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## Adsorption Chromatography of Dinitrophenylamino Acids on Silica Gel-Celite\*

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The technique of adsorption chromatography of dinitrophenylamino acids (DNP-amino acids) has been studied in this laboratory in order to separate N-terminal amino acids from the globins of various hemoglobins. The following method which gave good results is a modified method of GREEN et al. A mixture of silica gel and celite (2 to 1) is packed into a 15-cm. column with diameter of 0.9 cm. As developers mixtures of organic solvents such as acetic acid, acetone, ligroin, formic acid, ethyl acetate or benzene are used. In the above column and with the above developers, adsorption chromatography is carried out to separate various DNP-amino acids. This seems to be an effective method for qualitative and quantitative analysis of DNA-amino acids.

At present studies on the primary structure of the globins from various kinds of hemoglobins are being made in many laboratories<sup>1)2)4)5)9)13)</sup> in the aspect of comparative biochemistry<sup>6)</sup>.

The authors wanted to use the adsorption chromatography with silicic acid-celite as the adsorbent which was employed by GREEN et al. in 1952<sup>3)</sup> in order to separate and identify DNP-amino acids for the analysis of N-terminal amino acids. But since silicic acid employed by them was unavailable at the time, adsorption chromatography was carried out with silica gel in place of silicic acid, and this method gave relatively good results. In the chromatography by GREEN et al. the best result was obtained when the ratio of silicic acid to celite was 4 to 1, whereas in our experiment the ratio of silica gel to celite which gave the best result was 2 to 1.

Silica gel was formerly used by SANGER et al.<sup>8)</sup> for partition chromatography. However, the separation was rather inconstant due

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to the variety of silica gel, and this problem has been examined from many aspects<sup>7)</sup>. On the other hand, SCHROEDER et al.<sup>10)11)12)</sup> noticed that the adsorption chromatography with silicic acid was suitable for the separation of DNP-amino acids. Afterwards, GREEN et al. reported that more effective results could be obtained when a mixture of silicic acid and celite was used as the adsorbent. Our method in which silica gel was substituted for silicic acid gave a fairly good separation of 13 DNP-amino acids. This method might possibly be applied for the separation and identificaion of these DNP-amino acids.

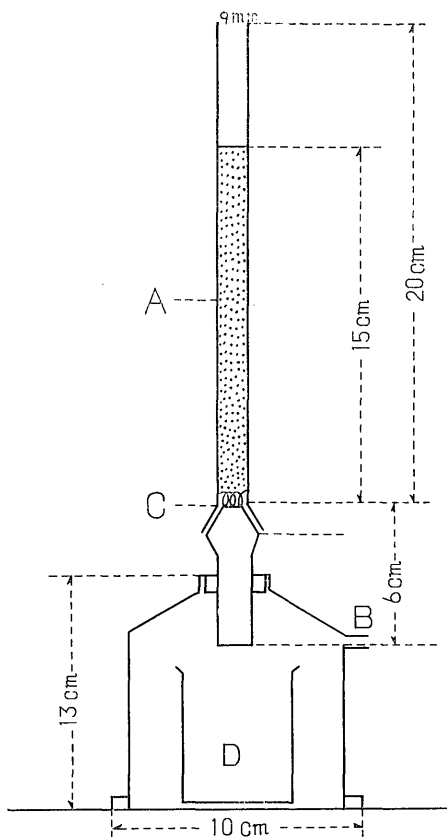
## MATERIALS AND METHODS

### *Adsorbent*

The adsorbent used in this experiment was 100 mesh silica gel manufactured for chromatographic analysis at the Mallinckrodt chemical Works (U.S.A.). The celite 545 was from the Wako Chemical Industry Ltd. (Japan). The proportions of silica gel to celite were examined,

namely 1 to 2, 1 to 1, 2 to 1 and 4 to 1, and the best result was in the proportion of 2 to 1.

