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5-THIO-D-GLUCOSE IS AN ACCEPTOR FOR UDP-GALACTOSE : D-GLUCOSE 1-GALACTOSYLTRANSFERASE

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SUMMARY

The glucose analog 5-thio-D-glucose, a potent inhibitor of glucose transport across membranes, was examined as an acceptor and/or inhibitor of lactose synthetase (UDP-galactose : D-glucose 1-galactosyltransferase, EC 2.4.1.22). Thioglucose was an effective acceptor for lactose synthetase with a K_m of 7.4 mM. Under identical conditions the K_m for D-glucose in this reaction was 5.4 mM. Thioglucose was 45 to 50% as effective an acceptor as D-glucose. Thioglucose acted as a pseudo substrate having a different K_m and V_{max}. Thus, thioglucose could be considered to be a competitive substrate for lactose synthetase. The product of the lactose synthetase reaction with thioglucose as an acceptor had a thin-layer chromatographic retardation factor slightly higher than that for lactose. Upon treatment of the reaction product with β-galactosidase, galactose and thioglucose were released. These observations suggest that the product of the lactose synthetase reaction with thioglucose was thiolactose.

The D-glucose analog 5-thio-D-glucose possesses a number of interesting properties. This analog has been shown to inhibit transport of glucose in kidney and diaphragm tissue slices (1) and to retard growth of a number of transformed cell lines in culture (2). Further, thioglucose reduced proliferation of Trypanasoma lewisi and Hymenilepis diminuta in rats (unpublished observations). Thioglucose administration has also been shown to inhibit spermatogenesis in male mice with no apparent affect on other body organs (3). Thioglucose inhibits spermatogenesis in male rats by interfering with RNA and protein synthesis (4, 5). Oral administration of thioglucose has also been found to inhibit lactose synthesis and secretory vesicle development in lactating rat mammary gland (6).

In light of this observed inhibition of lactose synthesis, the effect of thioglucose on partially purified lactose synthetase was examined. Thioglucose was examined as both an acceptor and an inhibitor of lactose synthetase and the product of the reaction was partially characterized.

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METHODS

Materials: Bovine α-lactalbumin, Escherichia coli β-galactosidase and UDP-galactose were from Sigma. Calcium phosphate gel, Cellex P (hydrogen form) and the ion exchange resin AG1-X8 (chloride form) were from Bio-Rad. [U-^{14}C] UDP-galactose (230 Ci/mole) was from Amersham/Searle. 5-Thio-D-glucose was from Pfanstiehl Laboratories and precoated silica gel G thin-layer plates were from Analtech. Ready Solv HP scintillation fluid was from Beckman Instruments.

Isolation of Galactosyltransferase: Fresh milk was obtained from the Purdue University Holstein herd and the galactosyltransferase of lactose synthetase (formerly called the lactose synthetase A protein) was isolated according to Fitzgerald et al (7) through the calcium phosphate gel step. The precipitate obtained by adjusting the active fraction from the calcium phosphate gel to 65% saturation with ammonium sulfate was used for all enzyme assays. This fraction was frozen and stored at -10 C; under these conditions activity was maintained for approximately 3 months.

Enzyme Assays: Complete reaction mixtures contained, in a final volume of 0.1 ml, the following: Tris-HCl, pH 7.4, 20 mM; $MnCl_2$, 20 mM; D-glucose or 5-thio-D-glucose, 20 mM; UDP-galactose (12.5×10^5 CPM/μmole), 0.25 mM; α-lactalbumin, 20 μg; and enzyme protein, 5 μg. Incubations were at 37 C for the indicated times and reactions were stopped by addition of EDTA to 200 mM. Contents of the reaction tube were placed onto a 0.5 x 8 cm column of Bio-Rad AG1-X8 resin packed in a Pasteur pipette. Columns were eluted with 2 x 0.5 ml of water and radioactivity in the eluate was measured in a Beckman liquid scintillation spectrometer (7, 8). To determine effects of acceptor concentration on reaction velocity, identical conditions were used except that the concentration of glucose or thioglucose was varied from 1.25 to 40 mM.

