UDPGLUCOSE 4-EPIMERASE FROM SACCHAROMYCES FRAGILIS : DESENSITIZATION WITH HEAT

Manju Ray and Amar Bhaduri+

Division of Biochemistry, Department of Pharmacy

Jadavpur University, Calcutta-700 032, India.

Received September 8.1975

SUMMARY : The allosteric kinetics exhibited by UDPglucose 4-epimerase from Saccharomyces fragilis changes over to a normal hyperbolic kinetics when the enzyme is heated at 41° for 2 mins. The native enzyme is completely insensitive to inhibition by UMP in the allosteric region. The desensitized enzyme is however, strongly inhibited by UMP at this low concentration. Apparently, desensitization by heat converts the enzyme to its ultimate catalytic form.

INTRODUCTION

We have recently reported that UDPglucose 4-epimerase (E.C. 5.1.3.2) from Saccharomyces fragilis exhibits an allosteric kinetics with UDPglucose as substrate (1). In an effort to desensitize the effector site, we have found that the allostericity can be completely abolished when the enzyme is subjected to mild heat treatment. Further, the native enzyme which is completely insensitive to inhibition by UMP at low concentrations of the substrate, is fairly strongly inhibited by the nucleotide, once the enzyme is desensitized by heat.

METHODS AND MATERIALS

All the biochemicals were obtained from Sigma Chemical

+ Present address : Visiting Scientist, Roche Institute of Molecular Biology, Nutley, New Jersey 07110, U.S.A.

Co., St.Louis, Mo., U.S.A. Highly purified UDPglucose 4-epimerase was either directly obtained from the same source or was prepared by following the procedure of Darrow and Rodstrom upto stage III of their method (2). When UDPgalactose was used as the substrate, the coupled assay procedure with UDPglucose dehydrogenase as the coupling enzyme was employed (3). The epimerisation catalysed by the enzyme with UDPglucose as the substrate was assayed by the two-step assay procedure described in our previous paper (1). Formation of UDPgalactose was measured both by estimating the decrease in UDPglucose content and by estimating the formation of UDP-galactose. All assays were carried out specifically in 0.1 M glycine-sodium hydroxide buffer since variations in cation concentration had significant effect on the rate of catalysis (unpublished observation).

RESULTS

Desensitization of epimerase by heat : Fig.1 shows the effect of mild heating on the kinetics of the enzyme with

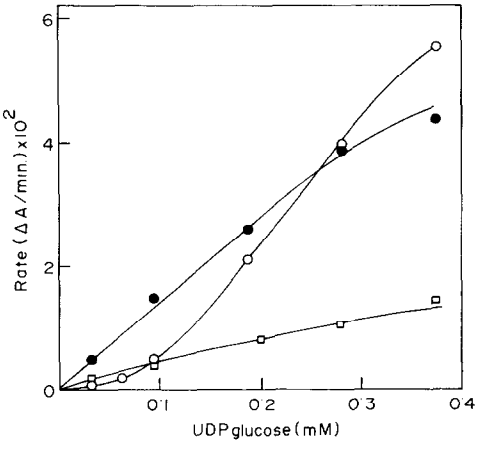

Fig. 1. Desensitization of epimerase by treatment with heat. The lyophilized enzyme was taken into solution with 0.1M sodium citrate pH 7.0. 0.5 ml of the solution containing 0.25 unit of epimerase, as assayed with UDPgalactose as the substrate, was exposed to heat on a water-bath. 0.1 ml samples were taken out at indicated time intervals. The incubation medium contained in a total volume of 1 ml, 40 μmoles of glycine-sodium hydroxide buffer pH 8.8, 10 μl of the native or treated enzyme and varying concentration of UDPglucose. Incubation time was 5 mins. UDPgalactose produced during this period was assayed by the two-step assay method. (○—○) indicate the rate with native enzyme. (●—●) and (□—□) indicate the rate with the enzyme heated at 41° for 2 mins and 5 mins respectively.

UDPglucose as the substrate. Heating at 41° for 2 mins, completely abolished the sigmoidicity and a virtually linear increase in velocity was observed upto 0.4 mM concentration of the substrate. The enzyme is apparently very sensitive to heat since the catalytic activity was drastically reduced on exposure to heat for 5 mins.

Effect of UMP on the native enzyme : UMP was previously shown to act as a strong competitive inhibitor for the yeast epimerase when UDPgalactose was used as the substrate (3,4). Similar inhibition by UMP was also noted for the lactating mammary gland enzyme (5). When we studied the effect of UMP on epimerization with UDPglucose as the substrate, we could observe inhibition by this nucleotide only at higher concentrations of the substrate (Fig.2). At lower concentrations of UDP-