Fig. 1 Chromatographic apparatus



### *Apparatus*

The apparatus used for this column chromatography is illustrated in Fig. 1. In this figure, A is a chromatographic tube into which the adsorbent is packed. The column of the adsorbent was 15 cm in height after the suction, which proved convenient for a later operation. The part of C was perforated with three small holes of 0.1 cm in diameter and after placing a small cotton plug on it, the adsorbent was poured in. A beaker was placed at D to collect the DNP-amino acid solution which had passed through the column.

### *Solvents*

Every solvent used in this

experiment was redistilled in order to remove water as completely as possible.

### Chromatographic Procedure

As DNP-amino acids are fragile in the sunlight, the whole procedure was performed in a dark room under fluorescent lamps. The adsorbent was packed into the chromatographic tube of the apparatus illustrated in Fig. 1. As described above, the height of the adsorbent was most suitable at approximately 15 cm. The top surface of the column was smoothed with a wooden stick and the adsorbent was prewashed in the following manner. With suction applied at B of the apparatus in Fig. 1., 5 ml of ether, 10 ml of a 1 to 1 mixture of ether and acetone, 2 ml of ether, 7 ml of ligroin and finally 7 ml of the developer described later were successively poured into the column. Then a 1-ml portion of DNP-amino acid solution was placed on to the column followed by suction, and developed by developers consisting of

Fig. 2 Chromatography with 8AA4AL

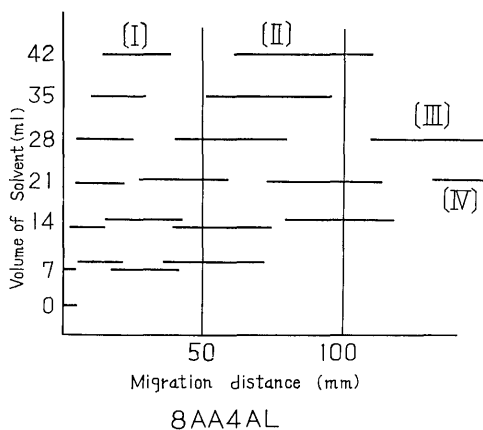


Fig. 4 Chromatography with 10AA4AL

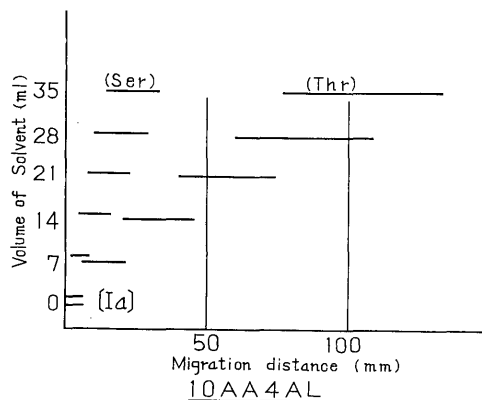


Fig. 3 Chromatography with 4AAB

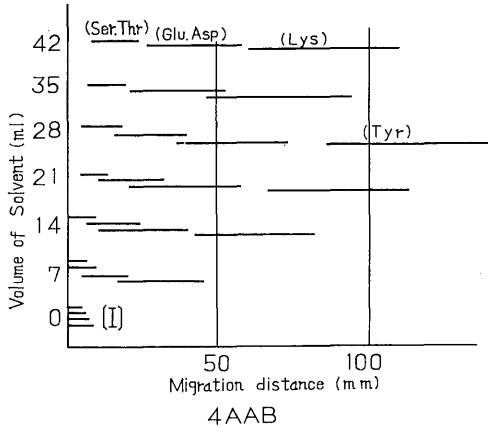
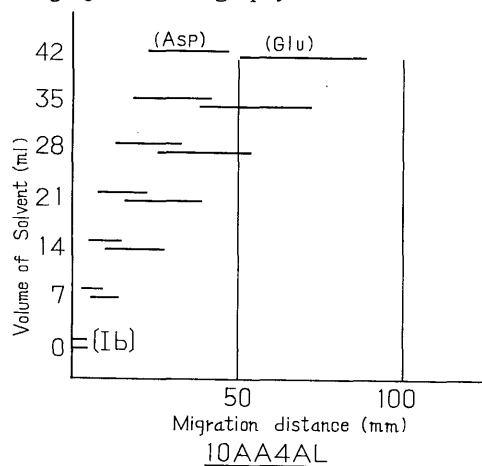


