Journal of Chromatography, 347 (1985) 1–10
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 993

MOLECULAR IMPRINTING OF AMINO ACID DERIVATIVES IN MACROPOROUS POLYMERS

DEMONSTRATION OF SUBSTRATE- AND ENANTIO-SELECTIVITY BY CHROMATOGRAPHIC RESOLUTION OF RACEMIC MIXTURES OF AMINO ACID DERIVATIVES

BÖRJE SELLERGREN, BJÖRN EKBERG and KLAUS MOSBACH*
Department of Pure and Applied Biochemistry, Chemical Center, University of Lund, P.O. Box 124, S-221 00 Lund (Sweden)
(Received June 20th, 1985)

SUMMARY

Substrate-selective macroporous polymers were prepared by a non-covalent imprinting procedure. By this method, acrylic polymers selective for D- or L-phenylalanine ethyl esters were prepared utilizing coulombic interactions, during polymerization, between the print molecule (D- or L-phenylalanine ethyl ester) and the carboxyl group carried by the vinyl monomers. These polymers, after removal of print molecules, were used in a chromatographic procedure to examine their enantio- and substrate-selectivity for some amino acid derivatives. The best separation factor, α, for D,L-phenylalanine ethyl ester was found to be 1.30. The selectivity persisted for several months after intermittent applications.

INTRODUCTION

During the last few years the resolution of racemic mixtures of amino acids and amino acid derivatives has been developed considerably[1–3], and the chromatographic techniques used allow in general complete separation of the enantiomers. Most of this work has been largely empirical and the mechanism of chiral recognition is not well understood[4,5]. Therefore it is often difficult to predict the elution order of different solutes and their enantiomers. These systems also have a low substrate selectivity and thus they often have resolving power for a number of different substrates[6], which in some cases may be beneficial, in others not. More recently, successful attempts have been made to design more efficient chiral stationary phases[7,8], permitting the prediction of the elution order from models of chiral recognition. Similarly, with the use of cyclodextrins and chiral crown ethers[9,10], more predictable enantio- and substrate-selectivities have sometimes been obtained. Nevertheless, there is no universal way to obtain a predictable enantio- and substrate-selectivity. We therefore decided to investigate whether our approach[11] producing polymers

0021-9673/85/$03.30

containing chiral cavities, based on molecular imprinting, would yield preparations which, when applied to chromatographic systems, would exhibit predictable enantio- and substrate selectivity.

Using different imprinting methods, batchwise racemic resolution of amino acids and amino acid derivatives has already been demonstrated. We have previously reported separation factors of about 1.1 using our imprinting method, which is based on pre-arrangement[12] of vinyl monomers by electrostatic interaction with the respective amino acid[11]. Other groups of workers have utilized covalent bonding of substrates and reported separation factors of about 1.4[13], and with a similar approach separation factors of about 1.04 have also been obtained[14].

The use of imprinted polymers for chromatographic separations has also been investigated. Thus for sugar enantiomers a separation factor of about 2.3 has been obtained[15,16]. Based on the substrate-selective acrylic polymers developed previously[12], similar preparations were subsequently applied to the chromatographic separation of synthetic dyes[17]. A new imprinting method based on organic silanes has also been developed[18], and tested in the chromatographic separation of some biomolecules. These are the only instances known to us of chromatographic applications using polymers containing specific cavities. One of the reasons there are so few, as already pointed out[15], is the importance of reaching a fast equilibrium; this is often difficult to obtain when using separation processes based on reversible covalent interactions. Therefore, since our method is based simply on an ionic interaction, chromatographic resolution is expected provided that the diffusion rate through the highly cross-linked network is high enough.

MATERIALS AND METHODS

Preparation of amino acid and peptide derivatives

Amino acids and Phe_3OH (PheOH is free acid of phenylalanine) were purchased from Sigma. Tritiated compounds were obtained from NEN and Amersham as were ^{14}C-labelled ethanol (NEN) and benzylamine (Amersham).

