UDPGLUCOSE-4 EPIMERASE FROM *SACCHAROMYCES FRAGILIS*: INTERACTION WITH SUGAR PHOSPHATES AT AN EFFECTOR SITE.

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Summary: UDPglucose-4 epimerase from *Saccharomyces fragilis* was found to be activated at low substrate concentrations by some metabolically related sugar phosphates. The stimulation of the enzyme activity showed a sigmoidal response to the increasing concentration of glucose-6 phosphate at a fixed substrate concentration. The activated enzyme was allosterically inhibited by UMP which otherwise acted as a strictly competitive inhibitor for the enzyme. The interaction with sugar phosphates was not accompanied by any change in the aggregation state of the molecule.

During the course of our study with UDPglucose-4 epimerase (E.C. 5.1.3.2) from goat liver we detected two forms of the enzyme in the liver, the catalytic activity of which were differently affected by certain sugar phosphates (1). In an effort to study these interactions more closely, a purified preparation of the enzyme from the yeast *S.fragilis* has been used for this work. We have found that G-6-P, G-1-P and Gal-6-P specifically stimulate the activity of the yeast enzyme. Further, the activation by G-6-P is possibly mediated through an interaction of this metabolite at a site other than the active site.

Materials and methods: The purified UDPglucose-4 epimerase from galactose-adapted *S.fragilis* was purchased from Sigma Chemical Co., St.Louis, Mo., U.S.A. All other biochemicals were obtained from the same source. UDPGdehydrogenase was a gift

from Sigma Chemical Co., St. Louis, Mo., U.S.A.

The lyophilized epimerase was taken into solution with 0.1 M sodium citrate pH 7.0 and was assayed by coupling the reaction with UDPG dehydrogenase. The assay mixture contained in a total volume of 1 ml, 100 μmoles of glycine buffer pH 8.8, 0.5 μmoles of NAD^+, 0.02 units of dehydrogenase where 1 unit was defined as that amount of enzyme that oxidized 1 μmole of UDPG per minute under standard conditions in presence of the requisite amount of the epimerase. The reaction was started with the addition of UDPGal. The rate remained linear between second and fifth minute. The unit of epimerase was defined as the amount of enzyme that converted 1 μmole of UDPGal to UDPG per minute. The usual specific activity of the preparations used were 5-10 units/mg. The highest specific activity reported for the yeast enzyme is about 50 units/mg (2).

Results: Activation of epimerase with G-6-P: The nature and the extent of activation of the yeast epimerase with varying concentrations of the substrate at two different concentrations of G-6-P are shown in Fig. 1. In both cases, the K_m decreased significantly but V_{max} remained unaltered. Increasing the concentration of G-6-P beyond 3 mM did not stimulate the activity further to any significant extent. G-1-P and Gal-6-P showed similar activation pattern with the enzyme. Phosphoglucomutase was not detectable in the assay medium. Fructose-6 phosphate, fructose-1 phosphate, mannose-6 phosphate, mannose-1 phosphate, glucosamine-6 phosphate and free sugars like glucose, galactose, mannose, fructose etc. had no stimulatory or inhibitory effect on the enzyme. None of the sugar phosphates had any effect on UDPG dehydrogenase,

