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IMPRINTING OF AMINO ACID DERIVATIVES IN MACROPOROUS POLYMERS

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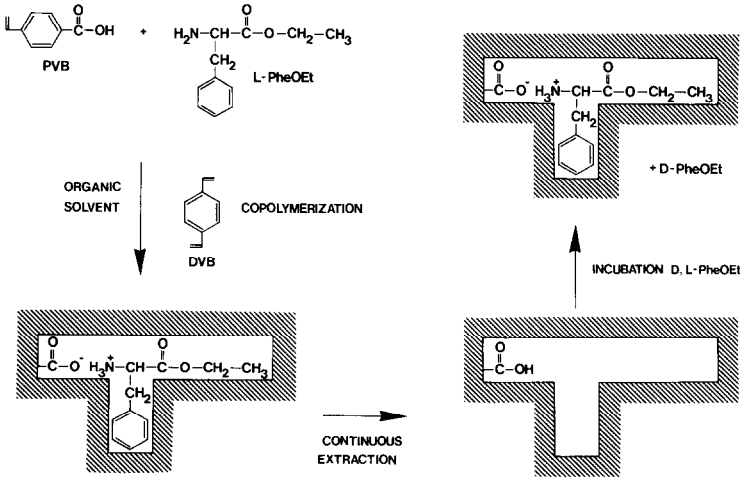
Abstract: Phenylalanine ethyl ester-selective polymers have been prepared using the ion-pair association of substrate and carboxyl-containing vinyl monomers in the polymerization step.

Preparation of polymers containing substrate-selective cavities shaped after the structure of a template molecule present during polymerization has proven to be possible in several studies.[1-4] Various names have been given to these approaches such as "template"[1,2] or "host-guest polymerization".[4] "Imprinting" has also been used to describe the technique employed.[4-6] In template polymerization it is a requirement that desired templates can be modified by vinyl substitution prior to their copolymerization with other vinyl monomers. It can be difficult to achieve such modifications, and elaborate organic chemistry is often needed to be able to derivatize wanted template molecules. It would therefore be desirable to use methods obviating the need of both template modification steps and associated hydrolyses steps of polymers required to remove polymer-bound template molecules after polymerization.[1-3] In this study, we would like to report on such an alternative approach to make substrate-selective polymers utilizing imprinting of small molecules in highly crosslinked polymers.[7]



SCHEME 1: PVB = paravinylbenzoic acid; PheOEt = phenylalanine ethyl ester; DVB = divinylbenzene.

Scheme 1 illustrates schematically the strategy used to prepare phenylalanine ethyl ester-selective polymers. A print molecule, D- or L-phenylalanine ethyl ester, is present at low concentration in a mixture containing, in addition to print molecules, vinyl monomers, initiator and an inert organic solvent. In this mixture, it is envisaged that substrate molecules would interact preferentially with carboxyl-containing vinyl monomers due to coulombic forces between positively charged amino groups of substrate and oppositely charged carboxylates of carboxyl-containing vinyl monomers. Specific complexes of substrate and vinyl monomers could therefore be formed due to electrostatic interactions during polymerization. After polymerization, loosely bound print molecules are washed from the polymers under very mild conditions by extracting the polymers with an organic solvent. The obtained polymers now contain imprints of added print molecules, and the formed cavities, shaped after the print structure, are equipped with carboxyl groups that can interact specifically with the amino function of rebound phenylalanine ethyl ester molecules.

Two different kinds of polymers specific for phenylalanine ethyl ester substrates were prepared in this study (Table 1). One series of polymers was made from styrene monomers and the other was made from acrylic monomers. A high concentration of crosslinking agents (divinylbenzene or ethyleneglycoldimethacrylate, 54 and 86 mole %, respectively) was present in the polymerization mixtures[8] in order to produce macroporous polymers of high rigidity.[1-3,10] Formed polymers were extracted with acetonitrile, and it was found that approximately 55 % of added print molecules were recovered from polymers prepared in the presence of carboxyl-containing vinyl monomers (paravinylbenzoic acid or acrylic acid).

Mixtures containing roughly equal amounts of D- and L-phenylalanine ethyl ester, differentially labelled with radioisotopes,[11] were applied to the prepared polymers. After sonication of the assay mixtures,[12] the latter were allowed to incubate at room temperature for at least 24 hrs. Measurements of radioactivity of supernatants were carried out to determine the amount of substrate free in solution and bound to the polymers at equilibrium. The ratio of apparent distribution coefficients (K_D/K_L) for the partitions of D- and L-substrate between polymer and solvent is expressed in Table 1 as the separation factor α. It could be shown that α-values determined for polymers prepared in the presence of D-print molecules were greater than one, indicating that the polymers were chiral and that they interacted preferentially with the D-form of the print substrate. This situation could be reversed and α-values determined for polymers prepared in the presence of L-print molecules were found to be less than one, indicating that these polymers were chiral as well, but in this case the produced polymers showed L-specificity in preference to D-specificity. On the other hand, the found resolution powers of reference polymers identically prepared in the presence of a D,L-mixture of phenylalanine ethyl ester molecules or in the absence of print molecules were practically zero, i.e. with determined α-values of roughly one indicating that no chiral polymers had been made during polymerization. Substrate-selective polymers were also prepared in the presence of styrene substituting paravinylbenzoic acid in the polymerization mixtures. It is interesting to note that these polymers had a low binding capacity for substrate (less than about 20 % compared to that of the corresponding carboxyl-containing print polymers). However, some chiral substrate binding was still found to be possible for such styrene-based print polymers.

