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BRIEF NOTE

MAPPING AND AMINO ACID ANALYSIS OF TRYPTIC PEPTIDES OF GLOBIN BY USE OF CELLULOSE THIN LAYER

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The fingerprinting method of peptides on filter paper[1] has been the most favored method of the structural analysis of abnormal hemoglobins. However, it needs the high potential electrophoretic apparatus and takes many days for completion of analysis. Recently we were successful in developing a clear and reproducible method for the separation of tryptic peptides of globin (α or β globin chains from the human hemoglobin) in a short period of time by using the cellulose thin layer and a simple ordinary electrophoretic apparatus. This paper will describe our new method and some of the results of the amino acid analysis of the peptide spots separated on the cellulose thin layer.

Chromagram Sheet (without fluorescent indicator) purchased from Eastman Kodak Co. was employed for this procedure. Aliquots of 300–500 μg of the soluble fraction of tryptic peptides[2] of β globin chain digested with TPCK-trypsin (Worthington Co.) was dissolved in a small amount of water (ca. two drops) and spotted on the sheet using a glass capillary at the point 5 cm distant from the anode side margin and 2 cm upward from the bottom side under constant blowing warm air with a dryer. This sheet was laid on a glass plate (20 × 20 cm) and set in a tank of cellulose acetate membrane electrophoresis. The bilateral (anodal and cathodal) edges of the sheet were connected with the electrophoretic buffer solution tanks with the wicks of filter papers (10 × 20 cm). The buffer solution used for electrophoresis was prepared by mixing pyridine, acetic acid and water (50 : 1 : 450, by vol., pH 6.8). The sheet was moistened well by spraying the buffer solution. An electric current of 300 Volts (6 mA) was constantly run at room temperature for two hours and a quarter. The sheet was taken out and dried, and then it was chromatographed by ascending method in which the upper phase of the mixture consisted of n-butanol, acetic acid and water (200 : 50 : 250, by vol.) was used as developing solution. The chromatography was ceased when the top of the developer reached 2 cm below the top margin of the sheet. The sheet was taken out and dried to be sprayed with cadmium-ninhydrin/acetone solution[3]. The separated peptide spots were colored pink (Figure).

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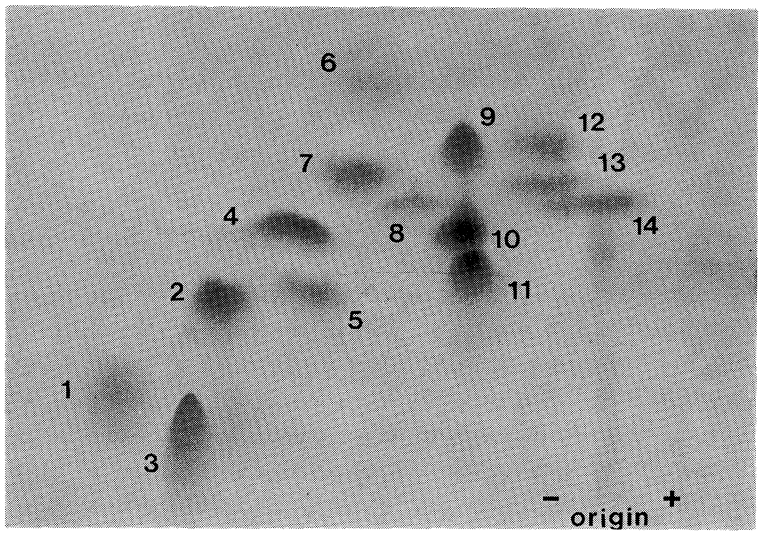

Figure. Mapping of tryptic peptides of β globin from human hemoglobin on cellulose thin layer (20×20 cm). Electrophoresis was done in the mixture of pyridine, acetic acid and water (pH 6.8), and then chromatography was followed by use of the upper phase of the mixture consisted of n-butanol, acetic acid and water.

