ENZYMATIC SYNTHESIS OF LACTOSE FROM URIDINE DIPHOSPHATE D-GALACTOSE AND D-GLUCOSE*

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Physiological evidence (see Folley, 1949), and the in vitro biosynthesis of lactose from D-glucose using mammary gland tissue slices (Grant, 1955; Malpress and Morrison, 1950; Heyworth and Bacon, 1955) and tissue homogenates (Kittinger and Reithel, 1953) suggested that D-glucose is a precursor of lactose. A possible pathway for the conversion of D-glucose into lactose via uridine diphosphate D-galactose (UDP-D-galactose) was indicated by the demonstration of uridine diphosphate D-glucose (UDP-D-glucose) and the enzyme UDP-D-galactose-4-epimerase (galactowaldenase) in mammary tissue (Caputto and Trucco, 1952); and by the observation that UDP-D-galactose-4-epimerase activity is increased in lactation (Maxwell et al., 1955). Gander et al. (1956; 1957) reported the synthesis of lactose 1-phosphate from UDP-D-glucose and α-D-glucose 1-phosphate (G-1-P) by enzyme fractions from bovine mammary tissue and proposed that lactose is formed according to the reactions:

$$\text{Uridine triphosphate} + \text{G-1-P} \xrightarrow{\text{pyrophosphorylase}} \text{UDP-D-glucose} + \text{pyrophosphate}$$

$$\text{UDP-D-glucose} \xrightarrow{\text{galactowaldenase}} \text{UDP-D-galactose}$$

$$\text{UDP-D-galactose} + \text{G-1-P} \xrightarrow{\text{galactosyl transferase}} \text{lactose-1-phosphate} + \text{UDP}$$

$$\text{Lactose-1-phosphate} \xrightarrow{\text{phosphomonoesterase}} \text{lactose} + \text{phosphate}$$

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In this synthesis G-1-P is the precursor of both the D-glucose and the D-galactose moieties. Isotopic experiments on perfused cow udders, however, have shown unequal labeling of the two hexose moieties of lactose (Wood et al., 1957) and suggest that another mechanism for lactose synthesis may exist. In the present paper evidence is presented that particulate preparations from guinea pig mammary gland are capable of catalysing the formation of lactose from UDP-D-galactose and D-glucose.

Mammary glands were obtained from lactating guinea pigs five days post partum. The animals were kept with their young until the time of the experiment to ensure that the glands were in an active state of lactation. The glands were chilled immediately upon removal, trimmed of extraneous fat and cut into small pieces by hand. The glandular tissue was teased out, connective tissue removed so far as possible, and the remaining material suspended in ten times its volume of ice cold 0.25 M sucrose. The suspension was homogenized at 0° for four consecutive periods of one minute each in a mechanically driven Potter Elvehjem homogenizer with intervals for cooling in between. The homogenate was filtered through four layers of cheesecloth and centrifuged at 0° for 30 minutes at 1,000 x g. The supernatant solution was filtered through glass wool to remove fat particles and then centrifuged at 50,000 x g for 1 hour at 0°. The deposit was washed by resuspension in 0.25 M sucrose, recentrifuged at 50,000 x g for 1 hour and then suspended in 0.1 M 2-mercaptoethanol-0.1 M Tris-HCl buffer pH 7.5; 1 ml of buffer was added to the particles obtained from 5 g of mammary tissue. The enzyme which catalyses the synthesis of lactose was associated with the particulate material, and only very slight activity was found in the supernatant solution obtained after the first centrifugation at 50,000 x g. Preparations from five different guinea pigs showed a similar distribution of enzyme activity. The particles retained the capacity to synthesize lactose for approximately one week during storage at 2-4°.

UDP-D-galactose-C^{14} was prepared by conversion of uniformly labeled D-galactose-C^{14} (Bean et al., 1953) to α-D-galactose 1-P using a D-galactokinase preparation from yeast (purchased from the Sigma Chemical Company) (Trucco et al., 1948) followed by treatment of the α-D-galactose 1-P with a UDP-D-galactose pyrophosphorylase preparation from Phaseolus aureus (Neufeld et al., 1957). UDP-D-galactose was separated from the reaction mixture by paper electrophoresis in 0.2 M ammonium formate buffer pH 3.6 and eluted from the paper.

