE 1. Glycohydrolase Enzyme Activities in Thai Plant Seeds (μmole/min/g dry seed)

Botanical Name	Common Name	α-D-Man	β-D-GlcNac	α-D-Gal	β-D-Gal	β-D-Fuc	β-D-Glc
F. Araceae							
Amorphophallus campanulatus Blume	บุก	0.11	NS	0.11	NS	NS	NS
F. Caesalpinaceae (Leguminoceae)							
Afzelia xylocarpa Craib.	มะค่าโมง	NS	NS	ND	NS	NS	NS
Cassia angustifolia Vahl.	มะขามแขก	NS	NS	NS	NS	NS	NS
Cassia bakeriana Craib.	กัลปพฤกษ์/Pink shower	NS	NS	ND	NS	NS	NS
Cassia fistula Linn.	คูน/Golden shower	NS	NS	NS	NS	NS	NS
Cassia floubunda Cav.	ขี้เหล็กอเมริกา	NS	NS	ND	NS	NS	NS
Cassia garrettiana Craib.	แสมสาร	NS	NS	NS	NS	NS	NS
Cassia surattensis	ทรงบาดาล	0.13	NS	NS	NS	NS	NS
Delonix regia Rafin	หางนกยูงฝรั่ง/Flame Tree	NS	NS	NS	0.15	NS	NS
Peltophorum inerme Llanos.	นนทรีทอง/Yellow Flame	NS	NS	ND	NS	NS	NS
Peltophorum dasyrachis Kurz.	นนทรีป่า/Jungle Flame	0.20	NS	0.16	NS	NS	NS
Sindora siamensis Teijsm.	มะค่าแต้	NS	NS	ND	NS	NS	NS
F. Caricaceae							
Carica papaya Linn.	มะละกอ/Papaya	NS	NS	ND	NS	NS	ND
F. Casuarinaceae							
Casuarina equisetifolia Linn.	สนทะเล/Ironwood	NS	NS	ND	NS	NS	NS
Casuarina junghuhniana Mig.	สนประดิพัทธ์/Pine Tree	NS	NS	ND	NS	NS	0.10
F. Compositae							
Helianthus annus	ทานตะวัน/Sunflower	0.20	0.10	0.24	NS	NS	NS
F. Cucurbitaceae							
Cucumis sativus Linn.	แตงกวา/Cucumber	0.11	NS	NS	0.35	NS	NS
Cucurbita moschata (Duch.) Poir.	ฟักทอง/Pumpkin	0.15	NS	NS	0.30	NS	NS
Luffa acutangula (L.) Roxb.	บวบเหลี่ยม/Gourd	NS	NS	NS	0.17	NS	NS
Momordica charantia Linn.	มะระ/Bitter melon	NS	0.12	NS	0.23	NS	NS
F. Faboceae (Leguminoceae)							
Arachis hypogaea L.	ถั่วลิสง/Peanut	0.14	NS	NS	NS	NS	NS
Glycine max	ถั่วเหลือง/Soybean	0.53	NS	0.21	0.17	NS	NS
Vigna radiata (L.) Wilzcek	ถั่วเขียว/Mung Bean	0.43	0.11	0.15	0.18	NS	NS
Vigna sinensis L. Saviex Hassk	ถั่วพุ่ม/Cow Pea	0.33	NS	0.12	0.28	NS	NS
Vigna sinensis Saviex	ถั่วดำ/Black Bean	0.11	NS	0.19	NS	NS	NS
F. Gramineae							
Sesamum indicum L.	งาดำ/Black sesame	0.31	NS	0.27	0.12	NS	NS
Sesamum spp.	งาขาว/White sesame	0.27	NS	0.49	0.20	NS	NS

