# LACTOSE AND D-GALACTOSE METABOLISM IN *STAPHYLOCOCCUS AUREUS*: PATHWAY OF D-GALACTOSE 6-PHOSPHATE DEGRADATION

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Summary: The pathway by which D-galactose 6-phosphate is degraded in *Staphylococcus aureus* has been elucidated. Galactose 6-phosphate is isomerized to tagatose 6-phosphate, which is phosphorylated with adenosine 5'-triphosphate, and the resulting tagatose 1,6-diphosphate is cleaved to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. The isomerase, kinase, and aldolase that catalyze these reactions are inducible and are distinct from the corresponding enzymes of glucose 6-phosphate metabolism.

The most common pathway for the metabolism of lactose involves hydrolytic cleavage to glucose and galactose, and further metabolism of galactose by the Leloir pathway (1): galactose → galactose-1-P → glucose-1-P → glucose-6-P. However, in *Staphylococcus aureus* (2) and certain other bacteria (3), lactose is phosphorylated with phosphoenolpyruvate prior to cleavage. In *S. aureus*, the resulting lactose phosphate is cleaved by a phospho-β-galactosidase to yield glucose and galactose-6-P (4). The pathway by which galactose-6-P is further metabolized has not been previously elucidated for any organism.

The present communication documents that galactose-6-P is metabolized in *S. aureus* by the pathway: galactose-6-P → tagatose-6-P → tagatose-1,6-$P_2$ → glyceraldehyde-3-P + dihydroxyacetone-P.

## MATERIALS AND METHODS

The organism used was *Staphylococcus aureus* NCTC 8511. Cells were grown aerobically at 37°C on a 1% peptone medium (5) supplemented with 1% sugar (lactose unless specified otherwise). Cell-free extracts were prepared by exposing cell suspensions to sonic vibration (10,000 Hz) for 20 minutes at 0°C in the presence of glass beads (80 to 125 μm diameter).

Crude extract was the supernatant fluid resulting from 20,000 rpm centrifugation in the SS-34 rotor of a Sorvall centrifuge.

D-Tagatose-6-P was synthesized from D-galacturonic acid (6-8). D-Psicose and D-tagatose were obtained as previously stated (9). Protein (10, 11), phosphate (12), aldohexose (13), and ketohexose (14), were determined by methods in the indicated references. Paper chromatography of sugars employed Whatman No. 1 paper, water-saturated phenol (9) as the solvent, and an orcinol spray (15) to reveal the location of ketohexoses. Trimethylsilyl derivatives of sugars were prepared and subjected to gas-liquid chromatography by standard procedures (16).

Assays for galactose-6-P and glucose-6-P isomerases, tagatose-6-P and fructose-6-P kinases, and tagatose-1,6-$P_2$ and fructose-1,6-$P_2$ aldolases are described in Table 1. The assays involving pyridine nucleotides were monitored at 340 nm with a recording spectrophotometer. The rate of product formation was constant with time and was proportional to enzyme concentration in the ranges used.

## RESULTS

In exploratory experiments with crude extracts, we were unable to detect ($< 0.2$ nmole per minute per mg of protein) a modification of galactose-6-P by reduction with NADH or NADPH, by oxidation with $NAD^+$, $NADP^+$, or 2,6-dichlorophenolindophenol, or by epimerization to glucose-6-P. Galactose 6-phosphatase activity was observed, but we could not detect a modification of galactose by reduction to galactitol, oxidation to galactonate, epimerization to glucose, or isomerization to tagatose. Furthermore, we could not detect either galactokinase (17) or galactose-1-P uridyl transferase (1) in extracts of cells grown either on galactose or lactose. Thus, many possible routes of galactose and galactose-6-P metabolism, including the Leloir pathway, could not be demonstrated in extracts of *S. aureus*.

