A New Method for the Attachment and Support of Active Enzymes

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A variety of methods for attaching enzymes to solid materials have been described (Crook, 1968; Manecke, 1968). Though these methods sometimes result in insoluble matrix-supported enzymes with relatively high activity, the procedures employed for preparing support polymers and attaching enzymes to them are elaborate and often difficult to repeat. We have developed an unusually simple method for preparing active insoluble polymer-supported enzymes.

The polymer used as an enzyme support is a random co-polymer of L-alanine and L-glutamic acid (10:1) prepared from the *N*-carboxylic acid anhydrides of L-alanine and L-glutamic acid benzyl ester. This polymer is now commercially available from the Pilot Chemical Co., Watertown, Mass., U.S.A. If allowed to swell first for 24 hr. in acetonitrile, the polymer is extremely wettable in aqueous buffer solution, forming a thick suspension. Enzymes may be attached to the water-swollen support polymer by using the condensing agent *N*-ethyl-5-phenylisoxazolium-3'-sulphonate (Patel, Lopiekes, Brown & Price, 1967) to form amide bonds between the carboxyl functions of the polymer and ϵ -amino groups on the enzyme surface.

Insoluble polymer-supported chymotrypsin was prepared by the following procedure. *N*-Ethyl-5-phenylisoxazolium-3'-sulphonate (300 mg.) (Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.) was added to a suspension of 100 mg. of acetonitrile-swollen support polymer in 5 ml. of 0.1 M-potassium phosphate buffer, pH 8.5, and the reaction stirred at 10° for 1 hr. Centrifugation of the reaction suspension yielded the insoluble activated polymer, which was resuspended in 5 ml. of 0.1 M-potassium phosphate buffer, pH 8.5, containing 500 mg. of chymotrypsin (Calbiochem Corp., Los Angeles, Calif., U.S.A.). After being stirred for 24 hr. the reaction suspension was washed and centrifuged ten times with buffer, pH 2.2, containing NaCl (1 M), to remove all unbound chymotrypsin. Several additional washings with distilled water removed the excess of NaCl and, after drying *in vacuo*, a product weighing 386 mg. was recovered. Polymer-supported trypsin was prepared by an identical procedure, which resulted in a product weighing 366 mg.

The polymer-supported enzymes were assayed by titrimetrically following the rates of the chymotrypsin-catalysed hydrolysis of *N*-acetyl-L-tyrosine ethyl ester and the trypsin-catalysed hydrolysis of toluene-*p*-sulphonyl-L-arginine methyl ester. The enzymic activities of the polymer-supported insolubilized enzymes are shown in Table 1.

Table 1. *Characteristics of polymer-supported trypsin and chymotrypsin*

Polymer-supported enzyme	Enzyme wt. (%)	Enzyme activity (%)
Chymotrypsin	74	38
Trypsin	73	43

Crook, E. (1968). *Biochem. J.* **107**, 1 p.Manecke, G. (1968). *Biochem. J.* **107**, 2 p.Patel, R., Lopiekes, C., Brown, S. & Price, S. (1967). *Biopolymers*, **5**, 577.