A NOVEL METHOD FOR IMMOBILIZATION OF CHICKEN BRAIN ARYLSULFATASE A USING CONCANAVALIN A

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SUMMARY: Chicken brain Arylsulfatase A (E.C.3.1.6.1) was immobilized by interaction with Concanavalin A. The immobilized enzyme retained its catalytic activity and this enzyme can be reused without appreciable loss of activity. The storage stability of bound and soluble enzymes was comparable and binding of enzyme to Concanavalin A increases its thermal stability. Kinetic studies indicated that bound enzyme shows similar anomalous kinetics as that of free enzyme but slight change was observed in relation to pH optima, K_m value and activation energy.

INTRODUCTION: Recent years there has been considerable interest in the preparation and properties of immobilized enzymes. The immobilization of enzymes is commonly achieved by covalent coupling of the enzymes with reactive insoluble matrices. All the aspects of immobilized enzymes are recently reviewed by Katchalski et al. (1). Bachhawat and Bishayee (2) observed that the Concanavalin A (ConA), a phytohaemagglutinin (3) forms insoluble complexes by interaction with lysosomal acid hydrolases. The present communication describes the immobilization of chicken brain arylsulfatase A on ConA and a comparison of some of the properties of the ConA bound enzyme with the soluble enzyme.

MATERIALS AND METHODS: The dipotassium salt of 4-nitrocatechol sulphate and soluble carbohydrate free ConA prepared according to the method of Bishayee et al (4) were obtained from Biochemical Unit, V.P. Chest Institute, Delhi, India. All other chemicals used were of analytical grade.

Assay of soluble and immobilized arylsulfatase A: The enzyme arylsulfatase A soluble and immobilized both were assayed according to the method of Farooqui and Bachhawat (5).

Protein determination: Protein was determined using the method of Lowry et al. (6) with crystalline bovine serum albumin as standard.

Preparation of enzyme: Chicken brain arylsulfatase A was prepared upto $(NH_4)_2SO_4$ fractionation step according to the method of Farooqui and Bachhawat (5). The $(NH_4)_2SO_4$ fraction was suspended in 0.02 M sodium acetate buffer, pH 5.0 and was further purified by heat treatment. The suspension was heated at 55 ($\pm$ 0.5)°C for exactly 5 minutes and then the solution was chilled quickly in ice. The denatured proteins were removed by centrifugation at 12,000 x g for 30 minutes and the heated supernatant which contained arylsulfatase A was used for immobilization by ConA. A portion of heat treated supernatant enzyme was dialyzed for 8 h against 500 volumes of 0.05 M sodium acetate buffer, pH 5.0 with three changes. The protein precipitated during dialysis was removed by centrifugation at 12,000 x g for 30 minutes and this solution was used for studying properties of free arylsulfatase A.

Immobilization of arylsulfatase A: The arylsulfatase A was precipitated by ConA by the procedure described by Bachhawat and Bishayee (2). The undialyzed heat treated supernatant which contains arylsulfatase A was incubated with ConA (1 mg of ConA per 2 mg of heat treated supernatant on protein basis) for 1 h at 37°C in presence of 1 M NaCl. This ConA to heat treated supernatant enzyme ratio was found to precipitate maximally heat treated supernatant arylsulfatase A. The pellets formed was centrifuged at 12,000 x g for 30 minutes and washed with 0.05 M acetate buffer, pH 5.0. Finally, the ConA-arylsulfatase A complex was finely suspended in 0.05 M sodium acetate buffer, pH 5.0 and used throughout these studies.

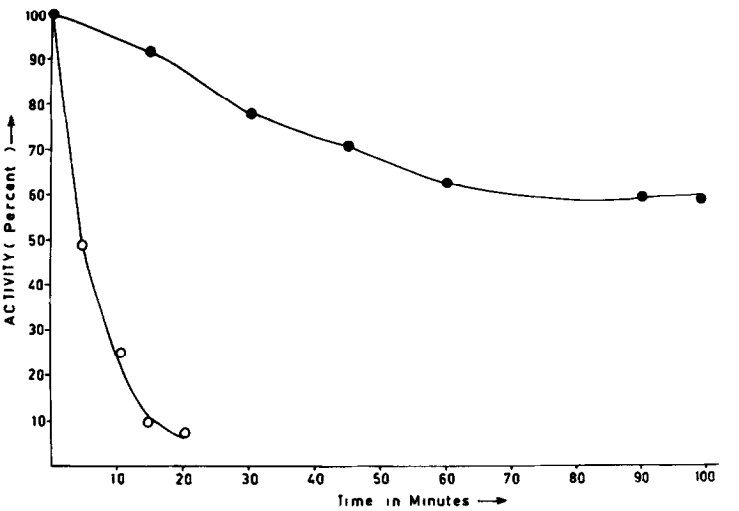

FIG.1. Thermal inactivation of soluble and ConA bound arylsulfatase A at 55°C in 0.05 M sodium acetate buffer, pH 5.0. Protein concentration used for both the enzymes was the same (1.1 mg/ml). Enzyme activity was assayed as described in the text.

ConA bound arylsulfatase A.
Soluble arylsulfatase A.

RESULTS AND DISCUSSION:

During this study it was observed that nearly 50-70% of arylsulfatase A binds with ConA and forms insoluble precipitate. The enzyme was active in the precipitated form since there was no release of arylsulfatase A in the supernatant under the usual assay conditions.

