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UDPGLUCOSE 4-EPIMERASE FROM SACCHAROMYCES FRAGILIS : ASYMMETRY IN ALLOSTERIC PROPERTIES LEADS TO UNIDIRECTIONAL CATALYSIS

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SUMMARY. UDPglucose 4-epimerase from Saccharomyces fragilis shows Michaelis kinetics with UDPgalactose as the substrate and allosteric kinetics with UDPglucose as the substrate. As a result of this allosteric asymmetry, when very low concentration of UDPgalactose is used as the substrate, a unidirectional catalysis takes place and the equilibrium is established only after UDPgalactose is first completely converted to UDPglucose. When desensitized enzyme is used or when allostericity is abolished with high concentrations of cation, the equilibrium is established in the normal fashion.

INTRODUCTION. UDPglucose 4-epimerase (EC.5.1.3.2) is an obligatory enzyme of galactose metabolic pathway. Unlike galactokinase and gal-1-phosphate uridyl transferase, epimerase is needed both for anabolism and for catabolism of the galactose moiety. Consistent with this physiological function, the enzyme has been found to catalyze a freely reversible reaction between UDPglucose and UDPgalactose in a wide variety of cells (1). We have recently shown that the epimerase from the yeast, Saccharomyces fragilis shows a distinct allosteric kinetics when UDPglucose is used as the substrate (2). With UDPgalactose as the substrate, however a typical hyperbolic kinetics is observed. Epimerase from yeast is thus a unique example of

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an enzyme catalyzing a freely reversible reaction but at the same time showing a distinct allosteric asymmetry with regard to its substrates. An immediate consequence of this allosteric asymmetry with UDPglucose as substrate is that the equilibrium is approached at different rates when widely varying concentrations of UDPglucose are used as substrates (2). We now report that when low concentrations of UDPgalactose are used as the substrate, the asymmetry in substrate kinetics from the two sides of the reaction, results in a unidirectional catalysis of UDPgalactose to UDPglucose. Thus, depending upon the concentration of the substrate and the enzyme present, conditions are developed where equilibrium is established only after the substrate has been completely converted to the product.

MATERIALS AND METHODS. All biochemicals, unless otherwise mentioned, were purchased from Sigma Chemical Co. U.S. UDPglucose 4-epimerase from Saccharomyces fragilis was purified upto stage 3 of the method described by Darrow and Rodstrom (3). In some cases, the highly purified lyophilized enzyme was also purchased from Sigma Co. The specific activities for the purified enzyme and the purchased enzymes were about 3-5 units/mg. The conditions for the coupled assay has already been described (2) and the specific activities and total units of enzymes used in different experiments are described on that basis. The extent of conversion of UDPgalactose to UDPglucose was estimated by the two-step assay procedure which was described in detail previously (2). The formation of UDPglucose as measured by difference in UDPgalactose content agreed very well with the value of UDPglucose obtained by initial direct estimation.

RESULTS. The contrasting pathways of approach to the equilibrium at two different concentrations of UDPgalactose are shown in Fig.1. The normal expected pathway of approach to the equilibrium was obtained with 0.3 mM concentration of UDPgalactose. However, when the concentration of UDPgalactose was substantially reduced (0.018 mM), UDPgalactose was completely converted to UDPglucose before the back reaction with UDP-

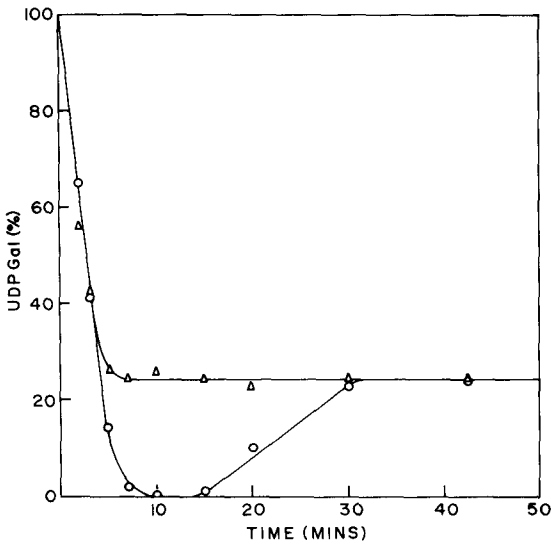

Fig.1. Approach to equilibrium with UDPgalactose as the substrate. (Δ–Δ) and (O–O) indicate the per cent of UDPgalactose left after specified reaction times using 0.3 mM and 0.018 mM UDPgalactose respectively. The other components of the incubation mixture in each tube were 100 umole of glycine-sodium hydroxide buffer, pH 8.7 and 0.025 unit of the enzyme in a total volume of 1 ml.

glucose as the substrate became significant and the equilibrium was gradually established. Apparently, because of the allosteric nature of the kinetics with UDPglucose as the substrate, at this low concentration of UDPglucose, the rate of conversion to UDP-galactose was negligible compared to the rate from the other side of the reaction. At this low concentration of UDPgalactose, the reaction catalyzed by the enzyme thus assumes a unidirectional character.

The conjecture that the unconventional pathway to equilibrium with low concentrations of UDPgalactose as the substrate is due to the allosteric properties of the enzyme was confirmed by the following experiment. We had previously shown that on mild heat-treatment the enzyme can be completely desensitized

and a strictly hyperbolic kinetics is obtained with UDPglucose as the substrate (4). When the desensitized enzyme was used as the control, the equilibrium was established in the normal expected manner even though in this case also very low concentration of UDPgalactose was used as the substrate (Fig.2). The slower rate of conversion of UDPgalactose to UDPglucose for the desensitized enzyme was due to its partial loss of activity on heating (4,5).

