Note

Dextran-bound AMP as the Allosteric Effector for Entrapped Threonine Deaminase

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We have reported the automated analytical systems consisting of immobilized enzyme or microbial cell tubes prepared with a photo-crosslinkable resin prepolymer.1-4 One of their applications is the assay of l-threonine in fermentation broths using biodegradative l-threonine deaminase (EC 4.2.1.16) of Escherichia coli ATCC 8739, which is characterized by a remarkable activation and stabilization by adenosine 5'-monophosphate (AMP).3 For the continuous measurement of l-threonine, however, AMP must be added to all the assay samples to retain a high and stable enzyme activity. Therefore, we have tried to co-immobilize both acetone-dried cells of E. coli as the enzyme source and polymer-bound AMP as the allosteric effector inside the gels of photo-crosslinked resin.

\[ N^6-[(6-aminohexyl)carbamoylmethyl]-AMP \] was synthesized according to the method of Lindberg and Mosbach,5 and this analogue was further bound to dextran T-40 (MW, 40,000) by the procedure of Lowe and Mosbach.6 Dextran-bound AMP (DX-AMP) thus prepared contained 34 μmol AMP/g dry dextran. The cultivation of E. coli and the induction of threonine deaminase were similar to those described previously.3 The activity of the enzyme was measured by the method of Kamihara and Tokushige,7 except that the pH of the potassium phosphate buffer was 7.0 and l-isoleucine was omitted from the reaction mixture. Acetone-dried cells of E. coli suspended in 0.9 ml of 50 mM potassium phosphate buffer, pH 7.0, were mixed with 1.0 g of a photo-crosslinkable resin prepolymer, ENT-1000, in the presence or absence of DX-AMP (corresponding to 6.8 μmol AMP) and immobilized as described previously.8

AMP and its analogues were tested for their activating effect on threonine deaminase using a free enzyme system (Fig. 1). The concentration of DX-AMP was limited to about 1 mM in the assay system due to its high viscosity. At 1 mM, the activity of DX-AMP was about 70% of that of free AMP. This decreased activity may be ascribable to the steric hindrance of the polymer moiety of DX-AMP and/or the partial degradation of the AMP moiety during the synthesis of DX-AMP. However, this value was considerably higher than that of the DX-AMP prepared by Mosbach and Gestrelius9 as the allosteric effector for glycogen phosphorylase b.

When E. coli cells were entrapped and used for the repeated reactions, the entrapped preparation without DX-AMP exclusively required the addition of exogenous AMP for its activity. This activity decreased rapidly over repeated reactions without AMP (Fig. 2). Conversely, the
preparation containing co-immobilized DX-AMP retained a relatively high activity over 40 batches of the reaction even when exogenous AMP was not added. The rapid reduction in activity during the first several batches may be due to a leakage of DX-AMP in the vicinity of the gel surface. The gradual decrease after 40 batches of the reaction would be ascribed to the inactivation of the enzyme itself.

The results mentioned above suggest that co-entrapment of polymer-bound allosteric effector(s) with enzyme within suitable gels would be useful for repeated or continuous reaction systems requiring such effectors.

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REFERENCES