Botanical Name	Common Name	α-D-Man	β-D-GlcNac	α-D-Gal	β-D-Gal	β-D-Fuc	β-D-Glc
F. Malvaceae							
Gossypium arborium L.	ฝ้าย A-25/Cotton	0.14	NS	0.13	0.17	NS	NS
Gossypium hirsutum L.	ฝ้ายศรีสำโรง/Cotton	0.28	NS	0.23	0.28	NS	0.13
Hibiscus cannabinus L.	ปอคิวบา/Kenaf	0.49	0.15	0.27	0.32	NS	NS
Hibiscus sabdariffa L. var *altissima*	ปอแก้ว/Thai jute	0.82	0.35	0.39	0.92	0.17	0.21
Hibiscus sabdariffa L. var *sabdariffa*	กระเจี๊ยบแดง/Red sorrele	1.15	0.40	0.40	0.63	0.11	NS
F. Meliaceae							
Malia azedarach Linn.	เลี่ยน/Chinaberry	NS	NS	NS	NS	NS	NS
Swietenia mahogani L.	มะฮอกกานี/Mahogany	0.28	NS	NS	0.12	0.12	0.12
F. Mimosaceae							
Acacia auriculaeformis Cunn.	กระถินณรงค์/Wattle	0.94	0.64	0.32	0.15	NS	1.06
Acacia catechu	สีเสียดแก่น	0.56	0.56	0.61	NS	NS	NS
Acacia farnesiana Willd.	กระถินบ้าน	NS	NS	0.16	NS	NS	NS
Albizzia procera Benth.	ถ่อน	1.24	0.14	NS	NS	NS	NS
Parkia speciosa Hassk.	สะตอ	NS	NS	NS	NS	NS	NS
F. Papilionaceae (Leguminoceae.)							
Dalbergia cochinchinensis Pierre	พะยูง/Blackwood	0.31	0.29	0.17	0.32	26.3	17.4
Dalbergia nigrescens Kurz.	ฉนวน	0.42	NS	NS	NS	2.28	1.18
Gliricidia sepium Steud.	แคฝรั่ง/Madre de Cocoa	0.50	0.16	0.61	0.28	1.06	NS
Sesbania grandiflora	แคบ้าน	0.37	NS	0.20	0.15	ND	NS
F. Pinaceae							
Pinus Khasya Royle.	สนสามใบ/Pine	NS	NS	0.15	0.15	NS	NS
F. Rutaceae							
Acronychia laurifolia Blume.	ยมป่า/Indian Mahogany	0.17	0.25	NS	NS	0.20	0.14
F. Solanaceae							
Capsicum annuum L.	พริกห้วยสีทน/Chilli	0.13	0.11	NS	NS	NS	NS
F. Tiliaceae							
Corcorus capsularis L.	ปอกระเจาฝักกลม/Tossa jute	0.50	0.48	0.20	0.74	0.47	0.30
Corcorus olitrorius L.	ปอกระเจาฝักยาว/Tossa jute	0.30	0.33	0.18	0.53	0.20	0.12
F. Verbenaceae							
Gmelima arborea Roxb.	ซ้อ	NS	NS	NS	NS	NS	NS
Tectona grandis Linn.f.	สัก/Teak	NS	NS	NS	NS	NS	0.22

ND = not determined; NS = not significant (<0.10 μmol/min/g seed); Man = mannosidase; GlcNac = N-acetyl-glucosaminidase; Gal = galactosidase; Fuc = fucosidase; Glc = glucosidase.

