J.Sci.Soc.Thailand, 17(1991) 137-140

SHORT REPORTS

RAPID SEPARATION OF HUMAN NORMAL AND ABNORMAL GLOBIN PEPTIDE CHAINS BY FPLC TECHNIQUE

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(Received 20 March 1991)

ABSTRACT

The separation of human abnormal and normal globin peptide chains by Fast Protein Liquid Chromatography (FPLC) system has been developed. The chromatographic conditions can separate α, β, β^E, and γ from each other in less than 30 minutes. This rapid separation reduces long time-consuming process of prenatal diagnosis of thalassemic disease.

INTRODUCTION

Carboxymethylcellulose column chromatography (CMC) developed by Clegg and co-workers[1] has been successfully used for the separation of β^E globin peptide. This peptide is an abnormal globin in which an amino acid at position 26 is changed from glutamine to lysine. Two chains of this peptide, two chains of α globin peptide, and heme groups form a tetrameric molecule of hemoglobin E, the most widely distributed abnormal hemoglobin of Thailand. Defective gene of this peptide combining with β-thalassemic gene produces the most severe form of thalassemic disease, β-thalassemia/hemoglobin E. The method is modified by using the concave gradient of sodium ions in eluting β^E separated from other normal globin chains. While this method has been used for chain labeling studies in prenatal diagnosis of thalassemia reliably and reproducibly, it takes 3 days to complete of diagnosis and requires a large amount of globin and fresh buffers. So high performance liquid chromatography (HPLC) technique is applied successfully to separate labeled normal globin chains.[2] Fast protein liquid chromatography (FPLC) is modified in our laboratory for the separation of α, β, β^E, and γ from each other.

Mono S HR 5/5, prepacked strong cation exchanger FPLC column is used with FPLC system in separation of adult globin chains from mixed denatured hemolysate without prior separation of globin. All α, β, β^E, and γ globin chains are eluted at separation pH of 6.1 clearly separated from each other in 30 min making the complete diagnosis within 1 day.

MATERIALS AND METHODS

EDTA blood samples were obtained from subjects with normal cord blood hemoglobin type and adult hemoglobin E heterozygote. Hemolysates were prepared according to the standard techniques.[3] Buffer A containing 20 mM sodium mono-hydrogen phosphate, 7.5 M urea, and 0.36% 2-mercaptoethanol was adjusted to pH 6.1 with 20% phosphoric acid and used as starting buffer. Buffer B containing 0.15 M sodium chloride in buffer A was used as limiting buffer. Both buffer solutions were filtered through a 0.2 micrometer-pore size membrane and degassed. Samples for injection were prepared by mixing 5 μl of each hemolysate with 1 ml of buffer A and 125 μl of 2-mercaptoethanol. The column was equilibrated for 25 min with buffer A at 1 ml/min. After 1 ml of sample was injected, a gradient was applied from 0 to 30% of buffer B in 5 min then from 30 to 75% of buffer B in 30 min at 1 ml/min. The eluate was monitored at 280 nm on chart recorder with 0.2 absorbance unit full scale.

RESULTS

Fig. I shows the chromatogram of denatured hemoglobin. Large first peak at the beginning of chromatogram has brownish yellow color of oxidized heme. In last 30 min, normal γ peptide was first eluted, followed by normal β, β^E, and normal α globin peptides. The identification of globin peptides were confirmed by Triton acid-urea polyacrylamide gel electrophoresis (data not shown).[4]

DISCUSSION

The suitable performance of the Mono S HR 5/5 on FPLC system at pH 6.1 in separating abnormal β^E, and normal β, γ, α-globin peptides provides a faster separation than CMC. An HPLC technique for separating normal β- and α-globin peptides has also been described but the problems occurred when Mono S column was used with the HPLC system.[2] Separation of abnormal β^E from other normal globin peptides by the FPLC system at separation pH of 6.4 is possible but normal β is co-eluted with normal γ globin peptides. After the separation pH is reduced to 5.7, normal β separates from normal γ but normal β is co-eluted with abnormal β^E globin peptides. The pH gradient between 4.2 and 9.4 was also applied but normal β was still co-eluted with normal γ globin peptides. So a separation pH of 6.1 was used that can separate α, β, β^E, and γ from each other. However, the position of another normal globin, δ is still not known. The amount of this normal globin peptide in fetal blood is very low, so it cannor produce the significant error in β/γ ratio determination. Fresh preparation of buffer is not further required. The storing of all buffers at $-20°C$ after use maintains the reproducibility of the results.

