PROPERTIES OF FREE AND IMMOBILIZED GLUCOSE ISOMERASE*

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Received 17 July 1973

1. Introduction

In recent years, a considerable amount of work has been carried out on immobilized enzymes [1–4], but relatively few studies are concerned with the immobilization of glucose isomerase (D-xylose ketol-isomerase EC 5.3.1.5) [5, 6]. Glucose isomerase (GI) was entrapped in polyacrylamide gel [5] and covalently bound to porous glass [6], but poor results were obtained for the instability and the low activity of these preparations.

The present communication describes the properties of the glucose isomerase from *Streptomyces griseolus* as free and immobilized form. A new method of immobilization [7], consisting in the physical entrapment of proteins in filamentous structures, was used to prepare insoluble derivatives of glucose isomerase. Optimal conditions for enzyme stability and activity in free and immobilized form are reported.

2. Materials and methods

S. griseolus CL71 was grown for 40 hr at 30°C in a medium (pH 6.7) composed of the following: enzyme digest of casein, 1%; yeast extract, 1%; glycerol, 1%; D-xylose, 1%; $CoCl_2 \cdot 6H_2O$, 0.024%; KH_2PO_4, 0.3%; $MgSO_4 \cdot 7H_2O$, 0.02%; NH_4Cl, 0.1%; $Na_2HPO_4 \cdot 12H_2O$, 0.6%.

Glucose isomerase was purified according to a modified Takasaki procedure [8]. Fibres with the enzyme were prepared according to the standard method developed in our laboratory [7]. 4 000–6 000 units of enzyme solution (approximately 40–80 mg of protein) per gram of polymer were entrapped.

Proteins were determined by the method of Lowry et al. [9]. The amount of entrapped protein was measured by total nitrogen determination.

The activity of the enzyme was followed by measuring polarimetrically the fructose formed. The reaction mixture contained: 0.5 M D-glucose; 5mM $MgSO_4$; 0, 1 mM $CoCl_2$; 50 mM triethanolamine buffer (pH 8.5) in a total volume of 3.6 ml. Addition of 400 µl of enzyme solution (approximately 20–30 units) started the reaction and the mixture was incubated at 70°C for 1 hr. One unit of activity is defined as the amount of enzyme which produces 1.0 mg of fructose under the above conditions. Specific activity is expressed in units per mg protein.

Isomerization of glucose to fructose by fibres was carried out by incubating at 45°C in a stirred water bath 2.5 g of wet fibres and 25 ml 60% (w/v) glucose solution containing 5 mM $MgSO_4$, 0.1 mM $CoCl_2$ and 0.2 M triethanolamine buffer (pH 8.5). Aliquots were taken at intervals and the optical rotation was measured. The isomerization extent after 6 hr measured the activity of the entrapped enzyme.

3. Results and discussion

A typical purification of glucose isomerase is summarized in table 1. Specific activity 10-fold higher than that published by Takasaki [7] was obtained in the first step (acetone ppt.) of the purification. This allowed the entrapment of the enzyme precipitated by acetone without any further purification and very

* Presented at FEBS Special Meeting Dublin, April 15–19, 1973.

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Table 1
Purification of glucose isomerase from *Streptomyces griseolus* CL71.

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)
1) Crude extract	3 650	87 500	24	–
2) Dialyzed extract	1 718	103 300	60	100
3) Acetone ppt.	800	97 000	121	94
4) DEAE-cellulose	212	56 000	263	54.4
5) Sephadex G-200	120	36 400	303	35.4

active fibres were obtained. The properties of the purified glucose isomerase from *S. griseolus* were found quite similar to those of the enzyme already reported [5, 8]. The value of the sedimentation coefficient ($s^{\circ}_{20\,w}$ = 9.51 X 10^{-13} sec.) was between those found for *S. albus* [8] and *Bacillus coagulans* [10].

The pH–activity profile, illustrated in fig. 1, shows a optimum between pH 8 and 8.5. Good activity and stability were found in triethanolamine buffer, pH 8.5.

Metal ions were found necessary for enzyme activity (see fig. 2).

