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Note

Separation of optical isomers by zwitterion-pair chromatography

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Pirkle *et al.*[1] reminded chromatographers that, to achieve chiral recognition or resolution of a chiral solute, it is necessary to arrange for a three-point interaction between the solute and an optically active component of the eluent or stationary phase. So far, attempts to use chiral ion-pairing agents or chiral bonded phases have been unsuccessful, except when an extremely rigid chiral ion-pairing agent has been used[2,3], or alternatively when one is chromatographing what are essentially diastereoisomeric complexes, such as the copper complexes of amino acids[4,5].

Recently, we developed the technique of zwitterion-pair chromatography, which has proved particularly applicable in the separation of zwitterionic solutes such as nucleotides[6,7], coenzymes[7] and drugs[8]. Our studies[7,8] have indicated that the enhanced retention provided by addition of a zwitterion-pairing agent arises from the formation of quadrupolar ion pairs. This interaction occurs within a "pH window" where both the pairing agent and solute exist as zwitterions and is maximal towards the centre of this window. We have postulated[8] that, since the formation of quadrupolar ion pairs provides two strong interactions between solute and eluent, it should be possible with only one further interaction, say through hydrogen bonding or Van der Waals' repulsion, to achieve chiral recognition using an optically active zwitterion-pairing agent added to the eluent.

Following this line of thought, we now report the separation of enantiomers of tryptophan and of glycylphenylalanine using L-leucyl-L-leucyl-L-leucine as a zwitterionic pairing agent.

EXPERIMENTAL

Liquid chromatography was carried out using a thermostatted photometer/-column oven unit (Shandon Southern Products, Runcorn, Great Britain), maintained at 25°C. The eluent was pumped by a single-piston reciprocating pump (Model 110; Altex, Berkeley, CA, U.S.A.). Columns were 125 × 5 mm I.D. (Shandon Southern Products) and were packed with ODS-Hypersil (Shandon Southern Products) using isopropanol as the slurry liquid followed by 200 ml of hexane pumped at the pressure of 7000 p.s.i.

Eluents were water–methanol (88:12), the pH being adjusted with 1 m*M* phosphate buffer (Na_2HPO_4 + H_3PO_4). Methanol was of HPLC grade (Rathburn Chemicals, Walkerburn, Great Britain). L-Leucyl-L-leucyl-L-leucine (LLL), DL-tryp-

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tophan, D-tryptophan, L-tryptophan, glycyl-DL-phenylalanine, glycyl-D-phenylalanine and glycyl-L-phenylalanine were obtained from Sigma (Poole, Great Britain): other reagents were of AnalaR grade (BDH, Poole, Great Britain).

Samples of solutes were dissolved in water–methanol (88:12) and introduced into the column by Rheodyne injection valve (Model 7120; Rheodyne, Berkely, CA, U.S.A.).

RESULTS AND DISCUSSION

We reported earlier[8] that LLL is strongly adsorbed from the above eluent by ODS-Hypersil, giving a surface concentration of 115 μmol/g or *ca.* 0.6 μmol/m^2, and that it could successfully be used as zwitterion-pairing agents for nucleotides and coenzymes (NADH, NaDPH). Accordingly, LLL has been used in this study as a chiral ion-pairing agent in an attempt to separate racemic mixtures of tryptophan and glycylphenylalanine.

When LLL is added to the eluent (pH 6.4) at a concentration of 2 m*M*, the single peaks for DL-tryptophan and glycyl-DL-phenylalanine split into doublets of equal area, as shown in Fig. 1A. The separation factors are 1.14 for tryptophan and

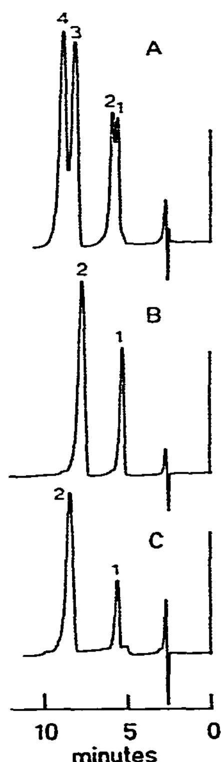

Fig. 1. A, Separation of racemic mixtures of tryptophan and glycylphenylalanine: Packing, ODS-Hypersil; eluent, water–methanol (88:12) containing 2 m*M* LLL and 1 m*M* phosphate buffer (pH 6.3); temperature, 25°C; flow-rate, 0.6 ml/min; detector, UV photometer, 254 nm. Peaks: 1 = glycyl-L-phenylalanine; 2 = glycyl-D-phenylalanine; 3 = L-tryptophan; 4 = D-tryptophan. B, Separation of L-tryptophan (2) and glycyl-L-phenylalanine (1). C, Separation of D-tryptophan (2) and glycyl-D-phenylalanine (1).

1.11 for glycylphenylalanine. Fig. 1B and 1C show chromatograms of the individual L- and D-forms, respectively, and establish that the L-form of each compound is eluted first.

It should be noted that, during this work, we observed that the presence of heavy metal ions seriously impaired the resolution of the enantiomers. This could have arisen from the formation of amino acid–metal ion complexes as reported elsewhere[4,5,9]. This view was supported by the observation that addition of copper ions to the eluent completely destroyed the capability of added LLL to enhance solute retention in normal zwitterion-pair chromatography. We have also noted that chiral resolution is impaired if reagents are not of AnalaR quality.

CONCLUSIONS

Our results provide the first example of enantiomer separations with use of ion-pairing agents in the absence of complexing metal ions. They add strong further confirmation to our basic hypothesis that zwitterion-pair chromatography is effected through formation of quadrupolar ion pairs in the stationary phase.

We believe that the method can be exploited in the resolution of a wide range of chiral amino acids.

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REFERENCES

1 W. H. Pirkle, D. W. House and J. Finn, *4th International Symposium on Column Liquid Chromatography, Boston, MA, May 7–10, 1979*, Abstract.
2 W. Lindner, J. N. Le Page, G. Davies, D. E. Seitz and B. L. Karger, *J. Chromatogr.*, 185 (1979) 323.
3 J. N. Le Page, W. Lindner, G. Davies, D. E. Seitz and B. L. Karger, *Anal. Chem.*, 51 (1979) 433.
4 H. Engelhardt and S. Kromidas, *Naturwissenschaften*, 67 (1980) 353.
5 V. A. Davankov, A. S. Bochkov, A. A. Kurganov, P. Roumeliotis and K. K. Unger, *Chromatographia*, 13 (1980) 677.
6 J. H. Knox and J. Jurand, *J. Chromatogr.*, 203 (1981) 85.
7 J. H. Knox and J. Jurand, *J. Chromatogr.*, 218 (1981) 341.
8 J. H. Knox and J. Jurand, *J. Chromatogr.*, 218 (1981) 355.
9 E. Grushka, S. Levin and C. Gilon, *J. Chromatogr.*, 235 (1982) 401.