Fig. 5 Chromatography with 10AA4AL



several organic solvents. For every 7 ml of the developers added, the migration distance on the column was recorded. Thus every DNP-amino acid could be identified quantitatively by its migration distance. The DNP-amino acid solution collected at D in Fig. 1 was concentrated to dryness and used for quantitative determination. The DNP-amino acids which were retained in the adsorbent were severed into zones. Every zone was eluted with a 1 to 4 mixture of ethanol and ether, and the solution was concentrated to dryness for quantitative determination. All the dried DNP-amino acids to be determined were dissolved in glacial acetic acid. The optical density was measured at the wave length of the maximum absorption at approximately 340 m $\mu$ . Hence the mol numbers of DNP-amino acids were calculated using their molar absorption coefficient.

## RESULTS AND DISCUSSION

when a mixture of 13 DNP-amino acids was put on the column and

Fig. 6 Chromatography with 2AA10AL

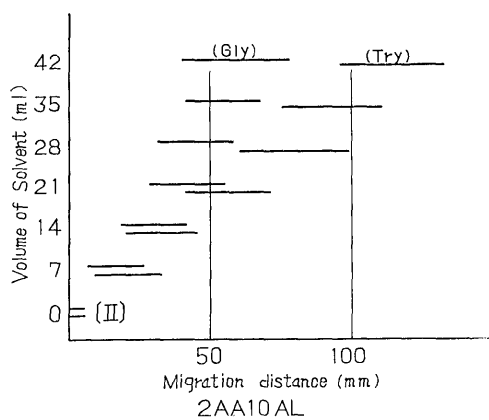


Fig. 8 Chromatography with 5AA5EL

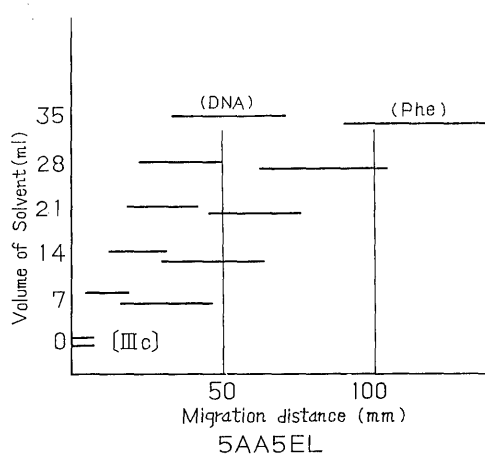


Fig. 7 Chromatography with 2F8EL

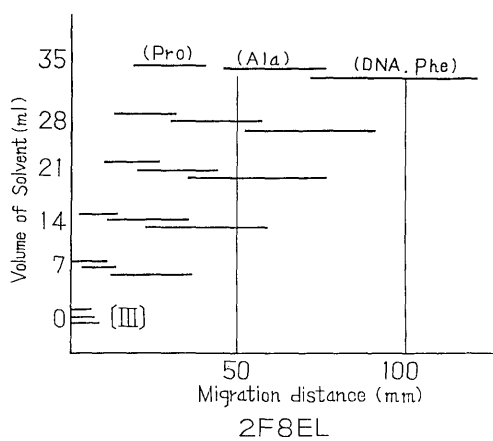
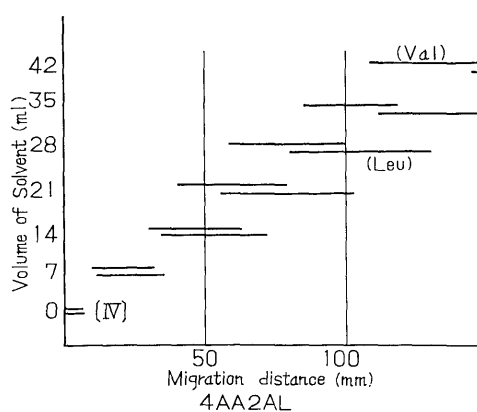