Product Characterization: Eluates from 5 reaction mixtures were combined and taken to dryness under a stream of nitrogen. The residue was dissolved in a small volume of distilled water and applied onto a silica gel G thin-layer plate; standard sugars were chromatographed on the same plate. The plate was developed in n-butanol : methanol : 0.03 M boric acid (5:3:1, v/v/v) (9). Sugars were visualized by spraying plates with 95% ethanol-5% sulfuric acid and heating in an oven at 100 C. Areas corresponding to thioglucose, galactose and the presumed thiolactose were scrapped from plates and treated with 1 ml of hyamine hydroxide. Radioactivity was then determined. A small amount of the reaction product was dissolved in 1 ml of 0.1 M sodium phosphate buffer, pH 7.0, containing 5 U of β-galactosidase (10). After 12 hr at 35 C the solution was evaporated under nitrogen and the residue was separated as above.

RESULTS AND DISCUSSION

The lactose synthetase reaction was linear with time for at least 5 min with either glucose or thioglucose as acceptor (Fig. 1b). After 5 min, only 45% as much radioactivity was incorporated with thioglucose as acceptor as with glucose as acceptor. The effect of acceptor concentration on the rate of product formation is shown in Fig. 1a. With either glucose or thioglucose, reaction rates were linear up to a concentration of 10 mM. Lineweaver-Burk plots of the data in Fig. 1a gave values for the K_m for glucose of 5.4 mM and

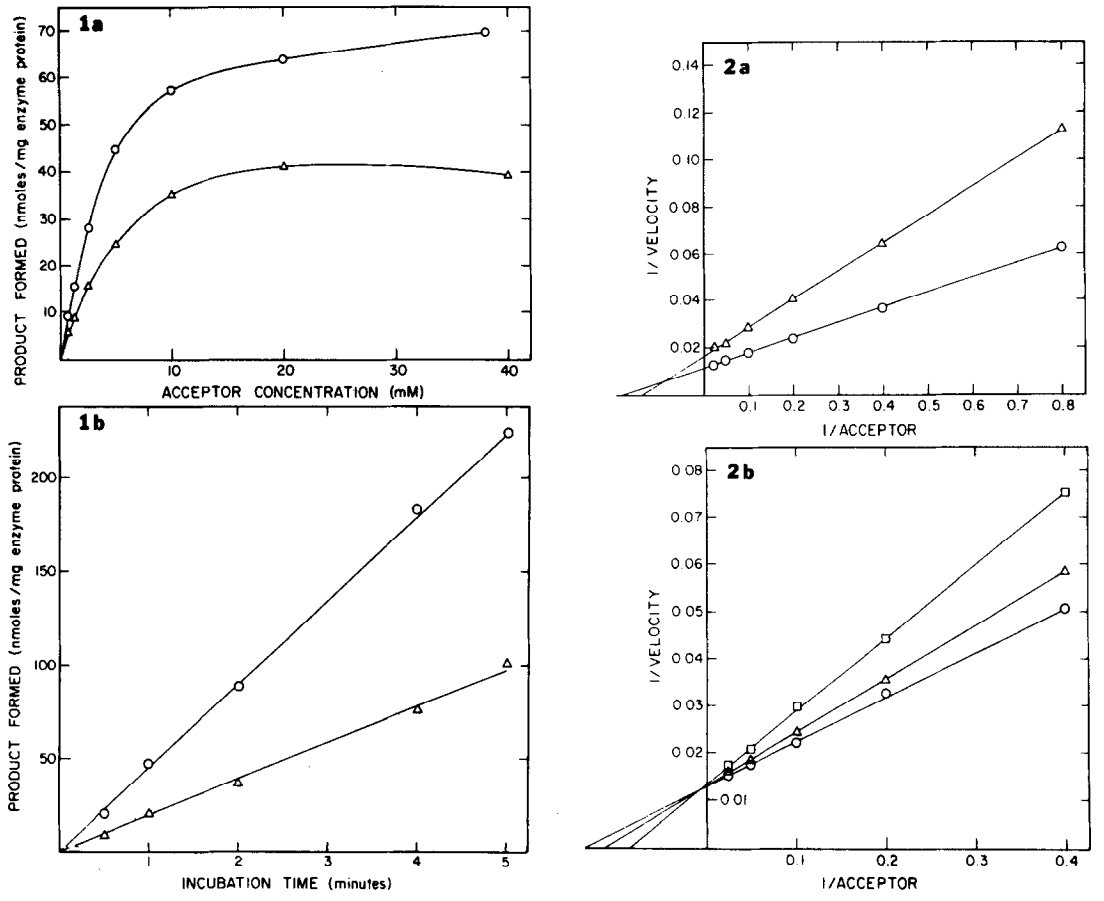

Figure 1. (a) The effect of glucose (○) or thioglucose (△) acceptor concentration on lactose synthetase activity. Incubation times were 4 min. Assay conditions are given in the text. (b) Linearity of the lactose synthetase reaction with D-glucose (○) or 5-thio-D-glucose (△) as acceptors. Reaction conditions are given in the text.

