Vol. 97, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 28, 1980 Pages 779-786

BACTERIAL DEXTRANASE IMMOBILISED ON ZIRCONIA COATED ALKYLAMINE GLASS USING GLUTARALDEHYDE*

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Received October 22,1980

SUMMARY

Bacterial dextranase has been immobilised on zirconia coated alkylamine glass through the process of glutaraldehyde coupling. The immobilised enzyme preparation exhibited 62% of the initial enzyme activity with a conjugation yield of 18 mg/g support. K_m of the immobilised enzyme exhibited a decline in its value as compared to the soluble enzyme while V_{max} remained unaltered. E_a of the enzyme was decreased upon conjugation. The soluble enzyme had its optimal pH at 5.4 while the alkylamine conjugated dextranase was optimally active in the pH range 5.2-6.2. The immobilised enzyme has also been characterised through its pI by a new method. The industrial importance of this work is discussed.

INTRODUCTION

Many studies have been reported on the preparation of immobilised enzymes to organic supports (1-4). Great interest in the use of water-insoluble enzymes has been shown lately in industry as these enzymes can be readily recovered from the reaction mixture. In recent years, studies have been reported on the immobilisation of enzymes to inorganic carriers (5-7). This paper describes the immobilisation of bacterial dextranase to an inorganic carrier i.e. zirconia coated alkylamine glass by a simple method of glutaraldehyde coupling. Immobilised dextranase has potential for application in food industry as well as in the preparation of glucose syrups and it is also an enzyme of biomedical importance (8).

*Communication No.2822 from the Central Drug Research Institute, Lucknow-226001, India.

0006-291X/80/220779-08$01.00/0

Inorganic carriers, in general, are not subject to microbial attack and the carrier does not change configuration over an extensive pH range or under various solvent conditions and is therefore easier to use in continuous systems and the enzymic preparations thus obtained are inexpensive, easy to manufacture and possess a long working life span.

MATERIALS AND METHODS

Bacterial dextranase and zirconia coated alkylamine glass of pore size 550A° were gifts from Beckman Labs. and corning Glass Works, USA respectively. Glutaraldehyde and dextran were purchased from E.Merk, W.Germany and Sigma Chemical Co., USA, respectively. All the other chemicals and reagents used were of analytical grade.

Soluble dextranase assay:- It was assayed according to the method of Janson and Porath (9) employing 50 µg of enzyme protein in acetate buffer of pH 5.4 (0.05 M) for 20 minutes with 2% dextran as substrate at 37°C in a metabolic shaker and the glucose released was measured using 1 ml of 1% dinitrosalicylic acid reagent (DNS) and the red colour was read at 525 nm.

Immobilisation of dextranase to alkylamine glass:- It was done according to Weetall (10). 1 g of zirconia coated alkylamine glass was activated by the addition of 10 ml of a 2.5% glutraladehyde with occasional shaking for one hour and the excess glutaraldehyde was washed off with water. 100 mg of bacterial dextranase was added in phosphate buffer of pH 7.0 (0.05 M) to 1 g of the activated support and kept overnight at 4°C for coupling. At the end, the unbound enzyme was washed off with acetate buffer of pH 5.4 (0.05 M) until no more enzyme activity could be detected and the immobilised enzyme was stored in the same buffer at 4°C. The immobilised enzyme was air dried and used for further experiments.

Immobilised dextranase assay:- 20 mg of alkylamine conjugated dextranase (on a dry weight basis) was employed/reaction mixture and the enzyme was assayed using 2% dextran as substrate at 37°C in acetate buffer of pH 5.4 (0.05 M) for 20 minutes in a metabolic shaker and the glucose released was measured using DNS reagent.

Determination of K_m, V_{max} and E_a:- K_m and V_{max} were calculated from the Lineweaver-Burk plots (11) by plotting 1/v vs 1/S while E_a was determined from the Arrhenius plots (12) by plotting log V vs 1/T.

Determination of isoelectric point (pI):- pI was determined by the method of Wills (13) employing suramin, a (poly)anionic compound as the inhibitor for both soluble and alkylamine

conjugated dextranases. The mid point on the pH range where the % inhibition varied from 0 to 100% was taken as the pI of the enzyme. Acetate buffer of 0.05 M in the pH range of 3.6-5.6 was employed for pH effect studies. Protein estimation was carried out according to Lowry et al (14).

RESULTS AND DISCUSSION

It may be seen from Table 1 that a high conjugation yield of 18 mg/g support has been obtained for the immobilisation of dextranase on zirconia coated alkylamine glass, while the enzyme retained 62% of its initial activity upon conjugation, the % activity lost being 38%. Thus, the three parameters mentioned above indicate that zirconia coated alkylamine glass was an acceptable support for dextranase.