Although the separation of normal globin peptides by HPLC with the reverse phase technique is excellent, the running costs are very high. The high prices of HPLC columns, acetonitrile, trifluoroacetic acid, and helium gas limit further development of separation methods for other abnormal globin peptides.

Success in the separation of α, β, β^E, and γ from each other establishes the FPLC technique as useful for prenatal diagnosis of thalassemia.

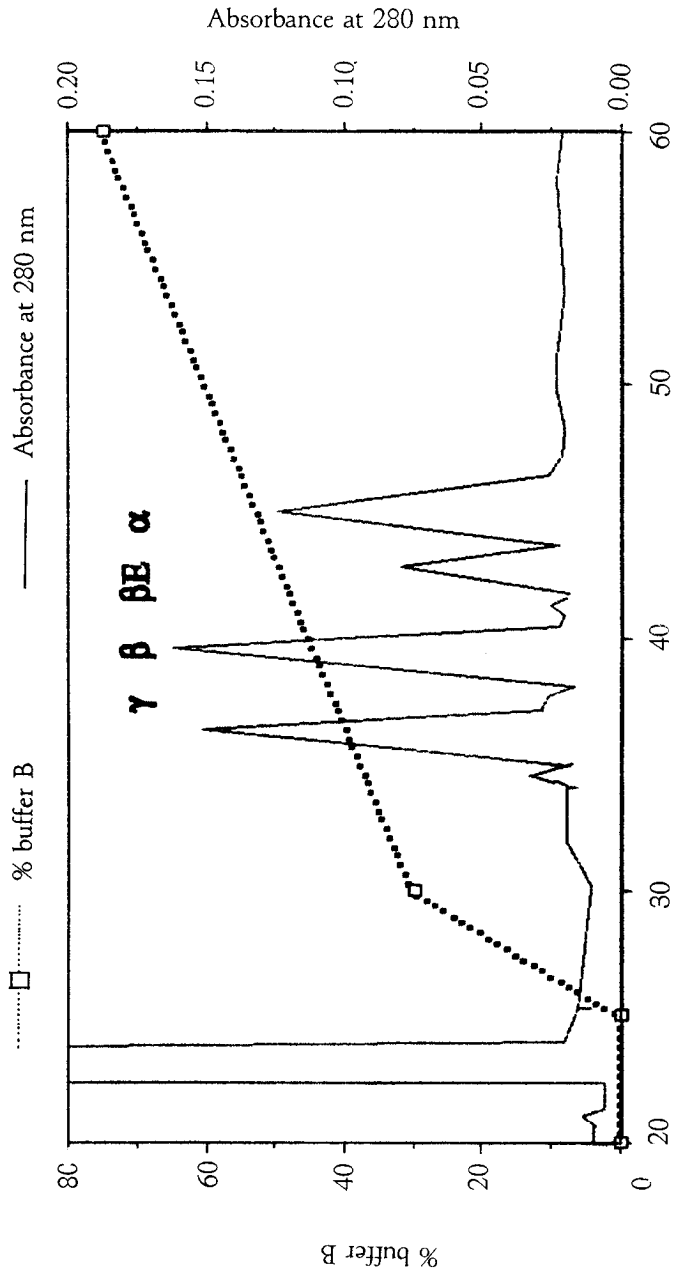

Fig. 1. Elution diagram of mixed denatured hemolysate from human erythrocytes with normal cord blood hemoglobin type and hemoglobin E heterozygote on Mono S HR 5/5 column and FPLC system. Column: 5 mm.×5cm. Eluent: 20 mM sodium monohydrogenphosphate pH 6.1, a step gradient from 0 M to 0.15 M sodium chloride. Flow rate: 1 ml./min. Fraction size: 1 ml.

ACKNOWLEDGEMENTS

I would like to thank Dr. Prawese Wasi for his support. This work was funded by CEC grant, program contract No. TS2.0131. TH(H). FPLC and Mono S HR 5/5 are the registered trademarks of Pharmacia.

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บทคัดย่อ

การแยกสายเปปไทด์โกลบินผิดปกติและปกติของคนโดยวิธี FPLC ได้รับการพัฒนา. เงื่อนไขในการแยกสามารถแยก α, β, β^E และ γ ออกจากกันภายในเวลา 30 นาที. วิธีการแยกอันรวดเร็วนี้ลดเวลาของการวินิจฉัยก่อนเกิดของโรคธารัสซีเมียลงได้.