Figure 2. (a) Lineweaver-Burk plot of the data in Fig. 1a. D-glucose (○) exhibited a K_m of 5.4 mM and thioglucose (△) a K_m of 7.4 mM. (b) Lineweaver-Burk plot of thioglucose as competitive substrate for lactose synthetase. Incubation times were 4 min. Assay conditions are given in the text. No thioglucose (○), 0.625 mM thioglucose (△), 1.25 mM thioglucose (□).

for thioglucose of 7.4 mM (Fig. 2a). This value for the K_m with glucose is very close to the value given by Fitzgerald et al (7).

Lineweaver-Burk plots revealed that, at low concentrations, thioglucose was a competitive substrate for lactose synthetase (Fig. 2b). The difference in the glucose (no thioglucose) activities in plots 2a and 2b was do to the

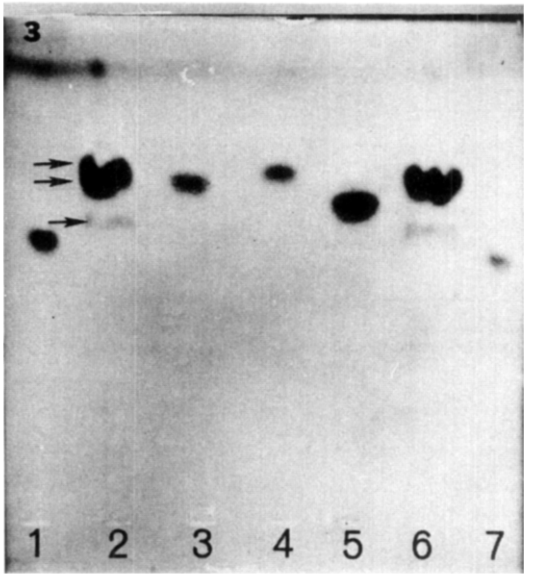

Figure 3. Thin-layer chromatogram of the product of the lactose synthetase reaction with thioglucose as acceptor. The silica gel G plate was developed in n-butanol : methanol : 0.03 M boric acid (5:3:1, v/v/v)and sprayed with 95% ethanol-5% sulfuric acid. Lanes 1 and 7, lactose; 2 and 6, reaction products with the positions of thiolactose (single arrow) and thioglucose (double arrow) shown; 3, glucose; 4, thioglucose; 5, galactose. An average of 90% of the incorporated radioactivity was found in the spot corresponding to thiolactose.

length of time the enzyme preparation was frozen. The same enzyme preparation was used for all assays, but the enzyme had been frozen for two weeks when plot 2b was made. Thus, thioglucose and glucose apparently interact at the same site within the enzyme.

By thin-layer chromatographic separation, the product of the lactose synthetase reaction with thioglucose as acceptor was found to have a retardation factor different from that of lactose (Fig. 3). When the product of the reaction with thioglucose as acceptor was incubated with β-galactosidase, about 50% of the radioactivity initially incorporated into product was recovered in the chromatographic region corresponding to D-galactose; longer treatment with the enzyme resulted in even greater recovery of radioactivity in the galactose region. This strongly suggests that the product of the react-

ion with thioglucose as acceptor has a β-linkage. While the positions of the linkage remain to be established, the product of the reaction has been tentatively named thiolactose.

Recently it was found that oral administration of thioglucose to lactating rats decreased lactose content of mammary tissue and inhibited maturation of secretory vesicles in mammary epithelial cells (6). It was postulated that administration of thioglucose may have prevented or inhibited glucose uptake into mammary epithelial cells, as previously demonstrated with other mammalian tissues (1). D-glucose is known to be required for lactose synthesis and indirect evidence suggests that it may also be necessary for secretory vesicle maturation (6, 11). Based on observations made in this study, it may be suggested that thioglucose could inhibit secretory vesicle maturation even if it is accumulated in mammary epithelial cells. Once accumulated, it could bind with the active site of the galactosyltransferase of lactose synthesis and in this mammer act as a pseudo substrate and diminish the rate of lactose synthesis.

Acknowledgements

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