Reactions which *could* be detected in crude extracts of *S. aureus* were ketohexose formation from galactose-6-P, galactose-6-P-dependent conversion

of ATP to ADP, and ATP-dependent formation of triose-P from galactose-6-P. These data were interpreted to indicate a pathway involving the isomerization of galactose-6-P to tagatose-6-P, phosphorylation of tagatose-6-P with ATP, and aldol cleavage of the resulting tagatose-1,6-$P_2$ to dihydroxyacetone-P and glyceraldehyde-3-P. Specific activities of the isomerase, kinase, and aldolase that catalyze these reactions, and the corresponding enzymes of the glucose-6-P pathway, in crude extracts of cells grown on several carbon sources are given in Table 1. Galactose-6-P isomerase, tagatose-6-P kinase, and tagatose-1,6-$P_2$ aldolase were specifically induced by growth of the organism on lactose or galactose, whereas the corresponding enzymes of the glucose-6-P pathway were constitutive.

Galactose-6-P isomerase was partially purified and separated from glucose-6-P isomerase by treating the crude extract with bentonite and chromatographing the supernatant solution on DEAE-cellulose (0 to 0.6 M KCl gradient). The product of the galactose-6-P isomerization reaction catalyzed by this enzyme preparation was dephosphorylated (9) for identification. Paper chromatography revealed the formation of a ketohexose that migrated with tagatose, but not with fructose, sorbose, or psicose. The time for half-maximal color development with cysteine-$H_2SO_4$ (9, 19) was also identical to that for tagatose but not for the other 2-ketohexoses. Gas-liquid chromatography of trimethylsilyl derivatives also confirmed that tagatose was the dephosphorylated isomerization product. Furthermore, non-dephosphorylated isomerization product served as a substrate for tagatose-6-P kinase.

Tagatose-6-P kinase was partially purified and separated from fructose-6-P kinase by ammonium sulfate precipitation (40 to 80% saturation), Sephadex G-100 chromatography, and chromatography twice on DEAE-cellulose (first a 0 to 0.5 M KCl gradient, then a 0.1 to 0.35 M KCl gradient). This preparation was used to prepare the product of tagatose-6-P phosphorylation. The product was purified by chromatography on Dowex 1-X10 (20). It eluted

TABLE 1. Specific Activities of Enzymes of Galactose-6-P and Glucose-6-P Metabolism in Crude Extracts of *S. aureus*.

| Enzyme Activity | Specific Activity (μmoles x $min^{-1}$ x mg $protein^{-1}$) | | | |
|---|---|---|---|---|
| | Sugar in Growth Medium | | | |
| | None* | Glucose | Lactose | Galactose |
| Galactose-6-P isomerase | 0.005 | 0.013 | 0.255 | 0.747 |
| Glucose-6-P isomerase | 0.633 | 1.31 | 0.504 | 0.576 |
| Tagatose-6-P kinase | 0.000 | 0.001 | 0.036 | 0.040 |
| Fructose-6-P kinase | 0.050 | 0.033 | 0.127 | 0.045 |
| Tagatose-1,6-$P_2$ aldolase | 0.000 | 0.000 | 0.028 | 0.065 |
| Fructose-1,6-$P_2$ aldolase | 0.095 | 0.162 | 0.094 | 0.109 |

*The peptone concentration was raised to 2% when no sugar was added.

Galactose-6-P isomerase assays (0.10 ml, 30°C) contained 6.8 μmoles of glycylglycine (pH 7.5), 0.6 μmole of $MgCl_2$, and 0.6 μmole of tagatose-6-P. After incubation for a timed period, the reaction mixtures were assayed for aldohexose formation. Glucose-6-P isomerase assays (0.15 ml, 30°C) contained 10 μmoles of glycylglycine (pH 7.5), 1.0 μmole of $MgCl_2$, 0.45 μmole of fructose-6-P, 0.10 μmole of $NADP^+$, and nonlimiting amounts of glucose-6-P dehydrogenase. With partially purified isomerase preparations (data not shown), the presence of the indicated enzymes was verified by using galactose-6-P as the substrate and measuring the formation of ketohexose, or glucose-6-P as the substrate and measuring fructose-6-P formation by coupling with fructose-6-P kinase, rabbit muscle aldolase, and α-glycerol-P dehydrogenase.