Thermal stability of free glucose isomerase was studied in order to establish the working temperature for the immobilized enzyme during long-term experiments. As shown in fig. 3, the enzyme is appreciably stable between 40°C and 60°C. A temperature of

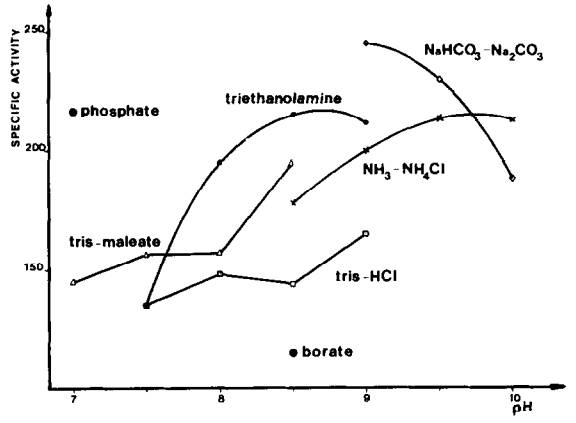

Fig. 1. pH–activity profile of soluble GI in different buffers. The enzyme activity (DEAE-cellulose eluate: 6.5 units/ml) was tested in 0.05 M buffers: (●—●—●) triethanolamine–HCl; (□—□—□) Tris–HCl; (△—△—△) Tris acid maleate–sodium hydroxide; (◇—◇—◇) $NaHCO_3$; (X—X—X) NH_3–NH_4Cl. For assay conditions see text.

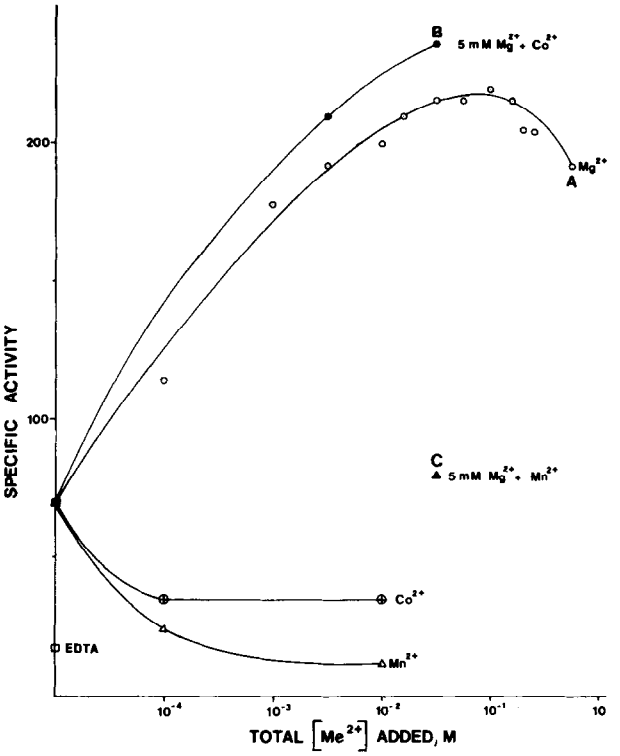

Fig. 2. Effect of metal ions on the activity of soluble GI. (DEAE-cellulose eluate). Activity of the undialyzed preparation ■. The following ions were added to the undialyzed preparations: Mg^{2+} ⊙, Co^{2+}⊕, Mn^{2+}△, Co^{2+} and 5 mM Mg^{2+}●, Mn^{2+} and mM Mg^{2+}▲. A sample was dialyzed 18 hr against 10^{-4} M EDTA and was assayed without additions ⊡.

45°C was therefore chosen for minimizing enzyme denaturation and substrate degradation. However under operating conditions progressive loss of activity was observed (fig. 4, a) which did not appear in parallel experiments in the absence of substrate. The rate of inactivation was increased by the time of contact of the enzyme with the same reaction mixture. Activity was scarcely lost when immobilized glucose isomerase acted under strictly anaerobic conditions or Na_2SO_3 was added (fig. 4, b). After 30 days, the 90% of the original activity was found. On the other hand the inactivation was enhanced when oxygen was bubbled through the reaction mixture. However oxygen did not affect the activity of enzymic fibres in buffer.

The above results suggest that the inactivating agent results from the interaction of the monosaccharide and the preparation under aerobic conditions.