TABLE 1. Batchwise resolution of racemic mixtures of phenylalanine ethyl esters after application of the mixtures to phenylalanine ethyl ester-selective polymers.

Polymer[1)]	Polymer specificity (print molecule)	Amount of D-ester bound to polymer (μmoles)	Amount of L-ester bound to polymer (μmoles)	Amount of D-ester found in supernatant (μmoles)	Amount of L-ester found in supernatant (μmoles)	Separation factor[3)] (α)
Styrene polymers	D-PheOEt[2)]	0.537	0.550	1.756	1.875	1.043 (1.061)
	L-PheOEt	0.498	0.554	1.795	1.871	0.937 (0.953)
	D,L-PheOEt	0.428	0.459	1.865	1.966	0.983 (1)
Acrylic polymers	D-PheOEt	0.637	0.578	2.246	2.119	1.040 (1.088)
	L-PheOEt	0.504	0.527	2.379	2.119	0.872 (0.912)
	None	0.585	0.567	2.298	2.130	0.956 (1)

1) For used polymerization conditions see (8).

2) PheOEt = phenylalanine ethyl ester.

3) α-Values within parenthesis are normalized values related to the α-values of reference polymers prepared in the presence of D,L-PheOEt (styrene polymers) or in the absence of print molecules (acrylic polymers).

Based on the found resolutions of the applied racemic mixtures, we feel that polymer substrate-selectivity observed in this study was indeed due to substrate binding to specific binding sites formed in the polymers after imprinting of substrate. It could be argued that print molecules remaining tightly bound in the polymers could interact with added substrate molecules and give rise to resolution of applied mixtures.[13] This possibility appears less likely, since it was found that polymers containing L-phenylalanine ethyl ester covalently bound to the polymer (polymers were prepared from divinylbenzene (55.5 mole %), ethylstyrene (41.6 mole %) and N-paravinylbenzoyl-L-phenylalanine ethyl ester (2.9 mole %) under otherwise identical polymerization conditions as described in reference (8)) were inefficient in resolving racemic phenylalanine ethyl ester mixtures.

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References and Notes.

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7. A brief account of part of this study was given at the Fifth International Symposium on Affinity Chromatography and Biological Recognition, Annapolis, Maryland, June 12-17, 1983.
8. Polymers based on styrene monomers were prepared from divinylbenzene (55.5 mole %), ethylstyrene (41.6 mole %) and paravinylbenzoic acid (2.9 mole %), and polymers based on acrylic monomers were prepared from ethyleneglycoldimethacrylate (90.5 mole %) and acrylic acid (9.5 mole %). Equal molar amounts of print molecules and paravinylbenzoic acid were used to prepare the former polymers, and in the preparation of the latter polymers the used concentration of acrylic acid was twice the concentration of print molecules. Styrene polymers were prepared after dissolving 111 mg of paravinylbenzoic acid (acid form), 145 mg of phenylalanine ethyl ester (free base), 3442 mg of divinylbenzene (Merck; this quality of divinylbenzene contains in addition to meta- and paradivinylbenzene (53.9 %) ethylstyrene (41 %) and diethylbenzene and residues (4.6 %) (9)) and 35 mg of azabis-(isobutyronitrile) (AIBN) in 4.8 ml of acetonitrile. The mixture was then placed in glass tubes. After degassing, the tubes were sealed under nitrogen and heated at 60 °C, 90 °C and 120 °C for 24 hrs at each temperature. Obtained polymers were ground and were then subjected to continuous extraction with acetonitrile in a Soxleth extractor for a minimum length of time of 24 hrs. Polymers based on acrylic monomers were prepared similarly.
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11. D-Phenylalanine-{C-1, ^{14}C}-ethyl ester (7.2×10^{-3} μCi/μmole) and L-{C-2, C-3, ^{3}H}-phenylalanine ethyl ester (12.7×10^{-3} μCi/μmole) were synthesized from phenylalanine and ethanol as described by J.P. Greenstein and M. Winitz in "Chemistry of the Amino Acids", Wiley, New York (1961), p. 925.
12. The assay mixtures contained D-{^{14}C}- and L-{^{3}H}- phenylalanine ethyl ester (added as free base), 0.5 g (dry weight) of polymer and 4 ml of acetonitrile (dried over molecular sieves).
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