Preparation of the ethyl esters: L-[^{3}H]PheOEt, D-PheO[^{14}C]Et, L-Trp[^{3}H]OEt, D-TrpO[^{14}C]Et and (L-Phe)$_3$O[^{14}C]Et followed standard procedures[19]. N-Ac-L,D-[^{3}H]PheO[^{14}C]Et, where Ac = acetyl, was made by acetylation[20] of a racemic mixture of radioactively labelled phenylalanine ethyl ester. L-[^{3}H]PheNHBzl, where Bzl = benzyl, was prepared by condensation of BOC-L-[^{3}H]PheOH, where BOC = *tert.*-butyloxycarbonyl, and benzylamine using N,N-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as condensation agent. Deprotection was carried out by removal of the BOC group with trifluoroacetic acid (TFA). D-PheNH[^{14}C]Bzl was prepared in a similar way by condensation of BOC-D-PheOH and ^{14}C-labelled benzylamine. N-Cap-L,D-[^{3}H]PheO[^{14}C]Et, where Cap = caproyl, was made by DCC/HOBt condensation in dimethylformamide (DMF) of BOC-CapOH and a racemic mixture of radioactively labelled phenylalanine ethyl ester, followed by deprotection in TFA. The tryptophan-containing peptide was built up by stepwise synthesis from the N-terminal end using the BOC group for temporary amino protection, and the carboxyl function blocked by its conversion into the methyl ester. All condensations were performed in DMF with DCC/HOBt as condensation agent. The radioactivity was introduced during the final condensation by using L-[^{3}H]PheOEt as nucleophile. All compounds were used as their free bases.

The compounds were characterized by thin-layer chromatography (TLC), NMR, UV and ninhydrin and all were at least 95% pure. Their specific radioactivities and TLC R_F values [silica plates eluted with chloroform–methanol (10:1)] were: D-PheO[^{14}C]Et, 15 000 dpm/μmol, R_F 0.77; L-[^{3}H]PheOEt, 45 000 dpm/μmol; D-TrpO[^{14}C]Et, 76 000 dpm/μmol, R_F 0.56; L-[^{3}H]TrpOEt, 29 400 dpm/μmol; (L-Phe)$_3$O[^{14}C]Et, 11 800 dpm/μmol, R_F 0.58; N-Ac-D-PheO[^{14}C]Et, 10 000 dpm/μmol, R_F 0.85; N-Ac-L-[^{3}H]PheOEt, 15 000 dpm/μmol; D-PheNH[^{14}C]Bzl, 238 000 dpm/μmol, R_F 0.35; L-[^{3}H]PheNHBzl, 30 854 dpm/μmol; N-Cap-D-PheO[^{14}C]Et, 10 000 dpm/μmol, R_F 0.23; N-Cap-L-[^{3}H]PheOEt, 15 000 dpm/μmol; (L-Trp)$_3$-L-[^{3}H]PheOEt, 31 209 dpm/μmol, R_F 0.27.

Preparation of the polymers

The vinyl monomer compositions of the polymerization mixtures were: polymer A, 90 mol % ethylene glycoldimethacrylate (EDMA, Merck), 10 mol % acrylic acid; polymers B and C, 83 mol % EDMA, 17 mol % acrylic acid; polymer D, 83 mol % EDMA, 4 mol % N-acryloylphenylalanine ethyl ester, 13 mol % acrylic acid. In all cases (except for polymer D) D- or L-PheOEt (the print molecule) was present in a concentration of about 4.5 mol %. In addition to the above polymers, reference polymers without print molecules were prepared. In a typical preparation (polymer B), 5643 mg EDMA, 432 mg acrylic acid, 289 mg PheOEt and 62 mg azobisisobutyronitrile (AIBN) in 8.24 ml acetonitrile were thoroughly mixed in a glass tube. After degassing, the tube was sealed under nitrogen and consecutively heated for 24 h at 60, 90 and finally 120°C (polymer C was heated for 24 h at 60 and 80°C). Afterwards the polymers were ground and then subjected to continuous extraction in acetonitrile for about 24 h.

To determine the recovery of print molecules, polymers where the print molecule, PheOEt, was replaced by ^{3}H-labelled PheOEt were prepared. Samples (50 mg) of these polymers were analysed before and after the extraction, by liquid scintillation counting (LKB 1217 rackbeta) following total combustion in a Packard Tri-Carb sample oxidizer. In addition to the radioanalysis, the extract was analysed by high-performance liquid chromatography (HPLC) on a C_{18} column (10 cm, Brownlee) using an Altex HPLC pump, a Gilson UV spectrometer and a LKB fraction collector. The eluent was methanol–0.05 *M* potassium phosphate buffer pH 6.0 (2:1) and the flow-rate was 0.47 ml/min. Detection was performed by UV spectroscopy and by radioanalysis of the collected fractions. The elution profile was rather inhomogeneous, but the peak of PheOEt was easily identified, $k' = 1.5$. The radioactivity profile showed two peaks: one for PheOEt and another with $k' = 3.6$ (also visible in the UV region), probably representing an impurity formed during polymerization. The fractions corresponding to the first peak gave a positive ninhydrin test, while those for the second peak gave a negative test. By estimating the peak areas it was found that for polymer B about 25% and for polymer C about 10% of the recovered print molecules had been converted.

