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ENZYME-ANALOGUE BUILT POLYMERS AND THEIR USE FOR THE RESOLUTION OF RACEMATES[1)]

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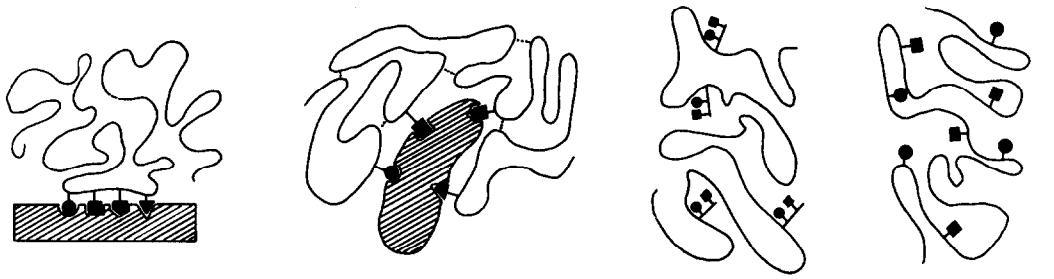
In the synthesis of polymers as enzyme analogues one of the main problems is the introduction of functional groups into the polymer in a definite steric arrangement. In natural hormones and enzymes the functional groups responsible for the interaction with a receptor or a substrate can be arranged into two types, which R. Schwyzer[2)] has called "continuate words" and "discontinuate words". In the first case, which is found in a number of hormones, the functional groups are located on the peptide chain one after another and interact with corresponding groups on the receptor (model A). In this way only two-dimensional information can be transferred, which is given by the functional group sequence on the chain. Contrary to the former is the "discontinuate words" arrangement, which is found in other hormones and in all enzymes. In this case the functional groups responsible for the specifity are located at quite different points on the peptide chain and are brought into spatial proximity by the specific folding of the chain. Here not only the functional group sequence on the chain but the peptide's tertiary structure, i.e. its topochemistry, is decisive (model B). In this case complex three-dimensional steric arrangements of the functional groups can be obtained.

To date the synthesis of enzyme analogues has been attempted by polymerisation or polycondensation of monomers with neighbouring functional groups (model A), or by grafting side chains with neighbouring groups onto a polymer (model C). Another method has been the copolymerisation of monomers containing different functional groups, thereby obtaining polymers in which functional groups are randomly distributed along the polymer chain and in space. The proximity of two different groups then occurs only on a purely fortuitous basis (model D).

This paper is dedicated to Prof. Dr. H. Hellmann, Marl, on the occasion of his 60th birthday.

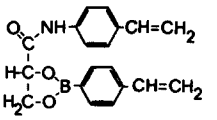


A. continuate words B. discontinuate words C. D.

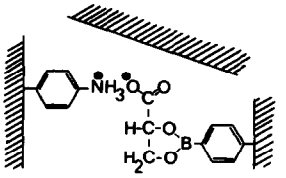
Since enzyme-analogue polymers prepared according to models A, C, and D show, with the exception of one example reported by I.M. Klotz et al.[3], only weak catalytic activity compared with natural enzymes[4], we have now tried to prepare synthetic polymers with functional groups in a "discontinuate words" arrangement. In doing this it was our idea, to bond the functional groups to be introduced,in the form of polymerizable vinyl derivatives, to a suitable template molecule. This compound should then be polymerized under conditions such that rigid, non-swellable polymers were formed. It was anticipated that after removal of the template the functional groups would be present in a fixed stereochemistry within cavities of the polymer and that the stereochemistry would correspond to the chemical structure of the template.

In order to test this idea, optically active compounds were chosen as templates, and such functional groups, which are known to undergo an easily reversible interaction with the template, analogous to the binding site interaction in natural enzymes. In the case when the functional groups, on removal of the template, remain exactly in the given steric arrangement, one would expect that the polymer would preferentially interact with that enantiomer of the template, which had been used originally. This means that the exact steric arrangement of the functional groups (and therefore the success of the process) would be indicated by the ability of the polymer to resolve a racemate of the template.

As examples the polymerisation of D-glyceric acid-(p-vinylanilide)-2,3-O-(p-vinylphenylboranate) (see E) [m.p. 143-45°, $[\alpha]_D^{20}$= +176,6° (dioxan)] and of D-mannitol-1,2;-3,4;5,6-tri-O-(p-vinylphenylboranate) [m.p. 158-60°, $[\alpha]_{436}^{20}$ = +144,0° (chloroform)] was studied. Both compounds were copolymerized by radical initiation with a high amount of bifunctional cross-linking agent in the presence of an inert solvent. Under these con-



E.



F.

ditions macroporous polymers can be obtained[5]. For our purpose the preparation of the polymer had to be somewhat modified, since the polymer chains after removal of the template should be extremely rigid and non-swellable in a number of solvents. The non-swellability of the polymer is necessary for the immutability of the spatial relationship of the functional groups in the macromolecule. On the other hand the polymer must permit good access to as many functional groups as possible.

E.g. the polymerisation of a solution of E (10 g) and a mixture containing 55% divinyl benzene isomers and 35% of ethylvinylbenzene (46.5 g) in acetonitrile (60 ml) with azo-isobutyronitrile (355 mg) was carried out in sealed tubes with a programmed temperature rise from 60 to 120°. The polymer obtained was ground and sifted to a main fraction having a grain size of 63 to 125 microns.

The splitting-off of the template was effected in the case of D-glyceric acid (polymer I) by heating the polymer at 70° with 20% HCl in methanol for 16 hours under N_2; in the case of D-mannitol (polymer II) with methanol/water 1:2 at 40° for 48 hours. With D-glyceric acid a maximum splitting yield of 30%, with D-mannitol of 80-90% could be obtained. The two polymers prepared in this way contained free boronic acid and amino groups, and free boronic acid groups respectively. They had a capacity for the uptake of glyceric acid and mannitol respectively that corresponded to the quantity originally split off. The glyceric acid taken up is bound to the polymer by a boronic acid diester linkage and an electrostatic interaction with the amino group (see F); the mannitol by boronic acid diester linkages.

On reaction of polymer I with D,L-glyceric acid in a batch procedure binding of the D-form was significantly better, and L-glyceric acid accumulated in the solution. The glyceric acid taken up by the polymer was easily split off to demonstrate an enrichment of D-glyceric acid. The resolving factor α, i.e. the ratio of the distribution coefficients between the polymer and the solution of the D and L-form, was 1.034 in case of D,L-

glyceric acid. The same polymer had an α-value for D,L- glyceric aldehyde of 1.036, and for D,L-glyceric acid methylester of 1.012. Polymer II showed an α-value for D,L-mannitol of 1.09. These values are apparently higher than those obtained with previous synthetic polymers containing a chiral residue either in the main chain, in the side chains or bound by electrostatic forces [6]. As expected the ability to resolve racemates was totally and irreversibly lost in solvents that swelled the polymer. Polymers prepared without strong cross-linking also had no resolving power.

This power of racemic resolution shows, that it was indeed possible to introduce functional groups into a polymer in a "discontinuate words" arrangement analogous to that in enzymes. By this method it may be possible to prepare synthetic polymers with much higher catalytic activity.

It should be mentioned that the described method for the resolution of racemates is based on a novel principle: In contrast to the usual resolution of racemates by interaction with a chiral molecule, no such species is required. Instead the resolution takes place in cavities, which are asymmetrical due to the particular spatial relationship of the functional groups. Steric limitations and hydrophobic interactions would possibly be contributory factors.

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