Fig. 9 Chromatography with 4AA2AL



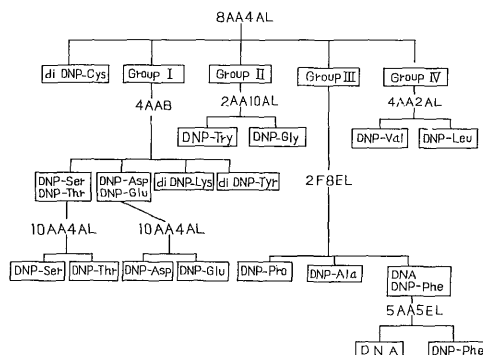
developed with 8AA4AL\*\*\*, these DNP-amino acids were divided into four groups as shown in Fig. 2. Group I included DNP-serine, DNP-threonine, DNP-aspartic acid, DNP-glutamic acid, di-DNP-lysine and di-DNP-tyrosine. The components of Group II were DNP-tryptophan and DNP-glycine. Group III contained DNP-proline, DNP-alanine, DNP-methionine, dinitroaniline and DNP-phenylalanine, and Group IV DNP-valine and DNP-leucine. When chromatographed with 8AA4AL, di-DNP-cystine remained near the top of the adsorbent.

Group I was eluted from the adsorbent and chromatographed with developer 4AAB. As a result the group was further separated into four parts of DNP-serine and DNP-threonine, of DNP-glutamic acid and DNP-aspartic acid, of di-DNP-lysine and of di-DNP-tyrosine as shown in Fig. 3. Among them the groups of DNP-serine and DNP-threonine, and of DNP-glutamic acid and DNP-aspartic acid were both developed with 10AA4AL, and the respective good separations were achieved as shown in Figs. 4 and 5. The DNP-glycine and DNP-tryptophan in Group II could be satisfactorily separated with 2AA10AL as shown in Fig. 6. Group III could be separated with developer 2F8EL into DNP-proline and DNP-alanine and then dinitroaniline and DNP-phenylalanine as shown in Fig. 7. The DNP-methionine which belonged to this group was destroyed considerably by the development with 2F8EL. The dinitroaniline and the DNP-phenylalanine could be separated from each other fairly well with 5AA5EL as shown in Fig. 8. Although the DNP-valine and DNP-leucine in Group IV could be separated with 4AA2AL as shown in Fig. 9, their separation were not as good as that of the others. However, the use of a larger amount of developer could achieve their complete separation.

In addition the most suitable solvent for dissolving every kind of DNP-amino acids should be selected. The authors used 15 AL or 5AAB to dissolve a mixture of 13 DNP-amino acids or a small DNP-peptides. For Group I was used 20AL or 4AA20AL, for Group II 2AA10AL, and for Groups III and IV 1AA10AL. Fig. 10. summarizes the above described separation of the DNP-amino acids.

Thirteen DNP-amino acids could be separated almost completely by this type of adsorption chromatography, and this method is considered to be satisfactorily usable for separating and identifying these DNP-

Fig. 10 Separation of DNP-amino acids by adsorption chromatography with silica gel-celite



amino acids. Furthermore this method could be applied also for separation of various small DNP-peptides.

### ACKNOWLEDGEMENT

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### FOOT NOTES

\*This report was presented at the 10th Kyushu Branch Meeting of the Japanese Biochemical Society.

\*\*\*This series of numerals and letters indicates the composition of a developer. The numerals represent volume percentages and the letters the component solvents. The developer 8AA4AL refers to a mixture prepared by placing 8 ml of acetic acid and 4 ml of a acetone in a graduated cylinder, filling the cylinder to 100 ml with ligroin, and mixing them. In this paper the following symbols are employed:

AA = acetic acid  
A = acetone  
B = benzene  
F = formic acid  
E = ethyl acetate  
L = ligroin

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