No fractionation of the polymer particles was performed before the batch incubation. This was carried out as described elsewhere[11]. As a complement to the supernatant analysis for the amount of bound enantiomers, the polymer itself was analysed. This was done by treating it with a highly alkaline solvating agent (Lumasolve from Lumac), neutralizing and then directly subjecting it to liquid scintil-

lation counting. In this way approximately the same α values were obtained as from the supernatant analysis.

Chromatographic procedure

The prepared polymers were milled and sieved under water in a Resch sieve and the fraction between 45 and 65 μm was used for packing the chromatographic columns. Of the printed polymers only those printed with L-PheOEt were used in chromatography, unless otherwise stated. Stainless-steel columns 200 × 5 mm (100 × 5 mm for polymer D) were packed with 0.8 g of polymer (0.4 g polymer D). The polymer suspension contained equal amounts of methanol and a 50% sucrose solution and the packing was carried out with a 50-cm packing column. Three column volumes of methanol-water (1:1) were then pumped through the column. Then the column was equilibrated in acetonitrile at a pressure always lower than 1000 p.s.i.

The chromatographic analysis was performed at 50°C using acetonitrile as eluent. The void volume was approximately 3.8 ml. The amounts of D,L-PheOEt injected for the experiments listed in Table I were: polymer A, 0.6 μmol; polymers B and C, 0.2 μmol; polymer D, 0.4 μmol; in a volume of 20 μl. The flow-rates were 15 μl/min on A and 75 μl/min on B–C. For the experiments listed in Tables II and III the amount of injected racemate was 0.3 μmol except for N-AcPheOEt (0.5 μmol) and PheNHBzl (0.4 μmol). The flow-rate was 80 μl/min, except for N-AcPheOEt and Phe_3OEt where it was 70 μl/min. The D- and L-enantiomers of the substrate were mixed in equimolar amounts before injection. In the case of PheOEt they were also injected separately.

The radioanalysis was performed by collecting eluted fractions and then counting the $^3H/^{14}C$ content in a liquid scintillation counter. In this way the relative amounts of the enantiomers could be determined. The elution profiles were then plotted and it was established that almost 100% recovery of substrate had been obtained. The void volume was determined by injecting acetone as an inert non-retained substance. Each chromatographic experiment was repeated several times and the mean retention was estimated from the maximum peak heights of the asymmetric peaks. The number of theoretical plates, n, was estimated after measurement of the peak width at half height, b, and the retention time, t, using the relationship $n = 8 \ln 2 (t/b)^2$.

RESULTS AND DISCUSSION

Preparation of polymers

Phenylalanine ethyl ester (PheOEt)-selective polymers, based on acrylic monomers, were prepared as described previously[11]. A mixture of the print molecule (D- or L-PheOEt) at low concentration, acrylic monomers and initiator in an inert organic solvent was allowed to polymerize in a sealed tube. During polymerization the print molecule and the acrylic monomers are supposed to interact by formation of ion pairs between the positively charged amino group of the print molecule and the negatively charged carboxyl group of the acrylic acid monomers. Using a high concentration of cross-linking agent (EDMA), macroporous rigid polymers are formed with sufficiently high mechanical stability for HPLC[16]. After polymerization, the print molecules can be removed by mild extraction of the polymers with acetonitrile,

a procedure that compares favourably with the more severe conditions required to displace covalently bound print molecules[13–15]. The polymers obtained are presumed to have specific cavities, formed by the print molecule (D- or L-PheOEt), which contain carboxyl groups that can interact selectively with re-added substrate.

In order to enhance the resolution in the batch procedure, different parameters were varied. In particular, the influence of the amount of carboxylic groups in the polymer was investigated (Table I). The amount of acrylic acid in polymer A was 10%, while in polymer B it was 17%, corresponding to cross-linking degrees of 90 and 83% respectively. The concentration of the print molecule was kept at a constant low level, with a molar ratio of carboxyl groups to print molecules of 2 in polymer A and 4 in polymer B. The recovery of print molecules after continuous extraction of the polymers in acetonitrile was exceptionally high, showing that almost all of the initially added print molecule could be removed. The small amount left in the polymer is possibly entrapped in the highly dense core of the polymer formed[21].