Darrow and Rodstrom (6) had previously shown that with UDPgalactose as the substrate, the catalytic activity of the epimerase could be substantially stimulated in presence of high concentrations (50 mM) of monovalent cations. We have recently observed that allosteric behaviour with UDPglucose as the substrate can be completely abolished in presence of high concentrations of cations (unpublished observation). Employing 50 mM Na^+ and at low concentration of UDPgalactose (0.02 mM) a shift in the pathway of approach to equilibrium, similar in nature to the heat-desensitized enzyme could be observed (Data not presented). This experiment, thus confirmed the conclusion of the previous experiment that the unidirectional catalysis of UDPgalactose to UDPglucose stems from the inherent allosteric asymmetry of the enzyme itself. This experiment also emphasizes the need for choosing a buffer that under experimental conditions should not be extensively dissociated to give large concentration of cation in the medium. In this respect, glycine-sodium hydroxide buffer (pK, 9.6) is particularly suitable for carrying out experiments at pH 8.7.

The nature of the detour pathway for the establishment of equilibrium was also dependent upon the concentration of

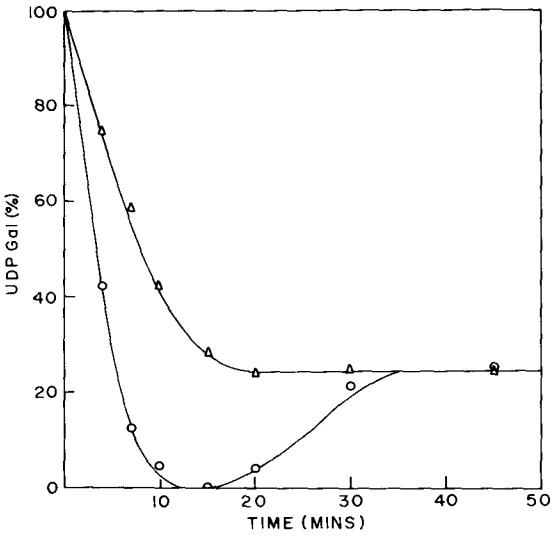

Fig.2. Approach to equilibrium with UDPgalactose as the substrate in presence of desensitized enzyme. The two incubation mixtures contained in a total volume of 1 ml, 100 umole of glycine-sodium hydroxide buffer, 0.018 mM UDPgalactose and either 0.025 unit of the native enzyme or 0.025 unit of enzyme after it was desensitized with heat at 41° for 2 mins (4). (o—o) and (Δ—Δ) indicate the per cent of UDPgalactose left after specified reaction times, using the native and the desensitized enzyme.

the enzyme present (Fig.3). When different concentrations of the enzyme were used with the same initial concentration of the substrate, UDPgalactose was completely exhausted at different rates and the equilibrium was finally established at different times. Obviously, the different rates of the back-reaction for the formation of UDPgalactose are not only dependent upon the concentration of UDPglucose but also on the concentration of the enzyme present. Thus, when very high concentration of the enzyme was present (ten times the maximum concentration for this experiment), the equilibrium was directly established (Not shown).

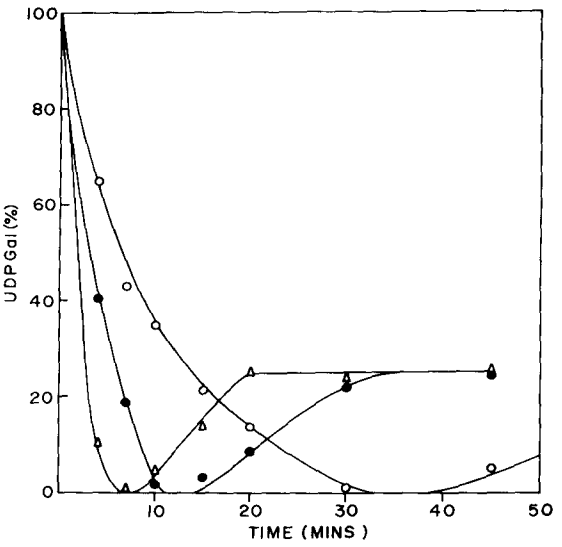

Fig.3. Approach to equilibrium with UDPgalactose as the substrate with varying concentrations of the enzyme. (o—o), (●—●) and (Δ—Δ) indicate the per cent of UDPgalactose left after specified reaction times using in a total volume of 1 ml 100 umole of glycine-sodium hydroxide buffer pH 8.7, 0.017 mM UDPgalactose and 0.01, 0.03 and 0.06 units of the enzyme respectively.

DISCUSSION. The fundamental role of some enzymes that catalyze irreversible and committed enzymatic steps of metabolic pathways is well documented and in some cases has been correlated with the physiological needs of the system. In contrast, the role of enzymes that rapidly establish equilibrium is not clearly understood. Krebs has shown that such enzymes can also make essential contributions to the regulation of some vital metabolic pathways (7,8). For example, in gluconeogenesis the steady-state concentration of pyruvate is largely dependent on the equilibrium and the concentration of metabolites for the lactic dehydrogenase system (8,9). Extrahepatic ketogenesis and propionate metabolism in liver and kidney may also be partially regulated by equilibrium enzymes (7). We now present the case of an equilibrium enzyme, which due to its inherent asymmetry in allostericity

will lead to unidirectional catalysis under certain restricted conditions. The equilibrium which is ultimately established is obviously the same irrespective of the path followed and is dependent only on the basic thermodynamic parameters. Whether this phenomenon is of any physiological significance and occurs in case of some other reversible enzymes needs to be explored in some detail.

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