Thermal stability of soluble and immobilized arylsulfatase A: Thermal inactivation of soluble and immobilized enzyme was determined at 55°C. The results obtained at 55°C are shown in Figure 1. At 55°C, a comparison of percentage activities with incubation time reveals that the immobilization of arylsulfatase A has enhanced its thermal stability. As shown in Figure 1 the free arylsulfatase A lost 95% of its activity in 20 minutes whereas the ConA bound arylsulfatase A retains 85% of its activity. Even after 100 minutes, ConA bound enzyme at 55°C retains 60% of its original activity.

This enhanced stability to thermal denaturation is consistent with results obtained by other workers (7). Also the optimum temperature for the desulphation of nitrocatechol sulfate by the enzyme arylsulfatase A was increased from 50°C to 55°C after immobilization on ConA.

Storage stability and reusability: The activity of soluble and immobilized arylsulfatase was redetermined after storage at 0-5°C for 30 days, in sodium acetate buffer (0.05 M, pH 5.0). Both the free and immobilized enzyme has found to be stable under this condition.

Reusability of the immobilized enzyme was studied as follows. The bound enzyme 3.45 mg protein was suspended in 0.5 ml of 0.05 M sodium acetate buffer, pH 5.0, and incubated at 37°C for 5 minutes with 0.5 ml of 12 mM 4-nitrocatechol sulphate in 0.4 M sodium acetate buffer, pH 5.0. After incubation, an aliquot (0.05 ml) of the incubation mixture was taken and directly added to 2.95 ml of 0.11 N NaOH, for assay. The rest of the incubation mixture was quickly cooled in ice. Then the mixture was centrifuged at 12,000 x g for 30 minutes. The pellet was washed once with 3 ml of 0.05 M sodium acetate buffer, pH 5.0 and again suspended in 0.5 ml of 0.05 M sodium acetate buffer, pH 5.0, and the enzyme activity was again measured as described above. This procedure was repeated six times. Even after six times repeated use of enzyme, it still had 82% activity in the pellet and this immobilized enzyme may be used many more time without appreciable loss in activity.

Kinetic properties: Both the soluble and immobilized arylsulfatase A showed anomalous time-activity relationship as reported earlier by Farooqui and Bachhawat (5) for soluble arylsulfatase A. In the presence of pyrophosphate, soluble and in-

TABLE 1. EFFECT ON KINETICS, OPTIMUM pH, K_m VALUE AND ACTIVATION ENERGY BY IMMOBILIZATION OF ARYLSULFATASE A

Parameters	Soluble Arylsulfatase A	Immobilized Arylsulfatase A
Kinetics	Anomalous	Anomalous
Optimum pH	6.0	5.5
K_m value	0.8 mM	1.4 mM
Activation energy	10.65 K Cal/mole	11.31 K Cal/mole

Standard assay conditions were maintained in measuring enzyme activity. K_m values for both enzymes were obtained by plotting $[S]/v$ Versus $[S]$ The activation energies were calculated in the temperature range 25-50°C. The time of incubation was 10 minutes.

soluble arylsulfatase showed a linear time-activity relationship.

A comparison of pH activity profile of the soluble with the immobilized arylsulfatase A reveals that two enzymes differ slightly in both the pH optimum and general shape of curve around that optimum pH. The pH activity profile of the immobilized arylsulfatase is broader at the optimal activity range and the optimum is slightly displaced (approx. 0.5 units) towards acidic pH values. Under the experimental conditions, the ConA has net positive charge. Therefore it can be considered as positively charged matrix. Furthermore, the binding of ConA to its determinant sugar (8) increases its net positive charge as reported elsewhere (9). A similar observation has been made with polycationic derivatives of trypsin (E.C.3.4.4.4) (10) and chymotrypsin (E.C.3.4.4.5) (10).

The K_m value and activation energy of immobilised arylsulfatase A were different from the soluble arylsulfatase A as shown in Table 1. The change in K_m value and activation energy may be due to changes in the conformation of the active site of enzyme,

induced by the binding of ConA to arylsulfatase A. Several other factors, (other than the change in conformation of active site) viz. the interaction between ConA bound enzyme and substrate takes place in a different environment from that existing in free solution, may also bring about the changes in K_m and activation energy values. There will be partitioning of the substrate between the support and the free solution so that the substrate concentration in the neighbourhood of the enzyme may be different from what it is in free solution. Also, the reaction in the immobilized enzyme may be to some extent controlled by the diffusion phenomenon (1,11). At the moment there are no definitive data to favour or eliminate unequivocally any of these possibilities.

It has been shown that number of enzymes, namely arylsulfatases A and B, acid phosphatase, β-N-acetyl hexosaminidase, β-galactosidase and β-glucuronidase (2) interact with ConA to form insoluble precipitates and these ConA-enzyme complexes retained their catalytic activities. It has also been shown that the interaction between these enzymes and ConA is mediated by the carbohydrate moiety of the enzymes (2). These observations along with the present observation suggest that any glycoprotein enzyme containing glucose or mannose as carbohydrate constituents can be immobilized with ConA. The enzyme can be obtained in free form by the dissociation of ConA-enzyme complex with α-methyl-D-glucoside or α-methyl-D-mannoside (2). However, one of the limitations of this technique is that this method cannot be utilized in the case of α-glucosidases and α-mannosidases, since the substrates for these enzymes will dissociate the complex.

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