TABLE I

COMPARISON OF THE PREPARED POLYMERS IN BATCH AND CHROMATOGRAPHIC EXPERIMENTS

In batch experiments[11], 6 μmol of D,L-PheOEt were equilibrated with 0.5 g of polymer. In chromatographic experiments the conditions were as described in Materials and methods. The L-PheOEt-specific polymers were prepared as described in Materials and methods. Polymer C was polymerized at lower temperature (80°C); D corresponds to polymer B but with no removal of print molecules, which are covalently bound to the polymer. n.d. = Not determined.

Polymer	*COOH/PM**	*Recovery** (%)*	*Batch experiments*		*Chromatographic experiments*		
			*Bound*** (%)*	$\alpha = K_L/K_D$	k'_D	k'_L	$\alpha = k'_L/k'_D$
A	2	95	22	1.093	0.170	0.187	1.10
B	4	90	41	1.080	0.370	0.475	1.28
C	4	95	n.d.	n.d.	1.372	1.512	1.10
D	4	0	n.d.	n.d.	0.292	0.292	1.00

* Amount of carboxyl-containing monomers in relation to the amount of print molecule (PM) present during polymerization.

** Of print molecules after extraction with acetonitrile.

*** Percentage of added D,L-PheOEt which is bound to the polymer after equilibration.

In order to examine the possible conversion of the print molecule by, *e.g.*, racemization, formation of diketopiperazine or linear peptides during polymerization, the extract was analysed. Based on polarimetric measurements, no racemization of the print molecule had taken place. On the other hand, HPLC analysis of polymer B indicated that about 25% of the recovered print molecules had been modified. The exact nature of the modified portion is not known, but according to the ninhydrin test it does not contain primary amino groups and thus could be the diketopiperazine of phenylalanine. By lowering the polymerization temperature from 120 to 80°C for polymer C, we found that only 10% of the print molecules had been modified. More-

over, the recovery was slightly higher, possibly due to a more flexible polymer backbone.

The rigid polymer particles obtained after extraction and drying could be dispersed in both water and acetonitrile, the hydrophilic behaviour becoming more pronounced with increasing carboxyl content.

Batch experiments

Both D- and L-PheOEt were used as print molecules. The average binding data obtained for several independent batch experiments are presented in Table I. The separation factors, α, were calculated as the ratio of the distribution coefficients, K_L/K_D, for the partitioning of the L- and D-forms between the polymer and the supernatant. This method of measuring the selectivity has a thermodynamic significance since it reflects the difference in the free energies of binding of the D- and the L-form according to: $d(dG) = -RT\ln \alpha$.

In all cases the α values were related to the corresponding values for reference polymers prepared in the absence of print molecules. The latter values were in all cases nearly equal to one. The α values in Table I are lower than those reported by Wulff *et al.*[13] and higher than reported by Damen and Neckers[14]. However, as recognition is based on only one defined interaction (non-covalent) the obtained selectivities seem quite good.

Obviously, the variation of the amount of carboxyl groups in the polymer has a greater effect on the binding capacity than on the selectivity. In other words, an increase in the number of carboxyl groups does not increase the non-selective binding (resulting in a lower separation factor). This behaviour might be explained by considering the ion-pair equilibrium during polymerization. On increasing the concentration of acrylic acid the equilibrium is shifted towards formation of the ion pair, thereby increasing the number of selective cavities. Another contributing factor to the preserved selectivity could be the lower degree of cross-linking in polymer B compared to polymer A. This could lead to a higher accessibility for the more selective cavities, because of the greater flexibility of the polymer chains in these polymers[22]. The ion-pair formation during polymerization would probably be more complete if a solvent with lower polarity were used[23], but this could affect the polymer structure, leading to a loss of the macroporous properties of the polymer.

In addition to the polymers described, imprinted polymers were prepared in the absence of acrylic acid monomers, thus omitting the ion-pair formation during polymerization. Interestingly, these polymers also showed some selectivity for the print substrate, although the capacity was very low. Thus, some form of selective cavities are formed even in the absence of the ionic interaction. The selectivities achieved are also interesting in view of the high recoveries obtained after extraction (90–98%). This is an additional indication that the recognition is not due to an interaction between the substrate and the chiral print molecules which have remained entrapped in the polymer, but instead due to incorporation of the substrate into the formed cavities.

