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Note

Chemical attachment of β-D-glucosidase to DEAE-cellulose

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Recently[1], we described the preparation of an insolubilised enzyme by the reaction of almond β-D-glucosidase with cellulose carbonate[2]. Two interesting features were noted. Firstly, the coupling process appeared to have a pH optimum of 7–8, *i.e.*, the most-active, solid-phase enzyme was prepared at pH 7–8; and secondly, the pH–activity profile of the solid-phase β-D-glucosidase was virtually identical with that of the native enzyme. We now report the attachment of the same enzyme to DEAE-cellulose, an experiment which has given significantly different results.

The activities of solid-phase β-D-glucosidases prepared from DEAE-cellulose carbonate at different pH values are shown in Fig. 1. The most-active preparations were obtained when coupling was performed at pH 9.0 and 10.0. This result is

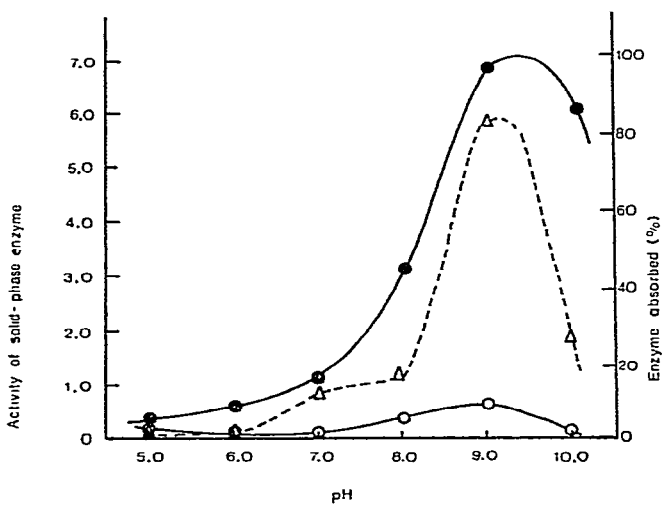

Fig. 1. Activity of solid-phase enzyme prepared from DEAE-cellulose carbonate and β-D-glucosidase at various pH values. The activity of each preparation is expressed in μmole of *o*-nitrophenol liberated per ml per h by 10 mg of complex, under the conditions described in the Experimental. ●, β-D-Glucosidase activity of product obtained from reaction of β-D-glucosidase with DEAE-cellulose carbonate; ○, β-D-glucosidase activity of controls obtained by incubation of DEAE-cellulose with β-D-glucosidase; △, fraction of β-D-glucosidase absorbed from solution by DEAE-cellulose.

strikingly different from that obtained with cellulose carbonate, for which the optimum pH of coupling lies between 7.0 and 8.0. Clearly, the presence of the diethylaminoethyl groups has shifted the pH profile upwards by approximately two pH units. That the enzyme was covalently linked to the support was indicated by the retention of activity even after exhaustive washing, and also by the observation that the controls, with DEAE-cellulose, gave very low, enzymic activities (Fig. 1).

In the earlier report, we attributed the pH profile of the coupling process for cellulose carbonate to a combination of several factors, namely, the stability of the cyclic carbonate groups at low pH and their instability at high pH, and the increasing number of reacting nucleophilic groups on the enzyme with increasing pH. Obviously, the presence of the DEAE-groups in the support has introduced a new factor.

The most likely explanation for the pH optimum of ~9.0 for the coupling process is that the enzyme is first absorbed on to the DEAE-cellulose by purely ionic forces. The isoelectric point[3] of almond β-D-glucosidase is of the order of 5.8. Above this pH, the enzyme will be progressively more negatively charged. Over the pH range used, the DEAE-cellulose will be positively charged, and therefore the absorption of the enzyme on to the support should lead to a high concentration of enzyme in the vicinity of the carbonate groups, which, in turn, should greatly enhance the rate of coupling.

Support for this explanation was obtained by a simple investigation (Fig. 1) of the absorption of the enzyme on to DEAE-cellulose, which showed that the amount of enzyme absorbed from the solution by the solid varies with pH, with the maximum at pH 9.0. It thus appears that the controlling factor in the coupling of β-D-glucosidase to DEAE-cellulose carbonate is the prior physical absorption of enzyme on to the carrier. The water-insoluble enzyme derivatives prepared from DEAE-cellulose carbonate were exhaustively washed with acetate buffer (pH 5.0) containing M