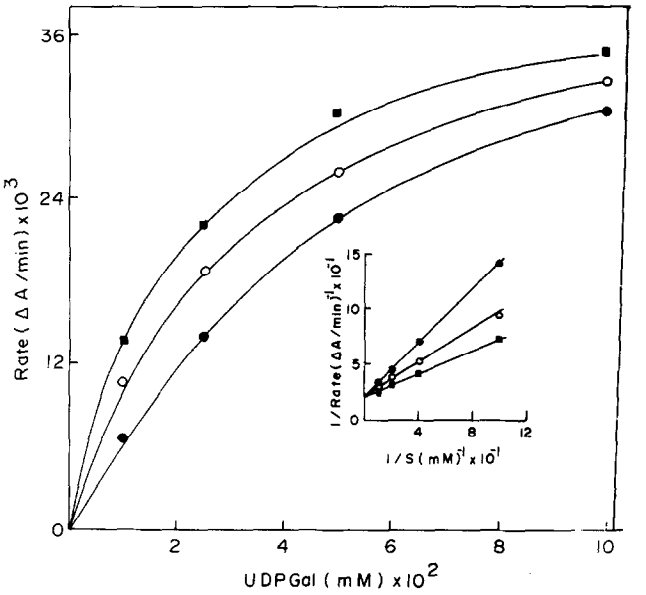

Fig. 1. Stimulation of activity of UDPglucose-4-epimerase in presence of glucose-6 phosphate.
The lower curve (-●-●-) indicates the activity of the enzyme in absence of glucose-6 phosphate. The upper two curves (-○-○-) and (-□-□-) record the activity of epimerase when assayed in presence of 1.5 mM and 3 mM concentrations of glucose-6 phosphate respectively. The inset shows the inverse plot of the same data.

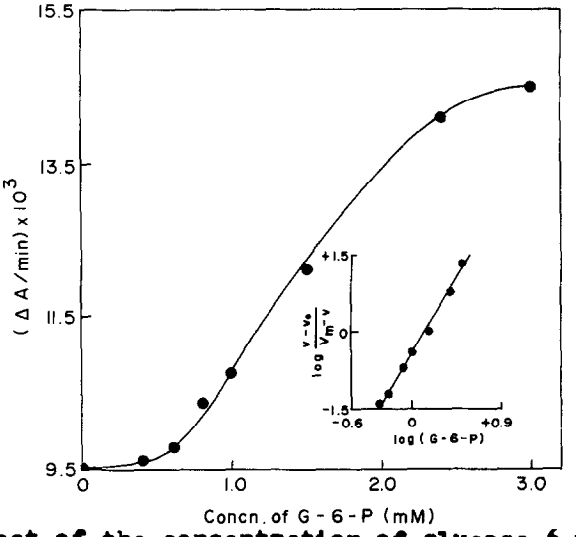

Fig. 2. Effect of the concentration of glucose-6 phosphate on the epimerase activity: Each assay mixture contained in a total volume of 1 ml, 100 μmoles of glycine buffer pH 8.8, 0.5 μmoles of NAD^+, 0.02 units of UDPG dehydrogenase and varying concentrations of G-6-P as is indicated in the figure. The reaction was started by addition of UDPGal, the final concentration of which in each case was 0.09 mM. In the inset, the Hill plot of the same data is shown. The V_m for this calculation is not shown in the figure.

the coupling enzyme. The inactive sugar phosphates could not counteract the stimulatory effect of G-6-P.

Effect of G-6-P concentration on epimerase activity: The stimulation of epimerase activity in presence of G-6-P, showed a sigmoidal response to the increasing G-6-P concentration (Fig.2). The shape of the saturation curve indicated that the binding of G-6-P with the enzyme might be a cooperative process. The Hill coefficient of this interaction was calculated to be 3.2 (Fig.2. Inset).

Inhibition with UMP: UMP acts as a strong competitive inhibitor for the yeast epimerase (3). (Fig.3A). When inhibition with UMP was however studied in presence of 3 mM G-6-P, the nature of inhibition clearly changed over to an allosteric one. Higher concentrations of UMP gave a more pronounced effect (Fig.3B). Darrow and Rodstrom had previously shown that cations like Na^+, K^+ and spermine had strong stimulatory effects on the epimerase activity (4). We found UMP inhibited the epimerase activity even in this case but the competitive nature of inhibition remained basically unchanged (Fig.3C).

Effect of G-6-P on reconstituted enzyme: On treatment with p-chloromercuribenzoate, the 120,000 M.W. yeast enzyme dissociates into two equal subunits and the bound NAD^+ is released in the medium. The inactive enzyme can be partially reactivated on incubation with mercaptoethanol and NAD^+. This reconstituted enzyme has the M.W. of the native enzyme but is only partially active (5). The kinetics of the reconstituted enzyme was found to be quite different in nature from that of the native enzyme. A weak but distinct sigmoidicity was observed at low substrate concentrations on several batches