Chromatographic experiments

Since the mechanism of recognition depends in part on ionic interactions, we expected the equilibration in the cavities to be fast enough for a chromatographic

separation. However, this is possible only if the diffusion through the highly cross-linked network, and the embedding of substrate into the cavities, is fast enough. The ionic interaction forces are highly dependent on the temperature and suitable retentions were obtained by thermostatting the column at 50°C. This also results in higher diffusion rates. To obtain a uniform packing the polymers were sieved under water to yield particle fractions of 45–65 μm. Acetonitrile, the solvent employed in the batch procedure, was used as eluent. Since labelled substrates were used, both radioactivity and UV detection were possible, permitting analysis of unresolved peaks. The separation factor, α, was determined as the ratio of the capacity factors for the L- and D-forms

$$\alpha = k'_L/k'_D$$

where $k'_L = (t_L - t_0)/t_0$. At equilibrium conditions, these chromatographic separation factors can be directly compared with those obtained in the batch experiments[1].

Typical elution profiles of the separately applied D- and L-phenylalanine ethyl ester are shown in Fig. 1. A separation factor of 1.30 could hereby be obtained. The number of theoretical plates was 200 for the L-form and 230 for the D-form. These values are about the same as for an inert substance such as acetone, implying a low column efficiency. When the racemic mixture was applied the UV profile showed only one unresolved peak with a plate number of 130. However, the separation factor, determined by the more sensitive radioactivity assay, was about the same as that obtained when the enantiomers were applied separately. As expected, the selectivity was reversed when polymers printed with D-PheOEt were used.

The separation could be optimized by varying the amount of substrate applied as well as the flow-rate. When the amount of substrate applied was decreased (0.6–0.2 μmol) the α value increased slightly, the lower limit of the former being set by the radiodetection sensitivity. This is likely to happen since the polymers contain a wide distribution of cavities with different selectivities[15]. Thus under non-saturating conditions the most selective cavities are occupied preferentially.

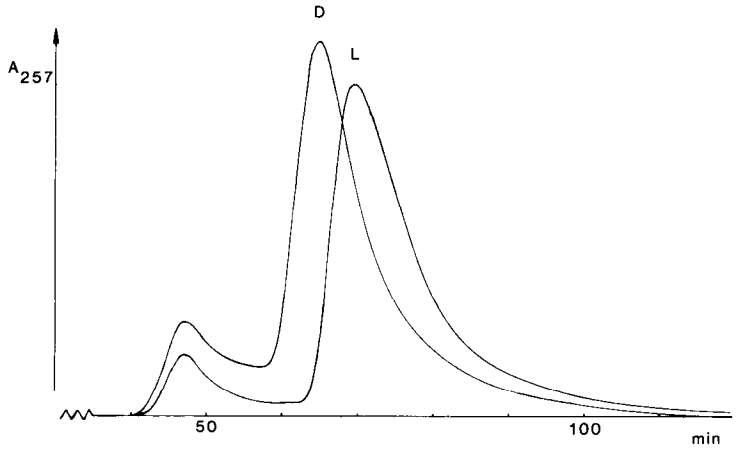

Fig. 1. Elution profiles of D- and L-PheOEt (0.2 μmol) injected separately on a column (200 × 5 mm) packed with 0.8 g of polymer B. The eluent was acetonitrile and the column was thermostatted at 50°C. Flow-rate: 80 μl/min. Separation factor, α = 1.29.

On varying the flow-rate, the optimum separation was found at 70–80 μl/min. Lower and higher flow-rates resulted in lower separation factors, possibly due to a kinetically controlled separation. Attempts to increase the resolution by the use of longer columns were also made, but these were unsuccessful.

As is seen from Table I, the separation factors obtained in chromatography are higher than those in the batch experiments. Polymers with four (polymer B, $\alpha = 1.28$) instead of two (polymer A, $\alpha = 1.10$) carboxyl groups per print molecule seemed to be more selective, although the latter were chromatographed under non-optimized conditions. This difference could be due to greater diffusion limitations introduced in polymer A by the lower flexibility of the polymer chain.