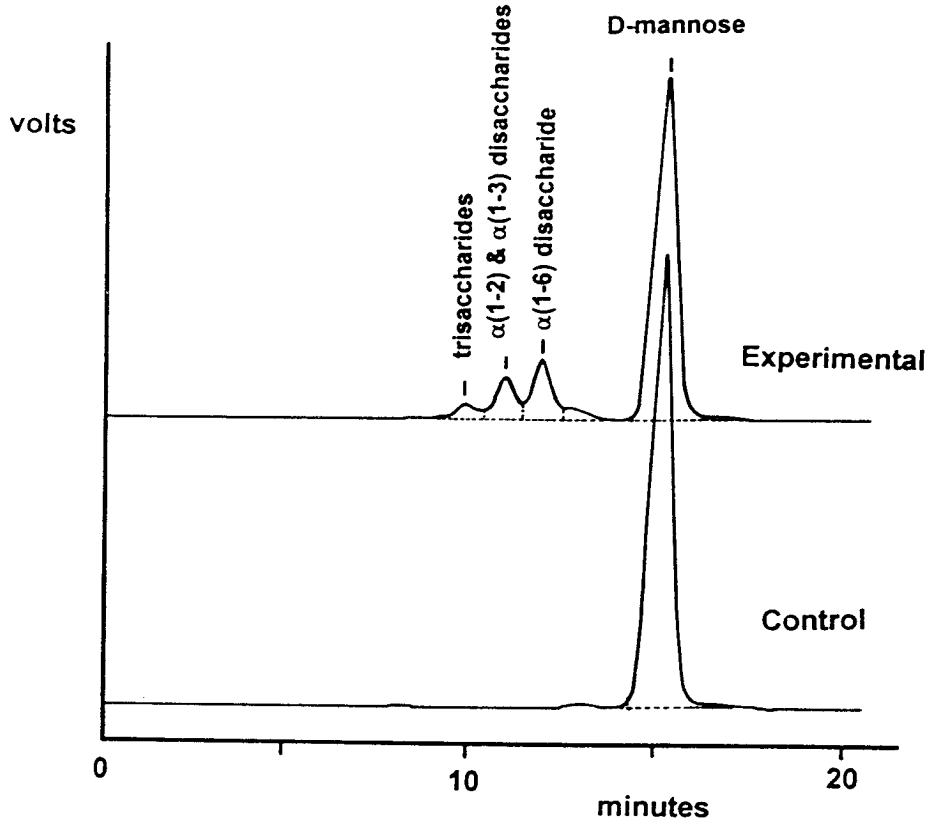

Fig. 1. H.p.l.c analysis of the oligosaccharide products obtained by incubating *Albizzia procera* Benth. ammonium sulfate fraction containing α-mannosidase with 50% (w/w) D-mannose at pH 5, 50° C for 7 days. *Experimental* and *Control* show reaction mixtures incubated with and without enzyme respectively.

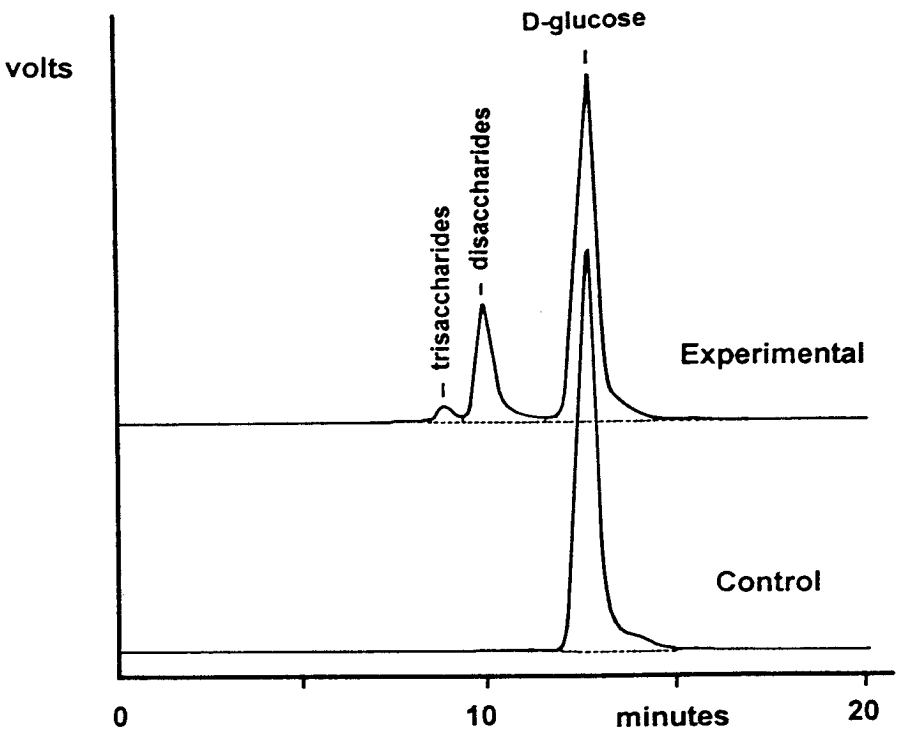

Fig. 2. H.p.l.c analysis of the oligosaccharide products obtained by incubating β-glucosidase and β-fucosidase from *Dalbergia cochinchinensis* Pierre crude extract with 50% (w/w) D-glucose at pH 5, 50°C for 7 days. *Experimental* and *Control* show reaction mixtures incubated with and without enzyme respectively.