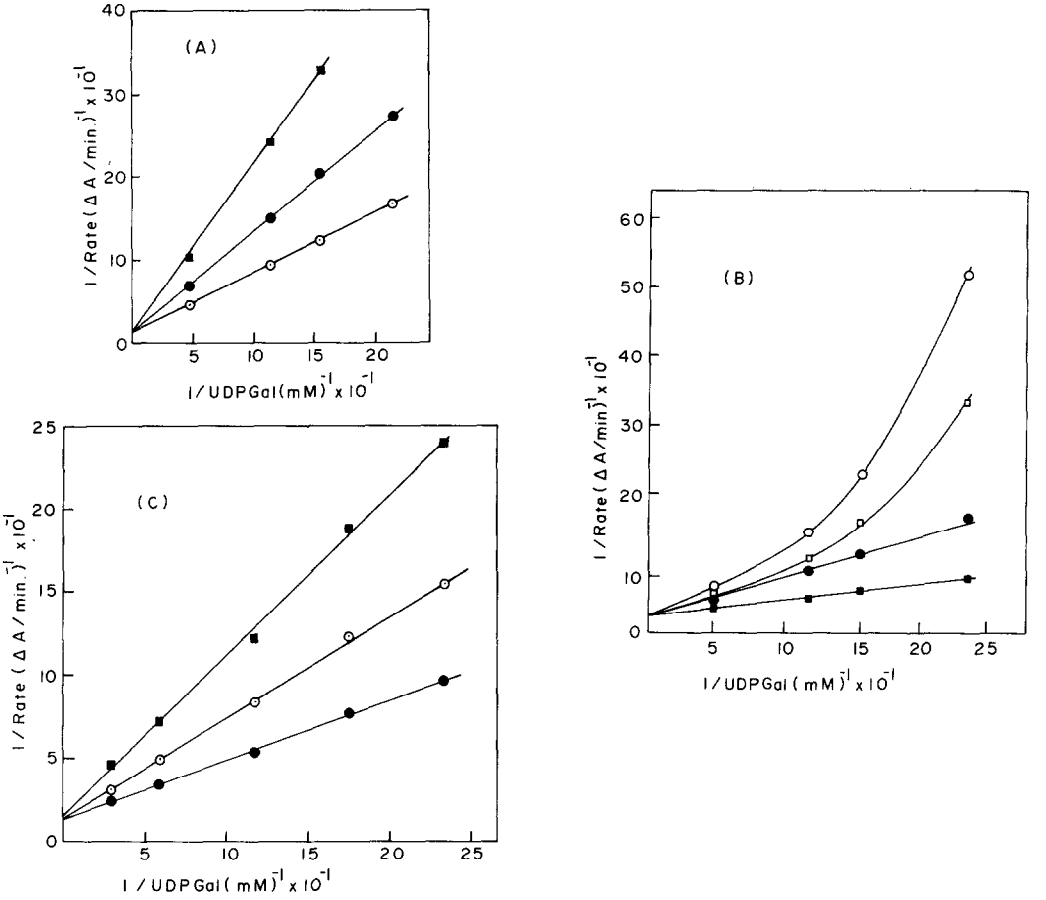

Fig. 3. Inhibition by UMP of UDPglucose-4 epimerase, in absence of either glucose-6 phosphate or cation (A), in presence of glucose-6 phosphate (B) and in presence of cation (C). (A) shows the Linweaver-Burk plot of the data at two different concentrations of UMP. The lowermost curve (-⊙-⊙-) stands for the rate of enzyme activity in absence of UMP. The upper two curves (-●-●-) and (-■-■-) were obtained when the enzyme was assayed in presence of 8 x 10^{-2} mM and 2 x 10^{-1} mM of UMP. In (B), the curve (-●-●-) indicates the activity of epimerase when assayed in absence of G-6-P and UMP. The lowermost curve (-■-■-) shows the activity of the enzyme when it is stimulated by the presence of 2 mM of G-6-P. The upper two curves, (-□-□-) and (-○-○-) show the nature of inhibition when the epimerase was assayed in presence of 2 mM G-6-P and two different concentrations of UMP. The concentrations of the inhibitor UMP were 8 x 10^{-2} mM and 2 x 10^{-1} mM respectively for the two curves. In (C), (-⊙-⊙-) represents the kinetics of the enzyme in absence of cation. When the activity was measured in presence of 50 mM of Na^+ in the form of sodium chloride, a curve represented by (-●-●-) was obtained. (-■-■-) indicates the Linweaver-Burk plot of the same Na^+ activated enzyme when it is inhibited by the presence of 2 x 10^{-1} mM UMP.

of reconstituted enzyme. In presence of G-6-P, there was a stimulation of activity and the nature of the curve changed from a sigmoidal to a hyperbolic one (Fig.4).