For the amino acid analysis of the peptide spots, the fluorescamine/acetone solution[4] was sprayed and the spots were encircled with a pencil under the illumination of ultraviolet lamp (365 nm). The encircled spots were cut out with a pair of scissors and the cellulose layer was scraped off by a spatula. One mililiter of 10 % acetic acid was added to the scraped-off powder, mixed well and allowed to stand at room temperature for 1 hour. The mixture was centrifuged at 3,000 rpm for 15 min and the supernatant was transferred to a container. This procedure was repeated two times. The combined supernatant was lyophilized and the residue was hydrolyzed in 1 ml of redistilled hydrochloric acid (6 N) under reduced pressure at 110°C for 20 hours in an usual way[5]. After drying up, the composition of peptides was analyzed by Yanaco automatic amino acid analyzer Model L-7. As shown in the figure, the peptide spots were distributed discretely with good separably on the sheet. On the basis of the results of amino acid analysis and the molecular ratio, the tryptic peptide number was able to be determined as shown in the Table.

The peptide spots number 6 and 7 showed were positive for tryptophan reaction.

TABLE. Results of amino acid analysis of the respective spots on the sheet and the identification of tryptic peptide number.

spot No. ×10-²μ mol	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Lys	1.145(1)	2.404(1)	2.164(1)	2.353(1)			1.957(1)	0.946(2)	1.977(1)	2.671(1)	1.965(1)	0.343(1)	1.234(1)	
His			2.158(1)	2.388(1)	2.551(1)			0.494(1)	1.989(1)		1.838(1)			
Arg						1.006(1)								1.578(1)
Asp				2.548(1)				1.731(3)	6.568(3)			1.301(3)	4.777(3)	3.846(2)
Thr						1.201(1)	2.637(1)			3.134(1)	2.399(1)	0.625(1)	1.464(1)	
Ser							2.212(1)	0.809(1)	2.350(1)			0.859(2)	2.597(2)	
Glu						1.267(1)				7.863(3)	4.717(2)	0.909(1)	1.867(1)	4.039(2)
Pro						1.107(1)				5.151(2)	2.186(1)	1.082(2)	2.785(2)	
Gly			2.426(1)	2.697(1)			2.683(1)	1.214(2)	4.388(2)			0.854(2)	3.259(2)	5.271(3)
Ala			2.097(1)	10.261(4)			5.198(2)	1.339(2)	4.541(2)	5.052(2)		0.661(1)	1.739(1)	1.895(1)
Cys														
Val		2.505(1)		5.631(3)		1.590(2)	2.867(1)	0.796(1)	2.137(1)	3.260(1)	1.750(1)	0.641(1)	1.815(1)	4.910(3)
Met												0.346(1)	1.243(1)	
Ileu														
Leu				2.385(1)		1.788(2)	2.692(1)	1.955(4)	7.672(4)		2.519(1)	0.395(1)	1.510(1)	1.743(1)
Tyr					2.256(1)	0.963(1)				2.259(1)				
Phe								0.543(1)	2.315(1)	3.362(1)		1.114(3)	3.774(2)	
peptide No.	Ⅷ	Ⅵ	Ⅶ	XⅣ	XⅤ	Ⅳ	Ⅱ	Ⅷ-Ⅸ	Ⅸ	XⅢ	Ⅰ	Ⅴ	Vox	Ⅲ

( ): indicated the molecular ratio.

By use of cellulose thin layer, it was possible to recognize an abnormal spot produced by the abnormal polypeptide chain with a very small amount of samples. In our experience, five sheets (tryptic peptide weight: ca. 2.5 mg in total) were satisfactory for collecting the peptide for amino acid analysis but, collection from 2-3 sheets (tryptic peptide weight: 1-1.5 mg) were also useful, since the recovery of peptide from the cellulose thin layer sheets is far better than that from filter papers. Tryptic peptide number III (Tp III) which is not seen on the filter paper fingerprint map was demonstrable clearly as a discrete spot on the cellulose sheet.

The sheet colored with cadmium-ninhydrin/acetone solution is kept in a good condition for a long period of time if it is wrapped with a salan wrap.

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