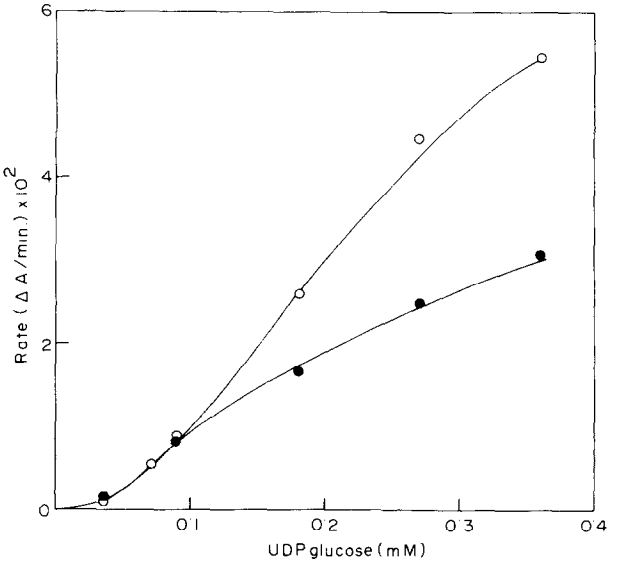

Fig. 2. Effect of UMP on the native epimerase with UDPglucose as the substrate. (o—o) and (●—●) indicate the kinetics with UDPglucose as the substrate in absence and in presence of 1.2 mM UMP. The incubation mediums for the assays were the same as described in the legend of Fig.1.

glucose (below 0.1 mM) no inhibition by UMP could be observed in several batches of the enzyme. Thus at 0.07 mM concentration of UDPglucose, even when 1.2 mM UMP was used (substrate to inhibitor ratio, 1:17) no detectable inhibition could be noted, whereas with the same concentration of UMP and with 0.27 mM UDPglucose as the substrate, an inhibition of about 50% was observed. In contrast, when 0.05 mM UDPgalactose was used as the substrate, 1 mM UMP could inhibit the enzymatic reaction by 40% (Data not presented).

Effect of UMP on the desensitized enzyme : After treatment with heat, the enzyme not only showed a normal hyperbolic kinetics but was also effectively inhibited by UMP at low concentrations of the substrate. Fig. 3 shows the effect of UMP on the desensitized enzyme when low concentrations of UDPglucose were used as substrates. At these low concentrations

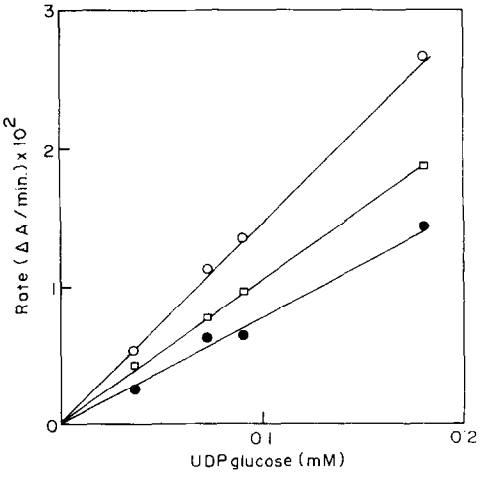

Fig. 3. Effect of UMP on the heated epimerase with UDPglucose as the substrate. 0.5 ml of the enzyme in 0.1M sodium citrate pH 7.0 containing 0.25 units of activity was heated for 2 min at 41°. (O—O) indicates the rate with the heated enzyme. (□—□) and (●—●) indicate the rates when the heated enzyme was assayed in presence of 0.6 mM and 1.2 mM UMP.

(below 0.1 mM) the native enzyme was completely insensitive to inhibition by UMP. But the desensitized enzyme showed about 30% inhibition in presence of 0.6 mM UMP with 0.07 mM UDPglucose as the substrate. Increasing the concentration of UMP resulted in an increased inhibition of the catalytic activity.

DISCUSSION

The kinetics of a large number of enzymes have been shown to shift from an allosteric one to a hyperbolic one on controlled heating. However, only in a few cases, the mechanism involved in this transition is clearly understood. UDPglucose 4-epimerase from S. fragilis is in some ways an unique enzyme in that it shows a normal Michaelis kinetics with UDPgalactose as the substrate but a distinctly allosteric kinetics with UDPglucose as the substrate (1). The cooperative kinetics with UDPglucose as the substrate indicates that the ultimate conformation of the enzyme is obtained only at higher concentrations of UDP-glucose. Apparently, this form is identical with the UDP-galactose-induced form, since at this high concentration, equilibrium can be established rapidly with either of the nucleotide sugars as the substrate. The identity or close similarity of these two forms is further confirmed by the inhibition by UMP of the native epimerase at higher concentrations of UDPglucose (Fig. 2). As exposure to heat results in inhibition by UMP even at low concentrations of UDPglucose as the substrate, desensitization by heat obviously transforms the native form either to the UDPgalactose-induced form or to a form which is conformationally very close to this form.

ACKNOWLEDGEMENT : This work was supported by grants obtained from Dept. of Atomic Energy, India.

REFERENCES

1. Ray, M., and Bhaduri, A. (1975) J. Biol. Chem. 250, 4373-4375.

2. Darrow, R.A. and Rodstrom, R. (1968) Biochemistry, 7, 1645-1654.

3. Ray, M. and Bhaduri, A. (1974) Biochem. Biophys. Res. Commun. 60, 1081-1089.

4. Pal, D and Bhaduri, A. (1971) Biochim. Biophys. Acta, 250, 588-591.

5. Tsai, C.M., Holmberg, N. and Ebner, K.E. (1970) Arch. Biochem. Biophys. 136, 233-244.