The product formed on incubation of UDP-D-galactose-C^{14} and D-glucose with the enzyme preparation was identified as lactose by 1) cochromatography with authentic lactose in two solvent systems, ethyl acetate : propanol : water (1:7:2 v/v) and ethyl acetate : pyridine : water (2:1:2 v/v) and 2) hydrolysis with E. coli β-galactosidase which is specific for a β-galactosyl linkage. Treatment of the hydrolysis products with sodium borohydride followed by electrophoresis in borate buffer pH 9 gave only one radioactive spot corresponding to galactitol; the lactose was therefore labeled in the galactose moiety. As shown in Table I incorporation of radioactivity into lactose was observed after 2 minutes incubation and increased up to one hour. Substitution of α-D-glucose 1-P for D-glucose in the reaction mixture also led to the formation of lactose; however, the amount of radioactivity incorporated was only about 20%, after one hour incubation, of that incorporated when D-glucose was used as a substrate. The presence of a phosphatase in the enzyme preparation was shown by hydrolysis of G-1-P-C^{14} when this compound was incubated with the mammary gland particles. The amount of hydrolysis observed was sufficient to account for the lactose formed from UDP-D-galactose-C^{14} and unlabeled G-1-P, assuming that D-glucose is the actual galactosyl acceptor. The possibility of small amounts of G-1-P formation from D-glucose cannot be excluded by the methods used, but attempts to detect the synthesis of C^{14}-labeled G-1-P from D-glucose-C^{14} under the usual experimental conditions were unsuccessful. Radioactive lactose 1-P could not be detected after electrophoresis of reaction mixtures

TABLE I

ENZYMATIC SYNTHESIS OF LACTOSE

The reaction mixtures contained 0.5 μmole UTP, 0.13 μmole $MgCl_2$, 5 μmoles Tris-HCl buffer pH 7.5, 0.005 μmole UDP-D-galactose-C^{14} (0.045 μc) and 25 μl of the mammary gland particle suspension. 0.5 μmole D-glucose was added to reaction mixture (1) and 0.5 μmole α-D-glucose 1-P was added to reaction mixture (2). The final volume of the mixtures was 4.5 μl. The reactions were carried out in capillary tubes (Porter and Hoban, 1954). The mixtures were incubated at 37° and the reactions were stopped by heating the sealed capillary tubes in a boiling water bath for 2 minutes. The neutral sugars were separated from the nucleotides and sugar phosphates by paper electrophoresis in 0.2 M ammonium formate buffer pH 3.6. Radioactive substances were located on paper by autoradiography, eluted from the paper and the mobile compounds were counted on aluminum planchets with a thin window Geiger-Müller counter. The neutral radioactive sugars were chromatographed on circular filter paper disks in ethyl acetate: propanol: water (1:7:2 v/v) and the radioactive bands eluted and counted.

Substrates	Incubation Time	% of total recovered radioactivity incorporated in lactose
(1) UDP-D-galactose-C^{14} + glucose	2	2
	10	16
	30	27
	60	55
	120	54
(2) UDP-D-galactose-C^{14} + G-1-P	2	0.3
	10	2
	30	5
	60	12

containing UDP-D-galactose-C^{14} and either unlabeled D-glucose or G-1-P. Lactose formation occurred when C^{14}-labeled UDP-D-glucose was substituted for UDP-D-galactose in the reaction mixture containing D-glucose, about 15% of the radioactivity being incorporated in one hour. The particles contain UDP-D-galactose-4-epimerase which would account for the transformation of UDP-D-glucose to UDP-D-galactose.

Incubation of the mammary gland particles with C^{14}-labeled UDP-D-galactose and N-acetyl-D-glucosamine in place of D-glucose led to the formation of a radioactive compound which moved faster than lactose in the solvent system used for chromatography. This compound was hydrolysed by

E. coli β-galactosidase and was indistinguishable from O-β-D-galactosyl-(1→4)-N-acetyl-D-glucosamine on co-chromatography with an authentic sample of this disaccharide. The considerable variation in the ratio of the radioactivity incorporated into lactose and into the disaccharide obtained with N-acetyl-D-glucosamine using different mammary gland preparations suggests that different enzymes are responsible for the synthesis of the two compounds.

The biosynthesis of lactose from UDP-D-galactose and D-glucose indicates that an enzyme is present in mammary gland which catalyses the formation of the disaccharide by the direct transfer of the galactosyl moiety of UDP-D-galactose to D-glucose. No evidence could be obtained for the formation of lactose 1-P as an intermediate in lactose synthesis. The demonstration of the direct synthesis of lactose from D-glucose is in agreement with the suggestion made by Schambye et al. (1957) that the D-glucose moiety of lactose originates from D-glucose in equilibrium with blood glucose and the galactose from hexose phosphates formed in the mammary gland.

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