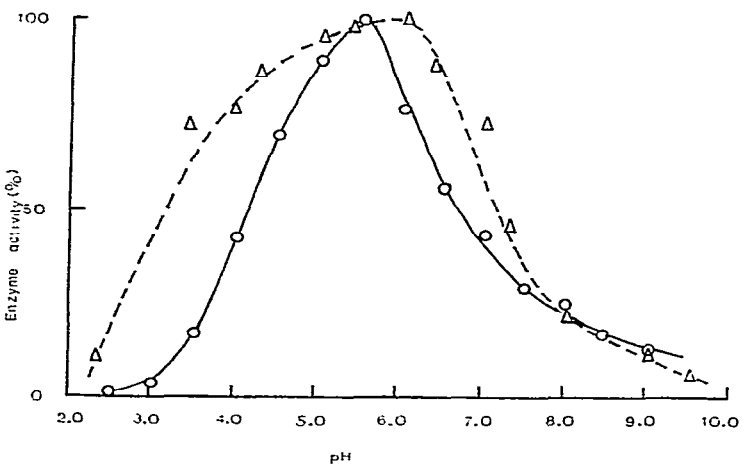

Fig. 2. pH–Activity profiles for free and bound β-D-glucosidase. The enzymic activity is given as % of the maximum; ○, free enzyme; △, enzyme attached to DEAE-cellulose carbonate.

sodium chloride and sucrose. This solution would be expected to remove virtually all of the purely physically absorbed enzyme, partly due to the high ionic strength and partly because, at pH 5.0, the enzyme should be positively charged.

The pH–activity profile of β-D-glucosidase coupled to cellulose was virtually identical to the profile of the native enzyme[1]. This is not surprising since the support is uncharged. Other workers have reported similar results[4,5]. The pH–activity profile of the enzyme when coupled to DEAE-cellulose, however, is markedly different. Fig. 2 shows the pH–activity profile of the solid-phase derivative, in addition to that of the native enzyme. The original, bell-shaped curve of the native enzyme has been appreciably broadened, although the pH optimum is not significantly changed. Goldstein *et al.*[6] have discussed the effect of polyelectrolyte supports on the properties of attached enzymes. They concluded that a polycationic support would shift the pH–activity profile towards the acid region, because the effective local pH in the vicinity of the carrier would be different from that of the exterior solution. The enhancement of the acid limb of the pH–activity profile in the present case is in accord with this view. The smaller difference on the alkaline limb cannot be so readily explained. Further study, on the effect of the polyelectrolyte support on the K_m and the V_{max} for the enzyme, clearly needs to be carried out.

EXPERIMENTAL

DEAE-cellulose carbonate. — Whatman DE32 cellulose (5.0 g) was treated with ethyl chloroformate (80 ml) in a cold (0°) mixture of methyl sulphoxide (150 ml), *p*-dioxane (7.5 ml), and triethylamine (40 ml). After 10 min, the reaction mixture was neutralised with conc. hydrochloric acid and poured into 90% aqueous ethanol (400 ml). The solid was filtered off, washed with 90% aqueous ethanol (200 ml), ethanol (200 ml), and ether (200 ml), and then dried *in vacuo*; yield 4.9 g. The infrared spectrum (Nujol mull) showed a broad band at 1790 cm^{-1} which probably represents both acyclic and cyclic carbonate groups. Because of the broadness of the band, it was not possible to determine accurately the degree of incorporation of cyclic carbonate groups.

Coupling of β-D-glucosidase with DEAE-cellulose carbonate. — The enzyme was coupled to the DEAE-cellulose carbonate in the same manner as described for cellulose carbonate[1]. DEAE-cellulose was treated similarly as a control.

Determination of β-D-glucosidase activity. — Each preparation of solid-phase enzyme was assayed for β-D-glucosidase activity, using *o*-nitrophenyl β-D-glucopyranoside as substrate, by the method described earlier[1].

Absorption of β-D-glucosidase on to DEAE-cellulose at various pH values. — β-D-Glucosidase (0.1 mg/ml in 0.2M acetate buffer, pH 5.0; 5.0 ml) was added to DEAE-cellulose (Whatman DE32 cellulose; 200 mg). The suspension was stirred for 15 min at 4° and then centrifuged, and an aliquot (0.10 ml) of the supernatant was taken and assayed for enzymic activity. This procedure was repeated by using β-D-glucosidase solution in the following buffers: 0.2M phosphate (pH 6.0, 7.0, and 8.0)

and 0.2M borate (pH 9.0 and 10.0). The percentage activity lost from the solution by absorption on to the solid was calculated for each pH value. The results are given in Fig. 1.

pH–Activity profiles for bound and free β-D-glucosidase. — The variation of the activity of β-D-glucosidase, free and attached to DEAE-cellulose carbonate, with pH was investigated by the method previously described. The results are shown in Fig. 2.

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