PREPARATION AND PROPERTIES OF UREASE CHEMICALLY ATTACHED TO NYLON TUBE

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1. Introduction

It has recently been shown that a water-insoluble enzyme, prepared by covalently attaching the enzyme to the inner surface of a polystyrene tube, can be used advantageously in auto-analytical methods based on the continuous flow through principle [1]. The present work describes a new method for attaching an enzyme to the surface of a nylon tube by linking free amino groups on the enzyme to amino groups on the surface of the nylon through the bifunctional reagent glutaraldehyde.

2. Experimental

Urease (EC 3.5.1.5) was extracted from Jack Bean meal by the method of Mamiya and Gorin [2]. The protein recovered from a second extraction of the meal with 0.014 M mercaptoethanol in 31.6% (v/v) acetone was lyophilised and used in all experiments. This material had a specific activity of 2.55×10^2 I.U. per mg and a K_m for urea of 3.5 mM. For the attachment of the enzyme to nylon, a 2 m length of nylon tubing made from 'Type 6' nylon (0.1 cm internal diameter, obtained from John Tullis, Tullibody, Alloa, Scotland) was first partially hydrolysed on its inside surface by perfusion for 60 min at 30° with 3 M HCl at a flow rate of 2 ml min^{-1}. The hydrolysis was arrested by washing through the tube with water, after which a 2.5% (v/v) solution of glutaraldehyde in bicarbonate buffer, pH 9.4, I 0.2 at 0° [3] was perfused through the tube for 15 min at 0° at a flow rate of 2 ml min^{-1}. The tube was then washed through at 0° with phosphate buffer, pH 8.0, I 0.05 at 0° [3] containing 1 mM EDTA and then perfused for 30 min at 0° with a 7.5% (w/v) solution of urease in the phosphate buffer containing 1 mM EDTA and 10 mM mercaptoethanol. The inside surface of the tube was finally washed free of any physically attached enzyme by perfusion in turn with 0.1 M $NaHCO_3$, 1.0 M NaCl and water. The tube was stored at 4° in water when not in use. Unless otherwise stated urease activity was determined at 25° in the presence of phosphate buffer, pH 7.0, I 0.05 at 25° [13]. In all cases the extent of urea hydrolysis was followed by determination of the ammonia by the method of Chaney and Marbach [4].

The protein content of the nylon tube-urease derivative was determined by hydrolysis of the urease-glutaraldehyde-nylon Schiff base in 3 M HCl for 60 min at 40°. The solubilised urease was then determined colorimetrically by the method of Lowry et al. [5], reference being made to a standard curve compiled by subjecting known amounts of the free enzyme to the same procedure. Repetition of the acid hydrolysis and subsequent analysis for protein in solution gave a negative result.

3. Results and discussion

The protein in the nylon tube-supported urease is believed to be covalently attached to the support, since prolonged subjection of the preparation to the washing procedure outlined above did not remove any enzymic activity from the tube. The protein content of the preparation corresponded to 62.5 μg of protein bound per metre length of tubing.

The effect of substrate concentration on the hydrolysis of urea by the nylon-tube supported urease was studied by perfusing urea solutions through the tube at predetermined flow rates. The results of this experiment (fig. 1) show the K_m of the nylon tube-supported urease for urea is approximately 3.5 mM and is independent of flow rate in the range 2.3–13.7 ml min^{-1}. Furthermore, these results show that the V_{max} for the hydrolysis of urea is 0.75 μmoles of urea hydrolysed per sec residence time per metre length of tubing and is also independent of flow rate in the same range.

The effect of pH on the hydrolysis of urea by urease free in solution and nylon tube-supported urease was studied at 25° at a substrate concentration of 50 mM. All assays were performed in phosphate-pyrophosphate acetate buffer, prepared by titrating an equimolar mixture of K_2HPO_4, $Na_4P_2O_7$ and sodium acetate to the approximate pH desired, then diluting the buffer solutions so that the concentration of all buffer ions was in each case 0.0125 M and finally checking the pH of the diluted mixtures. The results of this experiment (fig. 2) show that there is no significant difference between the pH-activity curve of the free enzyme and that of the nylon tube-supported derivative.

The effect of temperature on the hydrolysis of urea by both the free and the insoluble enzyme was studied at a substrate concentration of 50 mM at temperatures between 25 and 40°. Arrhenius plots for both enzymes were linear in this range and values for the activation energy of 9.6 and 9.2 kcal mole^{-1} were

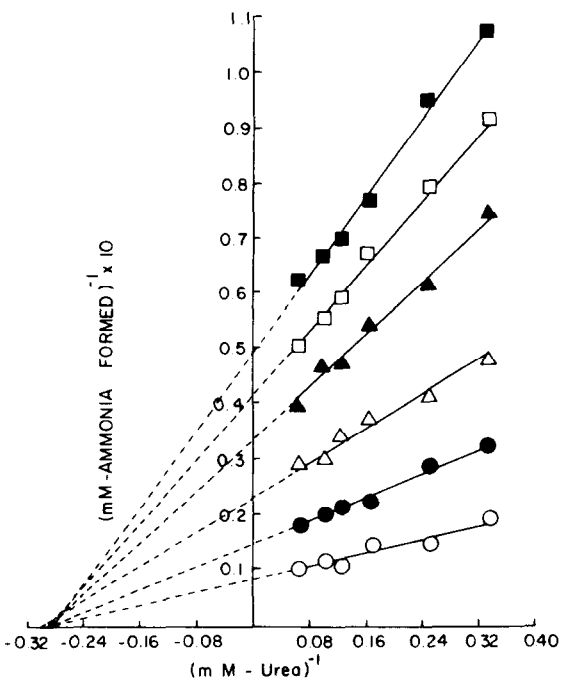

Fig. 1. The effect of substrate concentration on the hydrolysis of urea by nylon tube-supported urease at different flow rates. The assays were performed at 25° in phosphate buffer, pH 7.0, I 0.05, containing 1 mM EDTA flow rates (ml min^{-1}): ■—■ 13.7; □—□ 11.7; ▲—▲ 9.3; △—△ 6.5; ●—● 4.1; ○—○ 2.3.

