Enzyme Immobilization by Covalent Attachment to Novel Polymer Matrices Prepared by a Radiation Grafting Technique

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Summary By an irradiation process, *p*-nitrostyrene has been surface grafted onto a representative trunk polymer, polypropylene; conversion of the NO_2 substituent into a suitable functional group (–NCS) gives a water-insoluble matrix to which enzymes may be covalently bound whilst retaining enzymic activity.

WE report the use of a novel radiation grafting technique for the immobilization of the enzyme, trypsin, using polypropylene powder. The process appears to be of general application for the immobilization of a wide range of chemical and biochemical macromolecules (enzymes, enzyme inhibitors, co-factors, and immunochemicals) on a variety of trunk polymers. Using conditions of γ-irradiation which lead essentially to surface, as distinct from bulk copolymerization,[1] *p*-nitrostyrene has been grafted onto powdered polypropylene. Polypropylene is not subject to chemical or microbial attack as are natural polymers. Natural polymers have also been employed in radiation grafting,[2] however these grafts have never previously been used to insolubilize enzymes. By grafting *p*-nitrostyrene directly rather than grafting styrene and then nitrating with fuming nitric acid, depolymerization of the trunk polymer is avoided. Further, one nitro group per aromatic ring is obtained, rather than a distribution of non-, mono-, and poly-nitrated aromatic rings. Natural polymers (*e.g.* cellulose), synthetic polymers (*e.g.* polyacrylamide, ethylene–maleic anhydride copolymer), and glass have all been utilized for the insolubilization of enzymes by covalent bonding involving essentially chemical procedures.[3] However the most frequently used class of synthetic resins for this purpose are cross-linked polystyrene beads, in which a suitable functional group is present. When enzyme is covalently bound to such a matrix, it is distributed throughout the three-dimensional network. Thus the rate of enzymic catalysis by the bound enzyme is limited by

diffusion of substrate and product through the bead. These restrictions, particularly diffusional limitation, are reduced in the present radiation copolymer system where the functional group to which the enzyme is attached is on the surface of the polymer and thus readily accessible.

In the current work, polypropylene powder, as a suspension in a solution of *p*-nitrostyrene in DMF, was irradiated in a cobalt-60 source. Since grafting is proportional to dose (Table) and preliminary experiments showed that a graft of 30% was required for the present studies, the mixture was given 2 MRads. The nitro group in the recovered poly(*p*-nitrostyrene-g-polypropylene) was reduced to the amino form with stannous chloride–hydrochloric acid.[4]

TABLE

Radiation conditions for grafting of *p*-nitrostyrene to polypropylene

Total dose (MRad)[a]	% Graft[b]
0·5	11·9
1·0	17·5
1·5	24·0
2·0	30·2

[a] Polypropylene powder (5 g) suspended in 33% *p*-nitrostyrene in DMF (6 ml), irradiated in air at 200 kRad h^{-1} using a cobalt-60 source. [b] Homopolymer was removed from graft copolymer by Soxhlet extraction using $CHCl_3:C_6H_6$ (3 : 1) for 48 h followed by MeOH for 24 h and drying *in vacuo*. % graft is based on results of elemental analysis.

The amino derivative is a versatile polymer matrix which, theoretically, is capable of being utilized for the immobilization of biochemical macromolecules in a variety of ways. Thus, difunctional cross-linking reagents, such as glutaraldehyde,[5] *NN*-dicyclohexylcarbodi-imide[6] or *N*-ethyl-5-phenylisoxazolium-3′-sulphonate,[7] may be used to covalently bind the macromolecule to render it water-insoluble. Alternatively, the amino group may be converted into the diazo[8] or, as in the present work, the isothiocyanate derivative.[9] Using the latter procedure,[9] trypsin was covalently bound to the radiation surface-grafted co-polymer, poly(*p*-isothiocyanatostyrene-g-polypropylene), and its proteolytic activity determined using *NN*-dimethylhaemoglobin as substrate.[10] The covalently bound enzyme possessed 13% of the activity of an equal weight of native trypsin in solution at the same pH. Experiments are in progress to increase the conversion efficiency of the bound enzyme.

Compared with previous techniques for enzyme immobilization, the radiation copolymerization method for the preparation of the polymer matrix is simple, involves a one-step grafting operation, gives only a surface graft (so that the enzyme is readily accessible to soluble substrate), and is applicable to a wide range of polymeric supports of different chemical structures. It is potentially the most versatile of all techniques for such enzyme immobilization reactions.

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