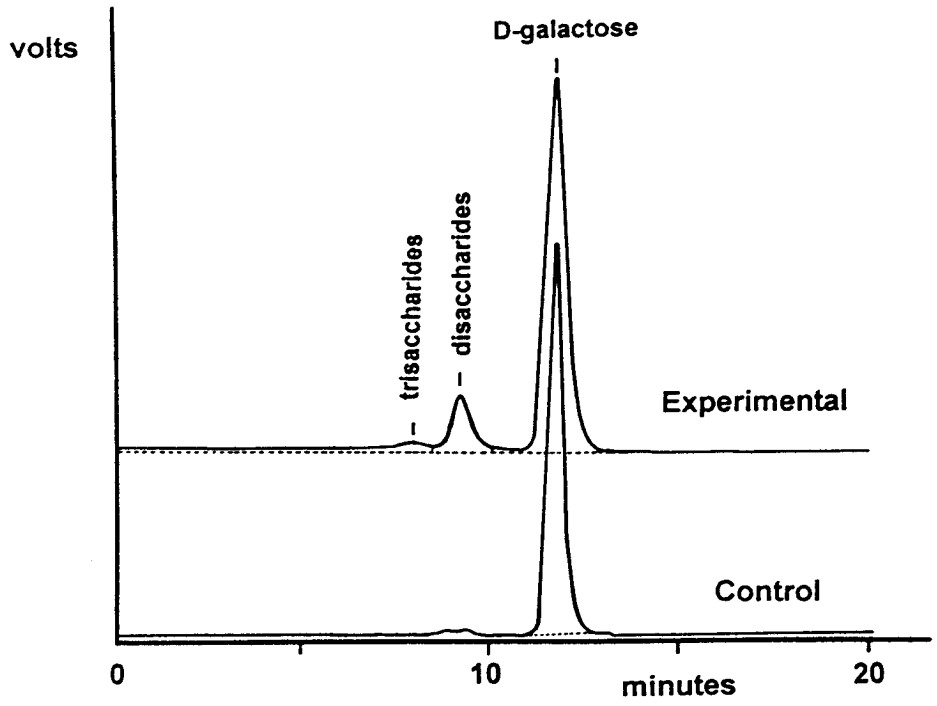

Fig. 3. H.p.l.c analysis of the oligosaccharide products obtained by incubating partially purified β-galactosidase from *Hibiscus sabdariffa* var. *altissima* with 50% (w/w) D-galactose at pH 5, 50° C for 7 days. *Experimental* and *Control* show reaction mixtures incubated with and without enzyme respectively.

disaccharide and trisaccharide peaks as a percent of the sum of the mono-, di-, and trisaccharide peak areas. In general, total synthesis was higher for α-D-mannosidase than for β-D-glucosidase and β-D-galactosidase (35-60%, 15-30% and 10-15% respectively).

DISCUSSION

Techniques for the reversal of enzyme action are well developed and some hydrolases and ligases are used commercially in reverse. Glycohydrolases are hydrolytic enzymes and synthesis of oligosaccharides with these enzymes can be carried out as an equilibrium-controlled or as a kinetically-controlled process. Equilibrium-controlled synthesis was first demonstrated by Bourquelot and Bridel in 1912[18]. In this approach, high concentrations of monosaccharide are used to shift the equilibrium towards synthesis, while high temperatures are used to accelerate the reaction so that equilibrium is reached. Kinetically controlled synthesis with glycosidases, also called the transglycosidation reaction, was reported in 1935 by Rabate[19]. In this approach an efficient glycosyl donor, such as a glycoside containing an aliphatic or aromatic aglycon[20], is reacted with high concentrations of acceptor. The yield of the synthesis product will depend on the rate of product formation compared to the rate of hydrolysis, and maximal yields of product require selection of suitable reaction time.

This study reports the screening of glycohydrolase enzymes from Thai plant seeds for possible use in oligosaccharide synthesis. Such screening studies necessarily involve some compromises to limit the experimentation to manageable proportions. Seeds were selected rather than other tissues since most seeds contain storage polysaccharides, and should consequently contain enzymes for hydrolyzing them. In addition, since there were no previous studies of which families would have higher levels of glycohydrolases, the selection of species screened represented those most readily available to us. Moreover, biological materials show variability, and the seeds obtained probably showed different degrees of viability. Enzyme levels may also depend on the germination state, but to simplify matters, only one condition of germination was studied. The pH used for the hydrolysis reaction was chosen as pH 5, since glycohydrolases from plants tend to have pH optima ranging from about 3.5 to 6.5.