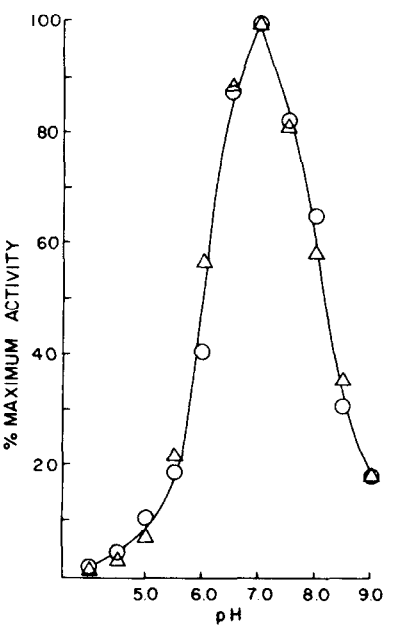

Fig. 2. The effect of pH on the hydrolysis of urea by urease free in solution (○) and chemically attached to a nylon tube (△). All assays were performed at 25° at a substrate concentration of 50 mM in the presence of phosphate-pyrophosphate-acetate buffer, prepared as described in the text.

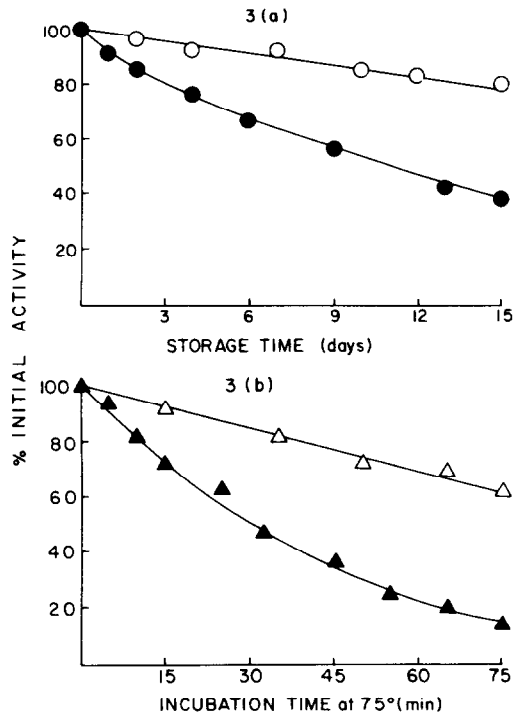

Fig. 3a. The effect of storage on the activity of urease free in solution (●——●) and chemically attached to a nylon tube (○——○). The free urease was stored at 4° in phosphate buffer, pH 7.0, I 0.05 containing mM-EDTA, and the nylon tube supported urease was stored in water at 4°.

Fig. 3b. The effect of temperature on the stability of urease free in solution (▲——▲) and chemically attached to a nylon tube (△——△). Experimental details are given in the text.

obtained for the nylon tube-supported urease and the free urease respectively.

The effect of temperature on the stability of urease free in solution and bound to the nylon tube is shown in fig. 3b. The data for the free enzyme were obtained by incubating urease (400 μg ml^{-1} in 0.05 M phosphate buffer, pH 7.0, containing mM-EDTA) at 75°; 0.05 ml aliquots were removed at intervals up to 75 min and pipetted into 1.0 ml of phosphate buffer, pH 7.0, I 0.05 at 25° [3] containing 1 mM EDTA and 50 mM urea for the determination of the residual enzymic activity. The data for the nylon tube-supported urease were obtained by incubating the tube at 75° while washing through with water at the same temperature. At intervals up to 75 min the residual urease activity was determined by perfusing the tube for 1–2 min with 50 mM urea in 0.05 M phosphate, pH 7.0 at 75° and determining the ammonia in the effluent. Fig. 3a shows the effect of storage on the activity of both urease preparations. Both these sets of data show that the nylon tube-supported urease is considerably more stable than the free enzyme.

This work shows that urease can be insolubilised by covalently attaching the enzyme to nylon. The Michaelis parameters of the insoluble preparation were independent of flow rate and the K_m was the same as that of the free enzyme, indicating that the reaction in the tube was not diffusion controlled [5]. The activation energy for the hydrolysis of urea by the bound enzyme was within 5% of that determined for the free enzyme, and the pH activity curves of both enzymes were similar, indicating that no drastic alteration in the kinetic properties of the enzyme had been incurred upon insolubilisation. The stability of the nylon tube-supported enzyme was, however, greater than that of the enzyme free in solution.

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References

[1] W.E.Hornby, H.Filippusson and A.McDonald, FEBS Letters 9 (1970) 8.
[2] G.Mamiya and G.Gorin, Biochim. Biophys. Acta 105 (1965) 382.
[3] S.P.Datta and A.K.Grzybowski, in: Biochemist's Handbook, ed. C.Long (E. F.N. Spon, London, 1961) p. 19.
[4] A.L.Chaney and E.P.Marbach, Clin. Chem. 8 (1962) 130.
[5] O.H.Lowry, N.J.Rosebrough, A.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.