LACTOSE SYNTHETASE IN MAMMARY GLAND OF THE CALIFORNIA SEA LION

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Summary. Mammary gland obtained from a lactating California sea lion, Zalophus Californianus, contained all the enzymatic activities required for synthesis of UDP-galactose from glucose. Galactokinase activity was absent and activity of galactose-1-phosphate uridyl transferase was extremely low. Galactosyl transferase activity was present, but there was negligable activity of lactose synthetase in either mammary gland or milk from the sea lion. α-lactalbumin, determined enzymatically, was not found in sea lion mammary gland or milk which accounts for absence of lactose from the milk of this animal.

In 1933, Schroeder (1) reported that an infant walrus that was fed cow's milk developed severe diarrhea, and attributed this reaction to hypersensitivity to milk protein. Pilson and Kelley (2) found no lactose in the milk obtained from the California sea lion, Zalophus Californianus, and subsequently, Sunshine and Kretchmer (3,4) demonstrated profound lactose intolerance associated with absence of lactase in intestine of California sea lion pups and adults. Other species of Pacific basin pinnepeds also had little or no lactose in milk and trace or absent lactase activity in intestine (4).

In this report, the biochemical basis for absence of lactose from milk of the California sea lion has been established by analysis of the enzymatic steps involved in lactose synthesis by mammary tissue and milk obtained from a lactating animal.

A lactating California sea lion was captured on the San Miguel Islands off the coast of Southern California, in early July, 1971. Mammary gland tissue, milk and other tissues were obtained fresh within 24 hours of capture and analyzed immediately or after several days of storage at -70°C. Hexokinase and galactokinase were determined according to the method of Heinrich and Howard (5); phosphoglucomutase (6), UDPG pyrophosphorylase (7), UDP - galactose epimerase (7), and galactose -1- phosphate uridyl transferase (8,9) were determined by established methods. All assays were linear with time and enzyme concentration. Appropriate corrections were made for activity in the absence of substrate and known interfering reactions. Lactose synthetase (glucose as substrate) and galactosyl transferase (N-acetylglucosamine as substrate) were determined by a modification of the method of Babad and Hassid (10)

The reaction mixture contained 2.5 μmoles glycylglycine, pH 8.0; 0.67 μmoles Mn Cl_2, 2.0 μmoles glucose or 1.25 μmoles of N-acetylglucosamine (NAG), ± 300 μg/ml purified bovine α-lactalbumin, 25 nmoles ^{14}C-UDP-galactose (0.4 μc/μmole), and 20% tissue hemogenate or whole milk in a final volume of 50 μl. Incubations were carried out for 15 min. at 37°C and the reactions stopped by boiling at 100°C for 2 min. The reaction mixtures were diluted with 250 μl H_2O and transferred to 0.6 x 3.0 cm columns of Dowex-1-Cl. The columns were eluted with 1.0 ml H_2O and the eluates containing ^{14}C-lactose were counted in a scintillation spectrometer after the addition of 10 ml of Bray's solution (11). Appropriate controls were included to correct for nonspecific hydrolysis of UDP-galactose, and in some experiments, 1 mM ATP or UTP was included to reduce the hydrolysis of ^{14}C-UDP-galactose (12,13). Enzymatic assay of α-lactalbumin in mammary gland homogenates and milk was performed according to the method of Fitzgerald et al.(14). Bovine A protein used in this assay was purified from skim milk through the carboxymethylcellulose-column-step (15). A standard curve with bovine α-lactalbumin was run with each assay.

TABLE I. Enzyme activities from mammary tissue of the rat and sea lion.

Enzyme+	Rat*	Sea Lion
Hexokinase	0.42 (0.4-1.4)‡	1.06
Phosphoglucomutase	1.90 (2.0-7.0)	3.15
UDPG Pyrophosphorylase	7.30 (7-29)	6.40
UDP-galactose epimerase	1.50 (1.0-8.0)	4.65
Galactokinase	0.37	0
Gal-1-P Uridyl transferase	0.0663	0.00016
Galactosyl transferase	0.176 (0.2-1.0)	0.078

+ All enzyme activities expressed as μmoles/min/gm wet weight.

* Values obtained in rats on the 9-10th day of lactation.

‡ Values shown in parentheses are those obtained by other investigators in rats at 8-21 days of lactation (7,16,17).

