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Note

Resolution of six polar DL-amino acids by chromatography on native cellulose*

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The resolution of amino acid racemates by paper chromatography was first reported more than three decades ago[1–4]. We have developed the method for separating D- and L-amino acids on cellulose columns and by thin-layer chromatography during biochemical investigations, and successfully resolved many DL-amino acids (proteinic and non-proteinic)[5–10]. However, six polar racemates (aspartic acid, glutamic acid, lysine, arginine, asparagine and glutamine) have remained unresolved. Recently, we have found novel elution mixtures that resolve not only the charged DL-amino acids, which was close to an isoelectric point for the respective amino acid, but also the uncharged ones (asparagine and glutamine).

The purposes of this note are to describe the resolution of the polar DL-amino acids, and to summarize all the resolutions of the proteinic DL-amino acids on cellulose columns by adding the present data to the previous results[8].

EXPERIMENTAL

The packed cellulose column (Merck, lot No. 2331; unmodified microcrystalline; column size: 250 × 0.85 cm I.D.; bed height: 240 cm) was first washed with 10 ml of 1 *N* hydrochloric acid, then neutralized with 30 ml of 0.01 *N* sodium hydroxide and equilibrated with the elution mixture as described below. The chromatograms, which were poorly resolved, were analysed by a computer to display a reasonable resolution profile as shown in Fig. 1. On the basis of the computer-analysed patterns, enantiomers with high optical purity were obtained on repeated rechromatography.

All the DL-amino acids (non-derivatized) were resolved with the elution mixture close to their isoelectric point. DL-Asp and DL-Glu (300 μg each) were thus resolved with an elution mixture containing acetonitrile, pyridine and $5 \cdot 10^{-3}$ *M* hydrochloric acid (5:5:2, v/v/v). The eluents were monitored after mixing with ninhydrin reagent. For DL-Lys (1 mg) and DL-Arg (500 μg), the mixture was acetonitrile–pyridine–$1 \cdot 10^{-3}$ *M* sodium hydroxide (2:2:1 for DL-Lys and 1:1:1 for DL-Arg), and for DL-Asn (200 μg) and DL-Gln (300 μg) it was acetonitrile–pyridine–water (2:2:1).

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RESULTS AND DISCUSSION

Fig. 1 shows the resolved chromatograms for each pair of D- and L-amino acids. Resolution of charged amino acids (Asp, Glu, Lys and Arg) was achieved with the elution mixture close to their isoelectric point, without which no resolution was obtained. For instance, D- and L-Asp were eluted at 7.5 h (acetonitrile–pyridine–$2 \cdot 10^{-3}$ M hydrochloric acid, 2:2:1) and at 10 h (acetonitrile–pyridine–$1 \cdot 10^{-3}$ M hydrochloric acid, 5:5:2), respectively, but no resolved chromatogram for D- and L-Asp was obtained. Under conditions close to their isoelectric points, therefore, it seems that the enantiomers of amino acids interact with cellulose slightly differently and might consequently be resolved as shown in Fig. 1. On the other hand, DL-Asn and DL-Gln were eluted with acetonitrile–pyridine–water, because they are polar but uncharged. It was already known that acetonitrile was very effective for resolving these DL-amino acids.

Since not all the racemates were completely resolved, the chromatograms thus obtained were analysed by a computer to show the elution pattern of the enantiomers (Fig. 1 inset). Applying the method employed here, we could establish the optical purity of the resolved enantiomers and give a strategy for rechromatography to enhance the optical purity of the peak.

We have previously resolved DL-amino acids on cellulose[5–10], and it is interesting to collect here all the data on cellulose column chromatography (Table I). Some of the data are comparable with those from other liquid chromatography methods involving a chiral stationary phase[11–16]. Since our method of chromatographic resolution has marked characteristics in comparison with other leading resolution techniques, as mentioned earlier[8], it should be improved further to give high resolution capability and hence make a contribution to a variety of different investigations.