Kinase assays (0.15 ml, 30°C) contained 10 μmoles of glycylglycine (pH 7.5), 1.0 μmole of $MgCl_2$, 0.5 μmole of ATP, 0.5 μmole of phosphoenolpyruvate, 0.05 μmole of NADH, 0.05 μmole of tagatose-6-P or 0.15 μmole of fructose-6-P, and nonlimiting amounts of pyruvate kinase and lactate dehydrogenase. Controls to correct for ATPase and NADH oxidase were minus the ketohexose phosphate.

Aldolase assays (0.15 ml, 30°C) contained 10 μmoles of glycylglycine (pH 7.5), 1.0 μmole of $MgCl_2$, 0.05 μmole of NADH, nonlimiting amounts of triose-P isomerase and α-glycerol-P dehydrogenase, and either 0.03 μmole of fructose-1,6-$P_2$ or 0.2 μmole of tagatose-6-P plus 0.5 μmole of ATP and a nonlimiting amount of rabbit muscle fructose-6-P kinase. The latter addition effected the synthesis of tagatose-1,6-$P_2$ (18). Controls to correct for NADH oxidase were minus the ketohexose phosphate. Since this assay gives a 2-fold amplification of the rate, the values in the Table have been divided by 2.

as a ketohexose diphosphate and had a phosphate to ketohexose ratio of 2. It was not cleaved by rabbit muscle fructose-1,6-$P_2$ aldolase, but was cleaved by tagatose-1,6-$P_2$ aldolase. A sample was dephosphorylated and the resulting ketohexose was identified as tagatose by methods described in the preceding paragraph. Therefore, the product of the kinase reaction must be tagatose-1,6-$P_2$.

Tagatose-1,6-$P_2$ aldolase was partially purified and separated from fructose-1,6-$P_2$ aldolase by protamine sulfate precipitation, treatment of the supernatant solution with bentonite, and chromatography of the bentonite supernatant solution twice on DEAE-cellulose (first a 0 to 0.5 M KCl gradient, then a 0.1 to 0.4 M KCl gradient). This preparation was used to identify the cleavage products of the tagatose-1,6-$P_2$. In the presence of α-glycerol-P dehydrogenase, 2 moles of NADH were oxidized per mole of tagatose-1,6-$P_2$. Since the aldolase was contaminated with triose-P isomerase, this observation was consistent with the formation of the expected products of aldol cleavage of a ketohexose diphosphate, namely, glyceraldehyde-3-P and dihydroxyacetone-P.

## DISCUSSION

It had previously been reported that galactose 6-phosphate is an intermediate in the metabolism of lactose in *S. aureus* (21-23). The present results establish that galactose-6-P is further metabolized through the pathway: galactose-6-P → tagatose-6-P → tagatose-1,6-$P_2$ → glyceraldehyde-3-P + dihydroxyacetone-P. This conclusion is based on the demonstration of galactose-6-P isomerase, tagatose-6-P kinase, and tagatose-1,6-$P_2$ aldolase activities in extracts of cells grown on lactose or galactose, the inducibility of these enzymes, and the apparent lack of enzymes that could function in alternative pathways of galactose or galactose-6-P metabolism. The isomerase, kinase, and aldolase that participate in the metabolism of galactose 6-P have not been previously reported to occur in any organism. In *S. aureus*, they are distinct and separable from the corresponding enzymes

of glucose-6-P metabolism. The facts that these enzymes are induced by galactose as well as lactose, and that galactokinase and galactose-1-P uridyl transferase could not be detected, suggest that galactose is also metabolized via galactose-6-P, presumably by being phosphorylated at C-6 with phosphoenolpyruvate.

Our demonstration that galactose-6-P is metabolized through tagatose-6-P and tagatose-1,6-$P_2$ in *S. aureus* is contrary to the preliminary results of Simoni and Roseman (24), who suggested that the pathway in this organism involves conversion of galactose-6-P to 6-phosphogalactonate.

A detailed description of the purification and properties of galactose-6-P isomerase, tagatose-6-P kinase, and tagatose-1,6-$P_2$ aldolase will be presented elsewhere. The extent of the occurrence of this new pathway in other organisms is currently under investigation.

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