IMMOBILIZATION OF GLUCOSE DEHYDROGENASE BY TITANIUM TETRACHLORIDE

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1. Introduction

A large number of methods for the immobilization of enzymes on a solid carrier has been described. Most authors use polyfunctional molecules (imines, diamines, bisaldehydes, bis-imidoesters) as coupling agents. Few procedures involving heavy metals have been published. Immobilization of enzymes on nylon fibres, microcrystalline cellulose and filter paper by means of transition metal salts has been described [1]. However, the enzymes could not be fixed to the inner surface of nylon tubing. We describe here a simple method for the insolubilization of glucose dehydrogenase (EC 1.1.1.47) [2,3] on nylon tubes using $TiCl_4$, $SnCl_4$, $MoCl_5$ and VCl_3 which yields immobilized enzyme of greater activity and stability than the more classical methods [4].

2. Materials and methods

Nylon tubing 2.6 m × 1.9 mm (Portex, Hythe, Kent) has been superficially hydrolyzed according to the method in [5]. The tubing was then air-dried at 45°C for 1 h. $TiCl_4$, 50 ml, 10% (v/v) in pentane have been pumped through the tubing at a speed of 1.7 ml/min. After drying of the tube (45°C, 2 h) this was rinsed with 250 ml coupling buffer (0.05 M Tris–HCl, pH 8.06, 17 mS cm^{-1}). Glucose dehydrogenase, 20 mg (260 U/mg protein, a gift from E. Merck, Darmstadt) was dissolved in 5 ml coupling buffer containing 3.3 mg NAD and this solution was circulated through the tubing at 4°C for 24 h and at a speed of 1 ml/min. It was then washed consecutively with 100 ml coupling buffer, 100 ml 1 M NaCl in 0.12 M phosphate buffer, pH 7.4 and 100 ml sodium citrate 0.5 M, pH 7.4. The empty tubes were stored at low temperature when not in use.

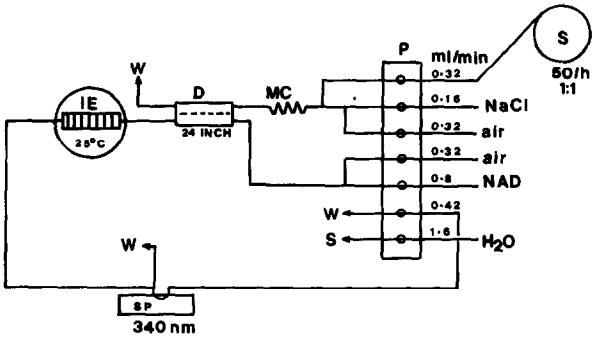


Fig.1. Diagram of continuous flow: S, sampler; P, pump; D, dialyzer (24 inch); IE, immobilized enzyme coil; W, waste.

Glucose concentrations were measured with a Technicon auto-analyzer II system using the tube prepared as above. Figure 1 shows the corresponding diagram of the continuous flow apparatus.

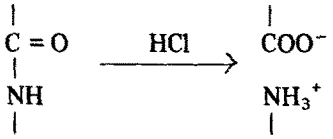
After use, the enzyme tube was washed as above, but substituting coupling buffer for the phosphate buffer.

3. Results and discussion

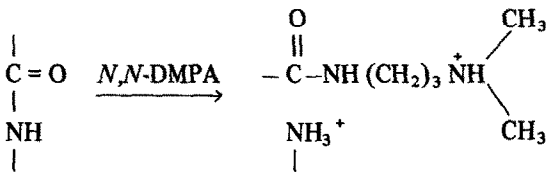
The overall reaction rate of the oxidation of glucose catalyzed by the prepared enzyme tube was measured as a function of pH. Figure 2 shows that maximum activity is obtained from pH 7.3–7.6. Below pH 7.3 the enzyme is inactivated irreversibly while the deactivation above pH 7.6 is partially reversible. This has been shown by preincubating the enzyme tube at pH > 7.6 for 25 min and measuring the activity at pH 7.41 thereafter.