The modification of the print molecule during polymerization does not seem to decrease the selectivity. Polymer C (analogous to polymer B but polymerized at lower temperature, resulting in less modification of the print molecules) showed a lower selectivity, $\alpha = 1.10$, than polymer B, $\alpha = 1.28$. This could be the result of the lower rigidity of the polymer formed, which in turn might adversely affect the stability of the cavities formed[21]. Furthermore, the high capacity factors obtained on this polymer indicate that non-specific binding is more pronounced, possibly due to the higher amount of unreacted end groups. This should also contribute to the lower α value.

Another polymer tested in these studies was prepared as a "blank" for polymer B by copolymerization of the actual print molecule. The latter, N-acryloyl-L-phenylalanine ethyl ester (4 mol % of vinyl monomers), was mixed with an excess of acrylic acid (13 mol %) and then cross-linked with EDMA (83 mol %) to give polymer D. Since the print molecule, *i.e.*, L-PheOEt, was not displaced, we used this preparation as a reference polymer in our investigations of enantiomer and substrate selectivity. As is seen in Table I, no selectivity was observed when using this polymer. This experiment was carried out as polymers prepared from chiral monomers, similar to ours, have been successfully used for chiral resolution[4]. Our results indicate that a similar recognition mechanism in our systems is highly unlikely, which again confirms the printing concept.

It should be mentioned that, when using eluents containing up to 10% water, the selectivity decreased only slightly, which seems promising for future experiments in water-based systems. Another important observation is that the resolving ability of the preparations persisted for several months with intermittent use, indicating the high stability of the polymers. Since polymer B showed the highest selectivity, $\alpha = 1.28$ under optimized conditions, it was used for the following investigation of substrate selectivity.

To investigate substrate selectivity and to some extent which part of the substrate molecule is the most important for recognition, we modified the print molecule substrate (PheOEt) at the chiral centre (Table II). The substrate selectivity was then investigated in two ways. First, as for PheOEt, the labelled D- and L-enantiomers of the substrates were prepared. The racemic mixtures of these were then chromatographed under the same conditions used for PheOEt, and the capacity factors and the α values were determined. For some substrates the specific radioactivity was rather low, requiring a higher amount of substrate to be applied on the column. If the amino group is blocked by acylation, resolution is lost, which confirms the importance of the electrostatic interaction for a chromatographic resolution. The other

TABLE II

CHROMATOGRAPHIC RESULTS FOR SOME RACEMIC AMINO ACID DERIVATIVES ON A L-PheOEt-SPECIFIC POLYMER B

Conditions as described in Materials and methods.

R_1–HN–CH(–R_2)–CO–R_3

Substrate	R_1	R_2	R_3	k'_D	k'_L	$\alpha = k'_L/k'_D$
PheOEt	H–	$C_6H_5CH_2$–	CH_3CH_2O–	0.370	0.475	1.28
TrpOEt	H–	3-Indolyl-CH_2–	CH_3CH_2O–	1.028	1.231	1.20
N-AcPheOEt	CH_3CO–	$C_6H_5CH_2$–	CH_3CH_2O–	0.222	0.222	1.00
N-CapPheOEt	$H_2N(CH_2)_6CO$–	$C_6H_5CH_2$–	CH_3CH_2O–	3.800	3.800	1.00
PheNHBzl	H–	$C_6H_5CH_2$–	$C_6H_5CH_2NH$–	1.200	1.200	1.00

variations made at groups R_1 and R_3 led to a loss of resolution, in contrast to variations in R_2 which only slightly affected the α value. The reason for this behaviour is not clear, but it seems that the size of the modified groups is important for recognition. Apparently, the derivatization of R_1 with caproic acid or R_3 with benzylamine results in a larger modification than the derivatization of R_2 with the side chain of tryptophan, the latter derivative resembling more closely the print substrate.

Considering the capacity factors, k', for the different substrates, it is obvious that a great deal of non-specific binding also occurs, since some of the unresolved substrates have higher capacity factors than PheOEt. This effect might be minimized by changing to a more polar eluent. The result should then be an elution order corresponding to the enantiomer selectivity, *i.e.*, PheOEt eluted last. However, in the present situation it is still possible to estimate the substrate selectivity from the elution order in Table II. Thus, for a certain substrate, the capacity factor, k', on the print column (polymer B) is compared with that on the reference column (polymer D). The ratio of these capacity factors has been taken as a measure of substrate selectivity (Table III). It is found that PheOEt and TrpOEt show about the same selectivity, while larger substrates such as Trp_3PheOEt and Phe_3OEt result in a considerably

TABLE III

SELECTIVITY FACTORS FOR SOME L-AMINO ACID DERIVATIVES USED IN CHROMATOGRAPHY

Conditions as described in Materials and methods.