Despite the above limitations, 6 glycohydrolase enzymes were found at significant levels in 38 out of the 50 species studied. One of the species studied, *Dalbergia cochinchinensis* Pierre, contained remarkably high levels of the enzymes β-D-glucosidase and β-D-fucosidase. α-Mannosidases were prevalent in many species, particularly in *Albizzia procera* Benth. β-D-galactosidase was found at moderately high levels in *Hibiscus sabdariffa* L. var. *altissima* and related species. Some of these enzymes that are present at high level may be useful, since it should be possible to isolate and purify them in adequate amounts. However, some species with moderate levels of enzymes, such as the bean family which contain α-mannosidases, may also be useful since most beans can be readily obtained in large amounts. Moreover, the availability of α-mannosidases from a variety of species is of interest, since enzymes from different sources may show different bond specificity, which may be useful for regiospecific synthesis, and approaches to improve regiospecificity have also been discussed[21].

In the present tests for oligosaccharide synthesizing activity, the equilibrium approach has been used, because of the simplicity of experimentation, requiring only the incubation of the crude extract or ammonium sulfate fraction with high concentrations of monosaccharide at elevated temperature for a prolonged period. The kinetic approach involves more detailed time-course studies, and selection of the appropriate concentrations of donor and acceptors. H.p.l.c. was selected as the analytical tool, since it provides quantitative data on the extent of synthesis, which t.l.c. cannot readily do. In the studies reported here using rather crude enzyme preparations, synthesis was demonstrated with α-D-mannosidase, β-D-galactosidase, and β-D-glucosidase. The extent of partial purification required before synthesis studies depends on the nature of the crude extract. With the β-D-glucosidase and β-D-fucosidase from *Dalbergia cochinchinensis* Pierre, which are actually the same enzyme (Svasti *et al.*, unpublished data), the concentration of enzyme in the crude extract was of a sufficiently high level to enable the crude extract to be used for synthesis studies without any other treatment. With enzymes from most other plant seeds, such as α-mannosidase from *Albizzia procera* Benth., crude extracts were too dilute and had to be concentrated by ammonium sulfate fractionation prior to use. In the case of *Hibiscus sabdariffa* L. var. *altissima*, moderate levels of both α-mannosidase and β-galactosidase were present in the crude extract. Thus, another DEAE-cellulose chromatography step was used in addition to the ammonium sulfate fractionation step, so as to remove α-mannosidase (which has a high synthetic capability) before studying the synthesis reaction with β-galactosidase.

Although the work described here has been performed with partially purified enzyme, studies to be reported elsewhere show that synthesis is also observed with purified preparations of the three enzymes. Total levels of synthesis varied with the enzyme, with α-mannosidases giving yields as high as 30-60% synthesis. Such high yields from reversing the reaction of an enzyme may seem surprising. However, high monosaccharide concentration not only shifts the equilibrium towards synthesis because of the monosaccharide concentration *per se*, but they also lower water activity, because in a 50% (w/w) monosaccharide solution, the ratio of the molar concentration of -OH groups from the sugar to the molar concentration of -OH groups from water molecules will be as high as 1:2.

In conclusion, the presence of six glycohydrolase enzymes has been demonstrated in the Thai plant seeds so far screened, and evidence has been presented to indicate that the hydrolytic action of some of these glycohydrolase enzymes may be reversed leading to a net synthesis of oligosaccharides. These studies provide a useful starting point for the selection of suitable enzymes for more detailed studies. Indeed, further studies are now in progress on the purifications and properties of some of these enzymes, as well as on improving the yields of synthesis and characterizing the structures of the oligosaccharide products formed.

ACKNOWLEDGMENTS

This research was supported by grants from the Chulabhorn Research Institute and the National Research Council of Thailand Thai-UK (NRCT-ROYSOC) Collaborative Program.