Table 1 gives a comparative data on the kinetic parameters of both soluble and alkylamine conjugated dextranase. It may be seen from this that there occured an alteration in the kinetics of the bound dextranase as expected for enzymes upon immobilisation (15). The Michaelis constant, K_m, for the alkylamine conjugated enzyme was decreased from 0.60%, to 0.40% indicating a significant increase in the affinity of the bound dextranase for its substrate i.e. dextran, while no alteration in the V_{max} was observed (Fig.1). When an enzyme is attached to a solid support, the kinetic pattern of reaction changes considerably leading to changes in the values of K_m and V_{max} (15). The changes in the kinetics of immobilised enzymes are controlled by four factors i.e. change in enzyme conformation, steric effects, microenvironmental effects and bulk and diffusional effects (15). In the present case, E_a (energy of activation) of the alkylamine conjugated dextranase exhibited a decline as compared to the soluble enzyme (9000 to 6500 calories) indicating an increase in stability and efficiency of the enzyme upon conjugation

TABLE 1

Kinetic parameters of soluble and alkylamine conjugated bacterial dextranase

Parameter	Soluble dextranase	Alkylamine conjugated dextranase
1. Conjugation yield	-	18 mg/g support
2. Loss in specific activity upon conjugation	-	38%
3. Specific activity retained	-	62%
4. K_m	0.60%	0.40%
5. V_{max} (μ moles glucose/ min/lit.)	620	616
6. Optimal pH	5.4	5.2-6.2 (plateau)
7. E_a (calories)	9000	6500
8. Optimal temperature	35°C	50°C
9. Thermal stability	Stable upto 50°C for one hour	Stable upto 60°C for one hour
10. Stability	-	Fully active if stored at 4° even after 20 days. Complete loss of activity occurs at 37° in 8 days.
11. Half-life	-	5 days at 37°C
12. pI (suramin)	4.3	4.3

K_m and V_{max} were determined from the Lineweaver-Burk plot by plotting 1/V vs 1/S while E_a was determined from the Arrhenius plots by plotting log V vs 1/T. pI was determined according to Wills as described under Materials and Methods. The two buffers used for pH effect studies were acetate (0.05 M) (pH 3.6-5.6) and phosphate (0.05 M) (pH 5.7-6.6). Experimental conditions are given in the text under Materials and Methods.

(Fig.2) (Table 1). The apparent activation energy of an immobilised enzyme differs from that of the soluble enzyme. As reported in the case of aminoacylase (16), chymotrypsin

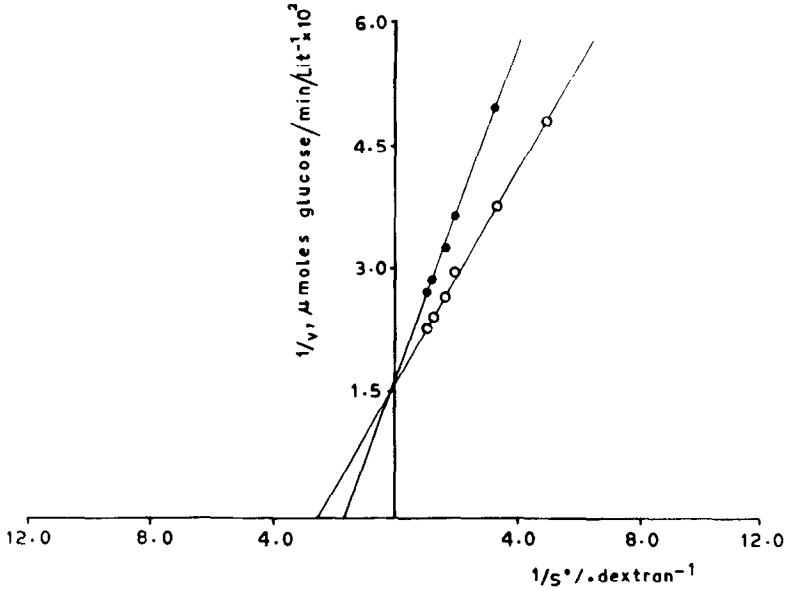


Fig.1 Lineweaver-Burk plot for the determination of K_m and V_{max}; soluble dextranase (•-•) and alkylamine conjugated dextranase (o-o).