The effect of temperature is shown in fig.3. The activity increases with temperature up to an optimum at 35–40°C (at pH 7.41) then decreases irreversibly. Although the temperature difference between the temperature of storing the tube (–70°C) and the analytic condition (35°C) is very high, the enzyme activity is not decreased after many freeze–thaw cycles. In order to gain more information as to how the enzyme is bound to the nylon matrix, the surface of the nylon tubing has been etched in two different ways:

1. Hydrolytic splitting of peptide bonds (tube A)



2. Nonhydrolytic splitting of peptide bonds (tube B)



(*N,N*-DMPA = *N,N*-dimethyl-1,3-diamine-propane)

Both tubes have been treated with $TiCl_4$ and enzyme solution in exactly the same manner. While tube A with free carboxyl groups did not lose its activity for 20 weeks, tube B with no free carboxyl groups lost 70% of its original activity. This leads to the assumption that the Ti (IV)-ion is coordinated through the carboxyl- and amino-groups of the nylon and the enzyme. A coordination to only the amino-groups is not sufficient. This finding agrees with the fact that titanium–amine complexes are slowly hydrolyzed in aqueous solutions [6].

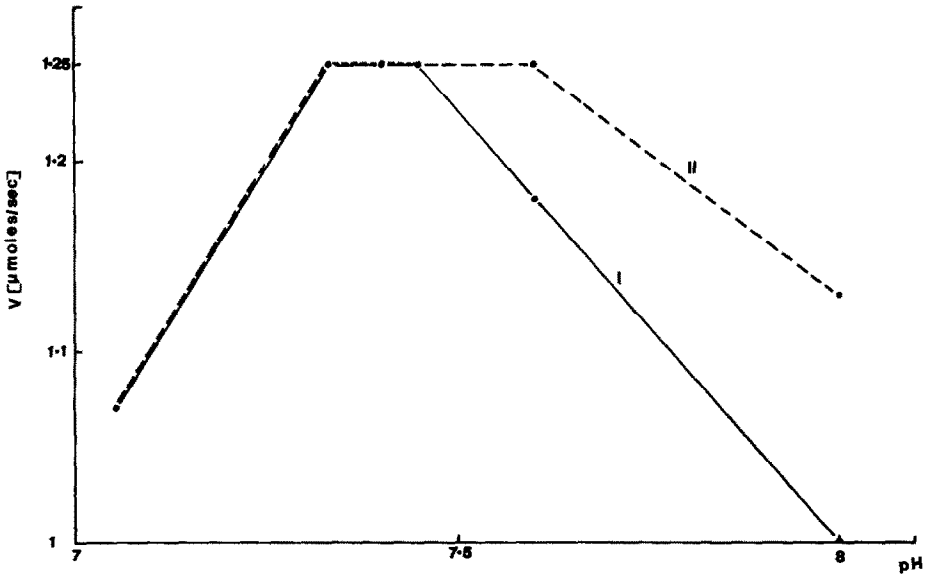


Fig.2. Activity as a function of pH. Glucose 27.8 mmol/l, NAD 44 mmol/l at 25°C. Curve I, activity measured at different pH values. Curve II, activity measured at pH 7.41 (phosphate 0.12 mmol/l) after preincubation at different pH for 25 min.

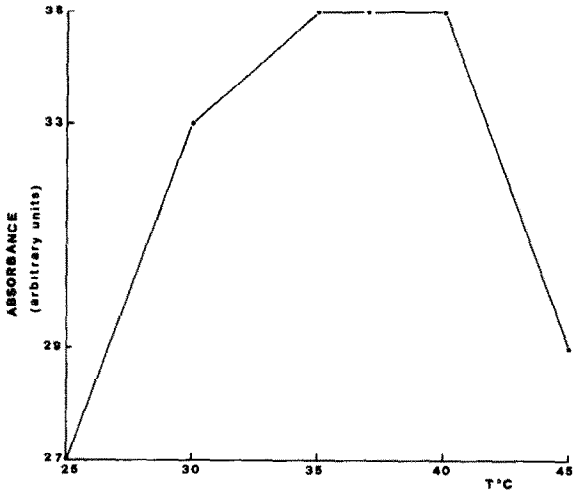
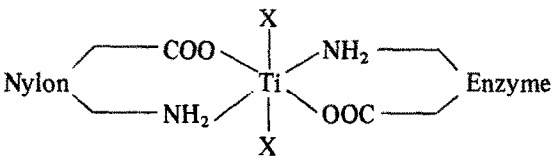


Fig.3. Effect of temperature on activity. Glucose 27.8 mmol/l, NAD 4.4 mmol/l, pH 7.41.

We therefore propose the following possible structure for the bound enzyme:



Taking into account the different factors like splitting of peptide bonds, concentration of metal salt, solvent, buffer, pH, time and temperature of immobilization, it should be possible to immobilize a large number of other enzymes. First results of successful immobilizations by optimizing the different factors have been obtained with glucose oxidase and urease.

Acknowledgements

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References

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