Table 1 shows the relative activities of enzymes, from lactating mammary gland of rat and sea lion, which participate in the synthesis of UDP-galactose from either glucose or galactose. Although galactokinase was absent and galactose-1-phosphate uridyl transferase barely detectable in sea lion mammary

gland, UDP-galactose can be formed from glucose by the hexokinase pathway. The activities of hexokinase, phosphoglucomutase, UDPG pyrophosphorylase and UDP-galactose epimerase were comparable to the values found in mammary gland tissue from the lactating rat. Galactosyl transferase activity (NAG as acceptor substrate) was also readily detectable in sea lion mammary gland. Analysis of sea lion liver, kidney, and red blood cells revealed in each case a complete absence of galactokinase and barely discernable galactose-1-phosphate uridyl transferase activity. The very low activity of galactose-1-phosphate uridyl transferase in sea lion liver confirms results previously obtained in this laboratory (3). However, Mathai et al. (18) reported that the activities of galactokinase and galactose-1-phosphate uridyl transferase in erythrocytes from the California sea lion were not greatly different than in humans. We are not able to explain these conflicting results.

Lactose synthetase (LS) is a complex enzyme system, requiring the presence of both galactosyl transferase (A protein) and α-lactalbumin (B protein). The function of α-lactalbumin is that of a "specifier" protein, which reduces the Km of galactosyl transferase for glucose and thus allows for synthesis of lactose at low concentrations of glucose (19). Table 2 compares the lactose synthetase activities of rat and sea lion mammary gland and bovine vs sea lion milk.

TABLE 2. Activities of lactose synthetase and galactosyl transferase in mammary gland and milk.

Tissue	Additions			
	Glucose	Glucose + α-LA*	NAG+	NAG + α-LA*
Rat mammary gland ‡	64.9	201	176	79
Sea lion mammary gland ‡	0 - 3	42-91	58-104	12-15
Bovine milk	70.7	63.8	4.7	--
Sea lion milk	0.7	34.7	62.0	--

* 300 μg/ml bovine α-lactalbumin.

\+ NAG = N-acetylglucosamine.

‡ Values represent mean of 3 different animals at 9-10 days lactation.

‡ Values represent range of 3-4 experiments. All activities are expressed in n moles/min/gm wet weight.

With glucose as the galactose acceptor, sea lion mammary gland and milk had negligible LS activity, whereas rat mammary gland and bovine milk had considerable activity. The addition of purified bovine α-lactalbumin markedly stimulated LS activity in sea lion and rat mammary gland, but inhibited LS in bovine milk, presumably because of the high endogenous content of α-lactalbumin in bovine milk (15,20). Galactosyl transferase activity (NAG as acceptor substrate) was present in all materials and was inhibited by the addition of bovine α-lactalbumin to mammary gland. The low activity of galactosyl transferase in bovine milk is most likely secondary to inhibition of activity by the high endogenous concentration of α-lactalbumin (19,20).

The extremely low activity of LS and the easily demonstrable presence of galactosyl transferase in sea lion mammary gland and milk suggest that α-lactalbumin is either absent or present in very low concentration. Results of a specific assay for α-lactalbumin activity are shown in Table 3. No detectable α-lactalbumin activity was found in sea lion milk or mammary tissue. The α-lactalbumin activity of sea lion milk is less than 1/500th that in the specimen of bovine milk analyzed, since a 1/500 dilution of bovine milk gave readily detectable α-lactalbumin activity, whereas undiluted sea lion milk showed no activity.

The absence of lactose from the milk of the California sea lion can be explained on the basis of an absence of α-lactalbumin activity in both mammary gland and milk. Because of potential species differences, the absence of

TABLE 3. α-lactalbumin activity in milk and mammary gland.

Tissue	Units*
Bovine milk	575
Sea lion milk	0
Rat mammary gland	100
Sea lion mammary gland	0

* Units correspond to μg of bovine α-lactalbumin equivalents per ml milk or gm wet weight mammary gland. Each incubation mixture contained 100 units/ml partially purified bovine A protein (15).

α-lactalbumin activity determined enzymatically with bovine α-lactalbumin as a standard does not prove the lack of a α-lactalbumin molecule. However, if α-lactalbumin is present in sea lion milk, it has lost its biological function or is present in extremely low concentration. Schmidt et al. (21) have recently reported that milk from the Northern fur seal contains low α-lactalbumin activity (at least 8 x greater than that from the California sea lion). Since Brew (22) has shown that the lactose content of milk in various mammalian species is proportional to the α-lactalbumin activity of that species, rather than its actual chemical concentration, the presence of a small amount of α-lactalbumin in the milk of the Northern fur seal suggests the possibility that the pinnepeds may have an altered α-lactalbumin molecule with low biological activity, rather than a complete absence of this protein.

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