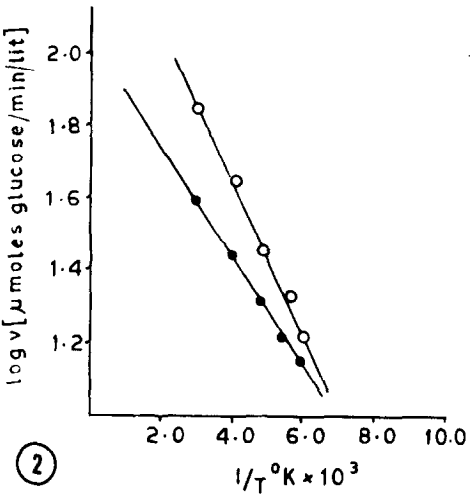
(17) and sweet potato β-amylase (7), dextranase also exhibited lower E_a values upon conjugation to alkylamine glass. According to Melrose (18), this may be attributed to:

a) the introduction of a strain in the enzyme, substrate or carrier

b) favourable electron flow between the reactants and polymer.

c) favourable changes of polarity or ionic strength in the vicinity of the active site.

This was confirmed by our results obtained on the increased thermal stability of the alkylamine conjugated dextranase. The increased thermal stability probably results from the prevention of conformational inactivation of the enzyme and the steric shielding that minimises attack by reactive solutes.



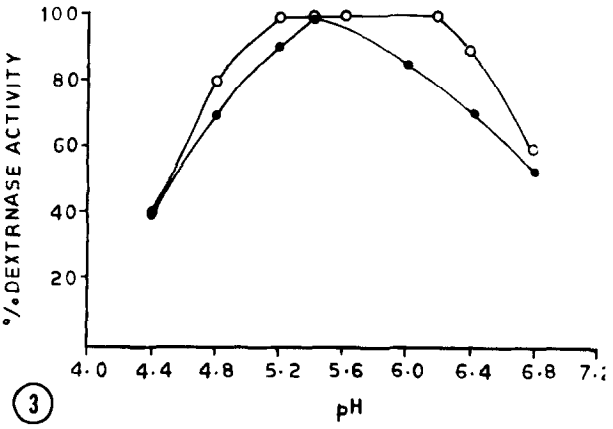


Fig.2 Arrhenius plot for the determination of E_a: soluble dextranase (o-o) and alkylamine conjugated dextranase (•-•).

Fig.3 pH-activity profiles for soluble dextranase (•-•) and alkylamine conjugated dextranase (o-o).

Bacterial dextranase is optimally active at pH 5.4 (Fig.3) while the alkylamine conjugated enzyme exhibited its optimal pH in the range of 5.2-6.2 (plateau) which in turn may be attributed to the loss of amino groups from the enzyme upon conjugation to alkylamine glass since glutaraldehyde coupling involves the amino groups of the enzyme for covalent bonding. The immobilised enzyme derivative showed optimum activity at higher pH values than the soluble enzyme indicating that the pH of the surface of the carrier was more acidic than would be expected due to the presence of the positively or neutrally charged zirconia residues. The pH-stability of an immobilised enzyme seems to depend primarily upon the nature of the surface of the carrier. (Poly-)anionic carriers tend to improve the stability of the enzyme conjugated in the alkaline region and enhanced stability is obtained with (poly-)cationic carriers on the acid side (15). These

changes are manifestations of alterations in the microenvironment. As may be seen from Table 1, the isoelectric point (pI) of dextranase remained unaltered upon its conjugation to alkylamine glass. This in part may be explained by the fact that unsubstituted amino groups in the zirconia coated alkylamine glass in the immediate environment of the fixed enzyme presumably maintain the pI of the immobilised enzyme to that of the soluble one.

The thermal stability of the alkylamine conjugated dextranase also was increased as it could withstand heat treatments upto 60°C for one hour while the soluble enzyme could withstand heat treatments only upto 50°C for one hour. The alkylamine conjugated dextranase was stable if stored at 4°C even after 20 days while complete loss of activity occured in 8 days at 37°C with a half life of 5 days which in turn may be attributed to heat denaturation.

To sum up, the most salient feature of the present communication consists in the characterisation of the immobilised dextranase through its isoelectric point as no method is available in the literature for the pI determination of immobilised enzymes due to their insoluble nature. This paper also reports an inorganic support i.e. alkylamine glass which is new for dextranase and which would facilitate the use of the immobilised enzyme in continuous systems as inorganic supports are not subject to microbial attack and the carrier does not change configuration over an extensive pH range.

ACKNOWLEDGEMENTS

Financial assistance in the form of a Senior Research Fellowship from the Council of Scientific and Industrial Research (CSIR), New Delhi, India is kindly acknowledged by one of us (V.Ramesh).

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