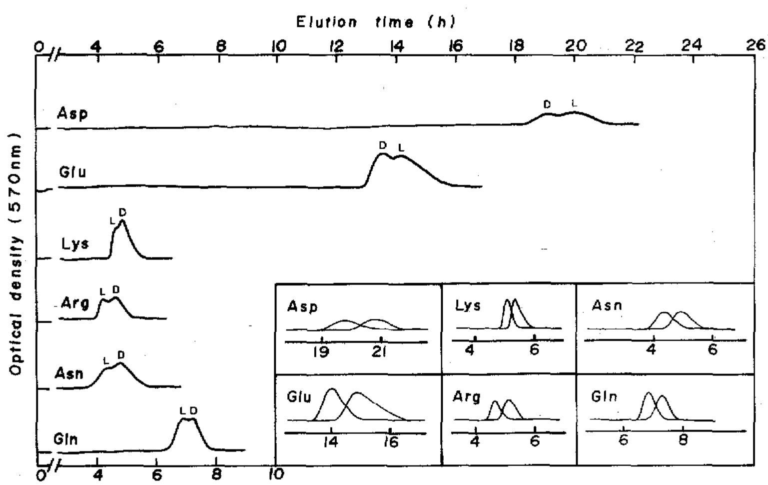

Fig. 1. Resolution of six polar DL-amino acids. Inset shows the computer-analysed patterns. Amino acid abbreviations are listed in Table I.

TABLE I

SUMMARY OF RESOLUTION OF ALL PROTEIN DL-AMINO ACIDS

DL-Amino acids		*Retention time (min)*			*Resolution factor**	*Resolution conditions***	*Identification method****	*Ref.*
Name	*Abbreviation*	*D (peak)*	*L (peak)*	*Trough*				
Aspartic acid	Asp	1184 (1183)	1242 (1243)	1213 (1213)	0.048 (0.049)	A–P–5 · 10^{-3} *N* HCl (5:5:2)	C	
Glutamic acid	Glu	844 (840)	882 (892)	863 (866)	0.044 (0.060)	A–P–5 · 10^{-3} *N* HCl (5:5:2)	C	
Lysine§	Lys	317 (321)	302 (302)	310 (312)	0.048 (0.061)	A–P–1 · 10^{-3} *N* NaOH (2:2:1)	C	
Arginine§	Arg	304 (306)	277 (276)	291 (291)	0.093 (0.103)	A–P–1 · 10^{-3} *N* NaOH (1:1:1)	C	
Asparagine§	Asn	286 (293)	252 (258)	269 (276)	0.126 (0.127)	A–P–W (2:2:1)	B,C	
Glutamine§	Gln	434 (436)	409 (408)	422 (422)	0.057 (0.066)	A–P–W (2:2:1)	C	
Serine	Ser	1263 (1263)	1337 (1348)	1300 (1300)	0.057 (0.066)	P–E–W (4:1:1)	A	8
Threonine§	Thr	1006 (1006)	927 (927)	966 (966)	0.082 (0.082)	P–E–W (4:1:1)	A	8
Cysteine	Cys	990 (989)	1040 (1043)	1015 (1016)	0.048 (0.053)	E–W (6:1)	B	8
Tyrosine	Tyr	596	674	635	0.123	P–E–W (4:1:1)	A	5,6,8,9
Histidine§	His	925	820	872	0.120	P–E–W (1:1:1)	A	5,6,8
Alanine	Ala	892 (892)	938 (938)	915 (915)	0.050 (0.050)	P–E–W (5.5:1:1)	B	8
Valine	Val	625 (625)	663 (663)	644 (644)	0.059 (0.059)	P–E–W (5:1:1)	B	8
Leucine	Leu	523 (515)	540 (542)	531 (529)	0.032 (0.053)	P–E–W (5:1:1)	B	8
Isoleucine	Ile	512 (512)	547 (547)	529 (529)	0.066 (0.066)	P–E–W (5:1:1)	B	8
Methionine	Met	634	695	664	0.092	P–E–W (5:1:1)	B	8,9
Phenylalanine	Phe	641	711	679	0.103	P–E–W (4:1:1)	A	5,6,8,9
Proline	Pro	710	770	740	0.081	P–E–W (3:1:1)	B	8
Tryptophan	Trp	380	435	413	0.133	P–E–W (1:1:1)	A	5,6,7,8,9

* Resolution factor = [D(peak) − L(peak)]/trough; values in parentheses were obtained by computer.

** P = Pyridine; E = ethanol; W = water; A = acetonitrile. All ratios are v/v.

*** A = C.D. spectra; B = co-chromatography, by which sample mixtures containing enantiomers in different ratios (*e.g.* D:L = 1:3, or *vice versa*) were eluted; and C = thin-layer chromatography on cellulose plates (Avicel SF, Funakoshi Yakuhin, Osaka, Japan), which was carried out by ascending development of D- and L-enantiomers alternately spotted at the origin.

§ L-Enantiomers were eluted faster.

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