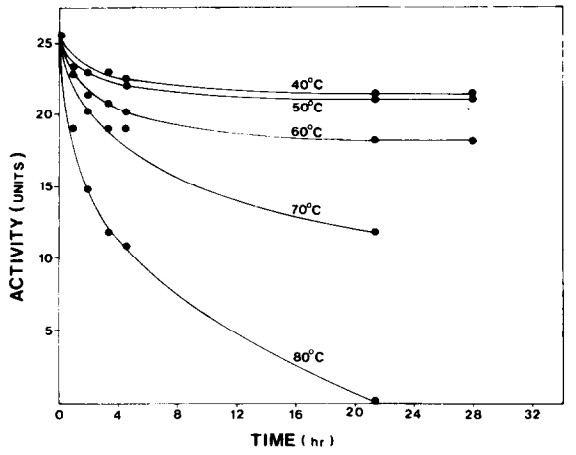

Fig. 3. Thermal stability of soluble GI. The enzyme (510 units/ml) was added to mixtures containing: 10 mM $MgSO_4$; 0.2 M $CoCl_2$ and 0.1 M triethanolamine buffer (pH 8.5) and was kept at the temperatures indicated. 2.0 ml aliquots were diluted with 2.0 ml 1 M glucose and the activity was tested for 1 hr at 70°C.

The mechanism of inactivation is at present investigated.

The storage stability of glucose isomerase fibres was found quite satisfactory. The enzymic fibres, stored one year at +4°C in a wet environment, retained their original activity. The fibres lost all the activity when they were exhaustively dried under vacuum: probably the enzyme precipitates irreversibly within the micropores of the fibres. Similar results were obtained by lyophilizing the enzymic fibres, but in this case a mechanical rupture of fibres with leakage of enzyme was responsible for loss of activity.

Not only the stability of the entrapped glucose isomerase was satisfactory, but also the activity. In fact the method of immobilization used, allowed to entrap the whole amount of protein added to the emulsion. Since the entrapped activity was completely recovered after dissolution of the polymer, the enzyme was not inactivated under spinning conditions. The activity displayed by the fibres was about 60% of the entrapped activity. The high activity of the glucose isomerase fibres allows to carry out the isomerization reaction at relatively low temperature avoiding undesirable side reactions, objectionable taste and formation of coloured products.

Acknowledgements

We thank Mr. Aurelio Barbaro and Mr. Mario Bettonte for skillful technical assistance.

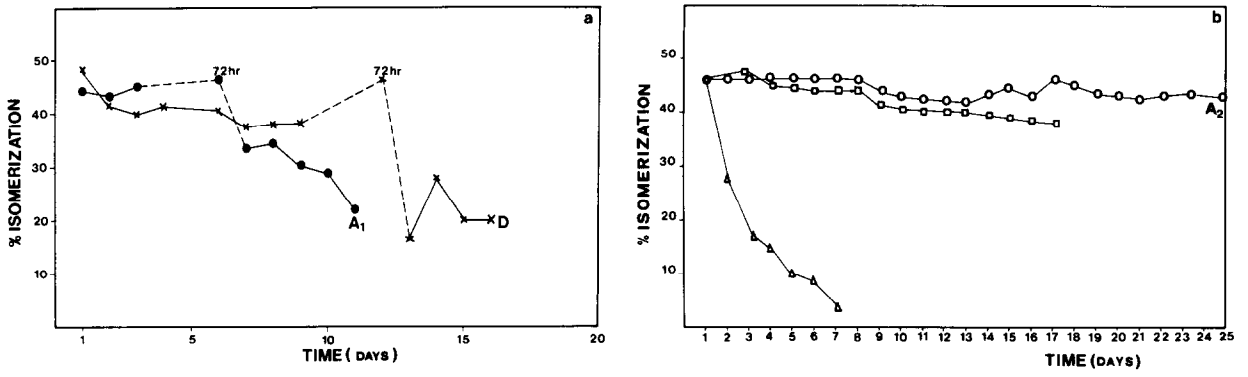

Fig. 4. Activity of entrapped GI in repetitive use. GI was entrapped in cellulose triacetate fibres at the acetone ppt. stage (A_1 6 670 u[a]/g TAC[b] ●—●—●), A_2 4 380 u[a]/g TAC ○—○—○) or after purification on DEAE-cellulose (D 8 950 u[a]/g TAC X—X—X) and was incubated for periods of 24 hr or 72 hr as indicated in the abscissa. At the end of this period the isomerization was measured and the reaction mixture was renewed. Other experimental conditions are described in text: a) kinetics carried out in aerobic conditions; b) kinetics carried out in anaerobic conditions. The inhibitory effect of the oxygen is shown with samples of the same preparation (A_2) in different operating conditions: reaction in anaerobiosis with 0.1 M Na_2SO_3 added to glucose mixture ○—○—○; reaction in aerobiosis with 0.1 M Na_2SO_3 added to glucose mixture □—□—□; reaction in vessel saturated with oxygen △—△—△. [a] Entrapped units. [b] Cellulose triacetate.

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