and GTP are the weakest inhibitors. (6) Mg^{2+} does not abolish the inhibition of chloramphenicol acetylation exerted by ATP.

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The preparation of trypsin chemically attached to nylon tubes

Although enzymes have been insolubilised by a wide variety of methods[1], the resulting products have so far only been used in the form of particles, both for continuously stirred reactors[2] and packed beds[3], in the form of porous sheets[4] and in the form of membranes[5]. The present work describes two new methods for the insolubilisation of an enzyme by its attachment to nylon, together with the novel use of a water-insoluble enzyme in the form of a tube.

The inside surface of a nylon tube (4.0 m long, 0.1 cm internal diameter) made of "Type 6" nylon (John Tullis, Alloa, Scotland) was partially hydrolysed by perfusion through the tube with 3.0 M HCl for 30 min at 30° at a flow rate of 2 ml/min, after which the hydrolysis was arrested by washing through the tube with water. At this stage the presence of liberated amine groups was confirmed by the red coloration produced on the inside surface of the tube, when a small portion of the latter was treated with a 0.1% (w/v) solution of 2,4,6-trinitrobenzenesulphonate in saturated sodium tetraborate. So as not to interfere in subsequent steps in the process, the amine groups were destroyed by perfusing the tube for 2 min with an ice-cold solution of 1% (w/v) $NaNO_2$ in 0.5 M HCl and then warming the tube to 40° and continuing the perfusion for a further 20 min. The destruction of the primary amine groups was confirmed by the inability of a small sample of the inner surface of the tube to form a red-coloured trinitrophenyl derivative with 2,4,6-trinitrobenzenesulphonate.

The tube was then cut into two equal lengths and to one portion benzidine was coupled to the carboxyl groups by perfusion through the tube at 10° with a

mixture of 1% (w/v) benzidine, 1% (w/v) dicyclohexylcarbodiimide in methylene chloride for 2 h at a flow rate of 1 ml/min. The tube was then washed by perfusion in turn with methylene chloride, acetone and water. The presence of covalently attached benzidine was established by the production of a red colour when a small portion of the tube was first diazotised and then treated with ice-cold alkaline β-naphthol.

The free carboxyl groups of the second portion of the tube were then treated to produce their acid hydrazide derivative: For this, the inside of the tube was perfused at 10° with a mixture of 5.0% (v/v) hydrazine hydrate, 1% (w/v) dicyclohexylcarbodiimide in dioxan for 2 h at a flow rate of 1 ml/min. The tube was then washed by perfusion with dioxan and then water.

For the chemical attachment of the enzyme to the inside surface of the nylon tubes both the benzidine and acid hydrazide derivatives were treated as follows. Both tubes were perfused at 0° with a mixture of 1% (w/v) $NaNO_2$ in 0.5 M HCl at a flow rate of 5 ml/min for a period of 20 min. Both tubes were then perfused with ice-cold 1 mM HCl until their effluent failed to give a positive reaction for nitrous acid by the starch–iodide test. This process was always effected immediately prior to the coupling of the enzyme. A 0.5% (w/v) solution of twice crystallised trypsin (Type I, Sigma Chemical Co., St. Louis, Mo., U.S.A.) in phosphate buffer, pH 8.0, I 0.1 at 0° (ref. 6) was then perfused through the tubes for 2 h at 0° at a flow rate of 1.0 ml/min. After this the tubes were washed free of any physically adsorbed protein by perfusion with solutions of 1.0 M NaCl, 0.5 M $NaHCO_3$ 1 mM HCl and finally water. Both tubes were stored at 4° in the dry state until required.

The tryptic activity of the tubes was determined by following the hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester (BAEE) when solutions of the substrate in Tris buffer, pH 7.8, I 0.1 at 25° (ref. 6) containing 0.25% (w/v) $CaCl_2$ were perfused through the enzyme tubes at predetermined flow rates. In each case the extent of hydrolysis was determined by measuring the absorbance at 255 nm of the effluent relative to that of the substrate solution, and using the data of SCHWERT AND TAKENAKA[7] the results have been expressed in terms of the concentration of BAEE hydrolysed on perfusion through the tubes.

The effect of flow rate on the hydrolysis of 0.5 mM BAEE by both nylon tube–trypsin derivatives is shown in Fig. 1. From a knowledge of the volume of each tube, the slope of the linear portions of the plots shown in Fig. 1 was used for the calculation of the specific activity of each trypsin–tube derivative. Values of 0.21 and 0.15 mM BAEE hydrolysed per min residence time per 2 m length of tube were obtained for the benzidine and acid azide derivatives, respectively. The values indicated for the two procedures were the average of two experiments performed simultaneously under the same conditions. In each case, for both benzidine and acid azide products, the values of the duplicates agreed to within 5%. The difference in specific activity of the two trypsin tubes may be due to either more trypsin being bound to the tube treated with benzidine or to a greater inactivation of the bound trypsin in the tube prepared by coupling through the acid azide derivative. Both trypsin–tube derivatives have been used over a period of 12 days without any significant decrease in their specific activity being observed.

Abbreviation: BAEE, α-*N*-benzoyl-L-arginine ethyl ester.

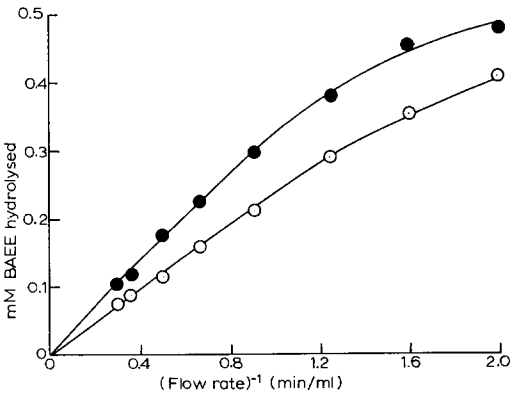

Fig. 1. The effect of flow rate on the hydrolysis of BAEE by trypsin chemically attached to the inside surface of nylon tubes. ●, nylon–benzidine–trypsin derivative; ○, nylon–acid azide–trypsin derivative. The assays were performed at 25° in Tris buffer, pH 7.8, I 0.1, containing 0.25% (w/v) $CaCl_2$ and 0.5 mM BAEE.

This work demonstrates that it is possible to chemically attach an enzyme to the inner surface of a nylon tube and to use the enzyme in this form. An important extension of this work could be the use of enzymes in this form for automated analytical procedures that are based on the continuous flowthrough principle.

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