Gel-filtration experiments: The epimerase was chromatographed on a Sephadex G-200 (1.6 x 22 cm) column in the presence and absence of 2 mM G-6-P using 0.1 M glycine buffer 8.8 as the eluting medium. Fumarase and alcohol dehydrogenase from yeast were used as reference proteins. Presence of G-6-P did not cause any shift in the positions of the any of the three enzymes with respect to the excluded front. When a similar elution experiment

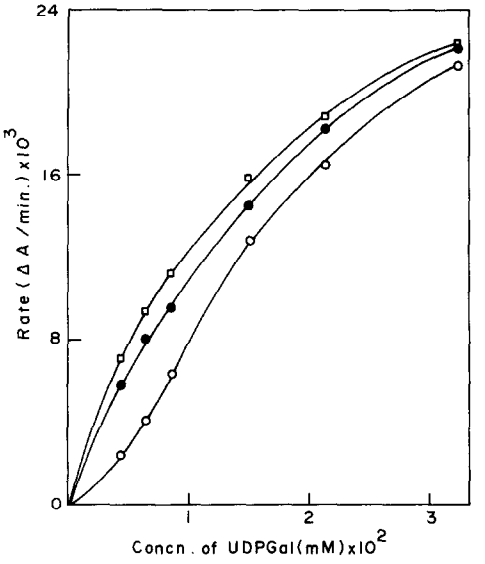

Fig.4. Effect of glucose-6-P on reconstituted enzyme. UDPglucose-4 epimerase containing 0.15 units of activity was taken in 0.01 M glycine buffer pH 8.8. The enzyme was completely inactivated by preincubation with 8 x 10^{-4} mM p-chloromercuribenzoate for 5 mins. The reconstituted enzyme was prepared by incubating 0.5 ml of inactive enzyme with 60 μmoles of mercaptoethanol and 1 μmole of NAD$^+$. The incubation was carried out for 45 mins at 25°C. Requisite amount of this reactivated enzyme was taken and assayed in the standard assay mixture. The lowermost curve (-○-○-) shows the kinetics of the reconstituted enzyme in absence of glucose-6-P. The upper two curves (-●-●-) and (-□-□-) show the kinetics in presence of 1.5 mM and 3 mM concentration of glucose-6 phosphate.

was carried out without G-6-P but in presence of spermine (15 mM), the presence of spermine caused the epimerase to move closer to the excluded front and to the elution peaks of fumarase and alcohol dehydrogenase, confirming the results obtained previously by Darrow and Rodstrom (5).

Discussion: In recent years the machanism of epimerization catalyzed by this enzyme has received considerable attention. Most of these efforts (6-9) have concerned with the presumed nucleotide-bound ketosugar intermediate, the existence of which was first postulated by Maxwell (10). In contrast to these detailed mechanistic studies with the enzyme very little is known about the nature of the protein or its interaction with related metabolites. Our experiments with sugar phosphates suggest for the first time that a site other than the catalytic site might be involved in influencing the activity of the enzyme.

The sigmoidal nature of activation with increasing concentration of G-6-P indicates that the metabolite might be binding at a site other than the active site (Fig.2). The strictly competitive nature of inhibition by UMP in absence of G-6-P shows that UMP binds directly at the active site. The shift of the kinetic curve with UMP in presence of G-6-P to an allosteric one strongly suggests that G-6-P binds at a site away from the binding site of UMP i.e. at a site other than the active site. In contrast, cations like Na^{+} probably bind directly to the active site and thus facilitate the binding of the substrate to the enzyme.

The reconstituted enzyme has the same dimeric form as

the original enzyme (5) but it showed much lower activity and a sigmoidal kinetics (Fig.4). Obviously the reconstituted enzyme failed to regain the conformation of the native enzyme. Addition of G-6-P brought about a significant change in conformation as was evidenced from the shift in the nature of the kinetic curve. The interaction of the native enzyme with G-6-P was however not accompanied by any change in the aggregation state of the molecule as was indicated by the gel-filtration experiments. The aggregation induced by spermine served as the control in this case.

Considering glucose as the starting hexose moiety, epimerase is the only enzyme of the galactose pathway which is involved in the anabolic route of galactose metabolism. The activation of the enzyme by G-6-P or G-1-P may thus be of some physiological significance though further work has to be done before the point can be established.

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