REFERENCES

1. Dey, P.M. (1984) *Adv. Enzymol.* **56**, 141-249.
2. Montreuil, J., Bouquelet, S., Debray, H., Fournet, B., Spik, G., and Strecker, G. (1986) in Glycoproteins (Chaplin, M.F. and Kennedy, F., eds.) pp. 143-204, IRL Press, Oxford.
3. Welply, J.K. (1989) *Trends in Biotechnol.* **7**, 5-10.
4. Rademacher, T.W., Parekh, R.B. and Dwek, R.A. (1988) *Ann. Rev. Biochem.* **57**, 785-838
5. Bucke, C. and Rastall, R.A. (1990) Chemistry in Britain pp. 675-678.
6. Paulsen, H. (1984) *Chem. Soc. Rev.* **13**, 15-45.
7. Schmidt, R.R. (1986) *Angew. Chem.* **98**, 213-236.
8. Nilsson, K.G. (1988) *Trends in Biotechnol.* **6**, 256-264
9. Kobata, A. (1979) *Anal. Biochem.* **100**, 1-14.
10. Dwek, R.A., Edge, C.J., Harvey, D.J. and Wornmald, M.R. (1993) *Ann. Rev. Biochem.* **62**, 65-100.
11. Ichikawa, Y., Look, G. C., and Wong, C. H. (1992) *Anal. Biochem.* **202**, 215-238.
12. Ajisaka, K., Nishida, H., and Fujimoto, H. (1987) *Biotechnol. Lett.* **8**, 421-424.
13. Rastall, R. A., Pikett, S. F., Adlard, M. W., and Bucke, C. (1992) *Biotech. Lett.* **14**, 373-378.
14. Hedbys, L., Larsson, P.-O., Mosbach, K. and Svensson, S. (1984) *Biochem. Biophys. Res. Commun.* **123**, 8-15.
15. Johansson, E., Hedbys, L., Mosbach, K., Larsson, P.-O., Gunnarsson, A., and Svensson, S. (1989) *Enzyme. Microb.Technol.*, **11**, 347-352.
16. Rastall, R. A., Rees, N. H., Wait, R., Adlard, M. W., and Bucke, C. (1992) *Enzyme Microb. Technol.* **14**, 53-57
17. Rastall, R. A., Adlard, M. W., and Bucke, C. (1991) *Biotech. Lett.*, **13**, 501-504.
18. Bourquelot, E. and Bridel, M. J. (1912) *J. Pharm. Chim. (Paris)* **7**, 569-573.
19. Rabate, M. J. (1935) *Bull. Soc. Chim. Biol.* **42**, 1715-1729.
20. Flowers, H. M. and Sharon, N. (1979) *Adv. Enzymol.*, **48**, 29-95.
21. Nilsson, K.G.I. (1987) *Carbohydrate Res.* **167**, 90-103.

บทคัดย่อ

จากการตรวจหาเอนไซม์ไกลโคไฮโดรเลสในเมล็ดพืชที่พบในประเทศไทยจำนวน 50 สายพันธุ์จาก 17 ตระกูล พบว่าตรวจพบเอนไซม์ อัลฟา-ดี-แมนโนซิเดส เบต้า-ดี- กลูโคซิเดส เบต้า-ดี- ฟิวโคซิเดส อัลฟา-ดี-กาแลคโตซิเดส และ เบต้า-ดี-เอ็น-อะซิทิล-กลูโคซามินิเดส ในปริมาณที่มีนัยสำคัญ (> 0.1 ไมโครโมล ต่อ นาที ต่อ กรัมเมล็ด) และ พบว่าเมล็ดพืช 38 สายพันธุ์ มีเอนไซม์อย่างน้อยหนึ่งชนิด จากนั้นได้ทดสอบความสามารถในการสังเคราะห์โอลิโกแซคคาไรด์ด้วยปฏิกิริยาย้อนกลับในสารละลายที่มีโมโนแซคคาไรด์ 50 เปอร์เซนต์โดยน้ำหนัก ที่ พีเอช 5 โดยใช้อุณหภูมิสูงและบ่มเป็นเวลานาน พบว่ามีการสังเคราะห์โอลิโกแซคคาไรด์เกิดขึ้นเมื่อใช้เอนไซม์ อัลฟา-ดี-แมนโนซิเดส จากเมล็ดถั่วเขียว เอนไซม์ เบต้า-ดี-กลูโคซิเดสจากเมล็ดพะยูง และเอนไซม์ เบต้า-ดี-กาแลคโตซิเดส จากเมล็ดปอแก้ว