Substrate (L-forms)	*k' (print)**	*k' (ref)***	*Selectivity factor, k' (print)/k' (ref)*
PheOEt	0.475	0.289	1.64
TrpOEt	1.231	0.787	1.56
Trp_3PheOEt	0.364	0.333	1.09
Phe_3OEt	0.125	0.111	1.13

* Capacity factor of the substrate on polymer B (imprinted with L-PheOEt).

** Capacity factor of the substrate on polymer D (reference polymer).

lower selectivity, indicating some form of size exclusion. This was also the result when injecting a mixture of PheOEt and Phe_3OEt.

CONCLUSIONS

The selectivity, both for the enantiomer of the printed substrate and for the substrate itself measured by comparison with substrate analogues, is ascribed to cavities formed in the molecular imprinting procedure applied. This "enzyme like" specificity appears remarkable considering that the sizes of the print molecule and of the monomers forming the cavity are almost identical, and that the recognition is based on only one defined interaction. In order to obtain a higher selectivity we are presently trying to improve the imprinting method. This can be done for example by optimizing the ionic interactions during polymerization, by using larger print molecules such as peptides or by allowing multiple interactions to take place during polymerization and subsequent resolution. Provided that the non-specific binding can be reduced, the chromatographic procedure has the advantage of being predictable. Thus it offers an easy way of designing a system where a certain elution order is desired. The fact that the polymer preparations described can be used in HPLC systems, with reproducible separations over several months, is also promising since the use of efficient chromatographic methods is necessary for better and faster separations.

REFERENCES

1 S. Allenmark, *J. Biochem. Biophys. Methods*, 9 (1984) 1.
2 D. W. Armstrong, *J. Liq. Chromatogr.*, 7 (1984) 353.
3 W. H. Pirkle and J. Finn, in J. D. Morrison (Editor), *Asymmetric Synthesis*, Academic Press, New York, 1983, p. 87.
4 A.-D. Schwangart, W. Backman and G. Blaschke, *Chem. Ber.*, 110 (1977) 778.
5 S. Yuasa, M. Itoh and A. Shimada, *J. Chromatogr. Sci.*, 22 (1984) 288.
6 A. Dobashi, K. Oka and S. Hara, *J. Am. Chem. Soc.*, 102 (1980) 7122.
7 W. H. Pirkle, M. H. Hyun and B. Bank, *J. Chromatogr.*, 316 (1984) 585.
8 V. Davankov, *Pure Appl. Chem.*, 54 (1982) 2159.
9 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.*, 22 (1984) 411.
10 G. D. Y. Sogah and D. J. Cram, *J. Am. Chem. Soc.*, 101 (1979) 3035.
11 L. Andersson, B. Sellergren and K. Mosbach, *Tetrahedron Lett.*, 25 (1984) 5211.
12 R. Arshady and K. Mosbach, *Makromol. Chem.*, 182 (1981) 687.
13 G. Wulff, W. Best and A. Akelah, *Reactive Polym.*, 2 (1984) 167.
14 J. Damen and D. C. Neckers, *Tetrahedron Lett.*, 21 (1980) 1913.
15 G. Wulff, W. Vesper, R. Grobe-Einsler and A. Sarhan, *Makromol. Chem.*, 178 (1977) 2799, 2817.
16 G. Wulff and W. Vesper, *J. Chromatogr.*, 167 (1978) 171.
17 O. Norrlöw, M. Glad and K. Mosbach, *J. Chromatogr.*, 299 (1984) 29.
18 M. Glad, O. Norrlöw, B. Sellergren, N. Siegbahn and K. Mosbach, *J. Chromatogr.*, 347 (1985) 11.
19 J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, Vol. 2, Wiley, New York, 1961, p. 925.
20 J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, Vol. 3, Wiley, New York, 1961, p. 2067.
21 W. Funke, *Chimia*, 22 (1968) 111.
22 G. Wulff, J. Vietmeier and H-G. Poll, *Nouveau J. Chimie*, 6 (1982) 681.
23 G. Schill, in J. A. Marinsky and Y. Marcus (Editors), *Ion Exchange and Solvent Extraction*, Vol. 6, Marcel Dekker, New York, 1974, pp. 1–57.