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Evidence for Specific Transport of Uridine Diphosphate Galactose across the Golgi Membrane of Rat Mammary Gland

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The inhibition of lactose synthesis by UDP-glucose, UDP-glucuronate and, less so, by UDP-*N*-acetylglucosamine was markedly smaller in preparations of 'intact' than of lysed vesicles derived from the Golgi apparatus of lactating rat mammary gland. This constitutes evidence for a specific, probably facilitated, transport of UDP-galactose across the Golgi membrane.

The concentration of UDP-galactose in mammary and other tissues is exceeded threefold or more by that of UDP-glucose (Murphy *et al.*, 1973; Keppler *et al.*, 1970), owing to the equilibrium of their interconversion by UDP-glucose epimerase (EC 5.1.3.2) (Maxwell *et al.*, 1962; Wilson & Hogness, 1964). Since UDP-glucose inhibits lactose synthase (EC 2.4.1.22) competitively with UDP-galactose, and shows an association constant approaching that of UDP-galactose itself (Morrison & Ebner, 1971; Khatra *et al.*, 1974; Kitchen & Andrews, 1974), the question arises whether this inhibition is also expressed within the intact mammary gland. A possible solution to this problem was suggested by our evidence for the location of lactose synthase within the lumen of the Golgi apparatus and of vesicles derived from it by homogenization (Kuhn & White, 1975). It appeared conceivable that the Golgi membrane selectively excludes UDP-glucose while permitting the free passage of UDP-galactose from the cytosol to the site of lactose synthesis. The experiments described below appear to support this scheme.

Experimental

Mammary vesicles were prepared, in 0.25M-lactose/50mM-glycylglycine/NaOH buffer, pH7.5, from the tissue of rats at 15–21 days of lactation. The synthesis of lactose was assayed by the incorporation of radioactivity from UDP-[^{14}C]galactose, the [^{14}C]lactose formed being mixed with carrier lactose and isolated by selective precipitation with ethanol/ether (3:1, v/v). Incubations were carried out with 'intact' vesicles, maintained iso-osmotic by the inclusion of lactose in the reaction mixture, or with vesicles previously lysed in 1% (w/v) digitonin solution. These techniques have been described in detail (Kuhn & White, 1975).

Results

Fig. 1(*a*) shows that, at a constant concentration of UDP-galactose (0.5mM), increasing concentrations of UDP-glucose progressively inhibited the synthesis of lactose, this being more so with lysed than with 'intact' vesicles. UDP-glucuronate also inhibited lactose synthesis (Fig. 1*b*), albeit at higher concentrations, and the same difference between 'intact' and lysed vesicles was apparent. With UDP-*N*-acetylglucosamine, which also inhibited (Fig. 1*c*), the difference between 'intact' and lysed vesicles was much smaller though apparently still in the same direction.

Before ascribing these differences to the integrity of the vesicles that were used for incubation, it was noted that the two types of incubation differed also with respect to the concentration of lactose and the presence of digitonin. Further, the concentration of α-lactalbumin that is retained within isolated mammary vesicles is not known, and is therefore not necessarily equal to that added to incubations with lysed particles. In control experiments, however, the activity of lactose synthase in the presence of UDP-glucose (3mM) and digitonin-lysed vesicles (28% of control) was unaffected by the addition of 0.2M-lactose such that iso-osmoticity was reached (27% of control). Further, the presence of UDP-glucose (3mM) inhibited lactose synthesis by vesicles lysed in 0.5% (w/v) Triton X-100 (activity 29% of control) equally to that by those lysed in 1% (w/v) digitonin (28% of control). Finally, UDP-glucose (2mM) added to lysed vesicles in the presence of 0.01, 0.02, 0.04 or 0.1% (w/v) α-lactalbumin resulted in mean rates (±S.E.M.) of lactose synthesis 45±0.7%, 42±1.2%, 32±0.9% and 25±0.3% respectively of that observed in the absence of UDP-glucose (all reactions were carried out in triplicate). Since the concentration of α-lactalbumin within the Golgi vesicles must be within or above this range, we conclude that the different susceptibilities of 'intact' and lysed vesicles to UDP-glucose are not accounted for by any differences in the concentration of α-lactalbumin.

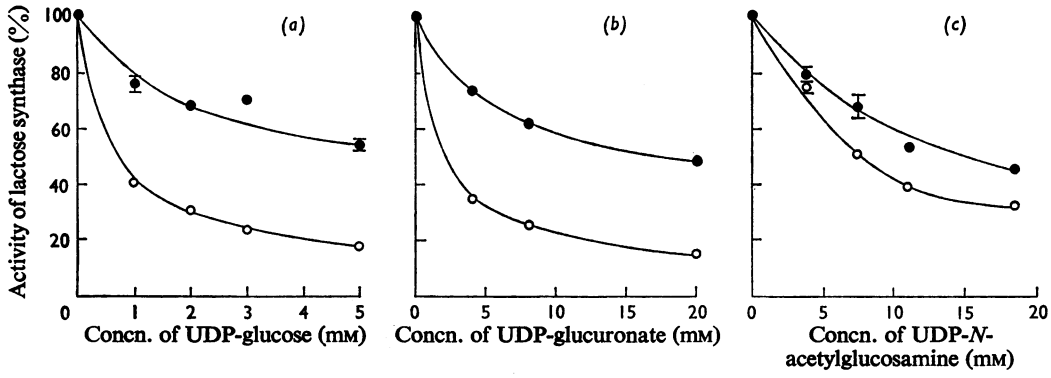

Fig. 1. *Inhibition of lactose synthesis by uridine nucleotides in the presence of 'intact'* (●) *or lysed* (○) *mammary vesicles*
All incubations contained 10mM-glycylglycine/NaOH buffer, pH7.5, 40mM-Tes [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid]/NaOH buffer, pH7.5, 7.5mM-$MnCl_2$, 10mM-glucose, 0.5mM-UDP-[^{14}C]galactose (0.5μCi/μmol) and UDP-glucose (*a*), UDP-glucuronate (*b*) or UDP-*N*-acetylglucosamine (*c*) at the concentrations shown. In addition, they contained either 'intact' vesicles (equivalent to 1.0mg of tissue) and 0.2M-lactose, or lysed vesicles (equivalent to 0.5mg of tissue), 0.2% (w/v) digitonin and 0.04% (w/v) bovine α-lactalbumin in a final volume of 50μl. Reactions were started by the addition of enzyme and the incubations were carried out for 20min at 37°C. Each point shows the mean result of six separate incubations carried out on two separate preparations; S.E.M. values are shown as vertical bars where they exceed the diameter of the symbol. Rates of lactose synthesis in the absence of inhibitor were typically equivalent to 0.12 and 0.52μmol of lactose/min per g of fresh tissue for 'intact' and lysed vesicles respectively, ignoring losses of enzyme during preparation.

The possibility remains that a small amount of UDP-glucose epimerase, carried over from the cytosol into the present vesicle preparation, was acting on added UDP-glucose to generate unlabelled UDP-galactose. This would have lowered the specific radioactivity of the UDP-galactose and have created an apparent inhibition of lactose synthesis. However, the incubation conditions were not optimal for the activity of the epimerase. Moreover the degree of inhibition shown by UDP-glucose with lysed vesicles was very comparable with that for the purified lactose synthase of human and bovine milk (Morrison & Ebner, 1971; Khatra *et al.*, 1974) and was not less when four times less lysate was used (results not shown). In any case, greater inhibition occurred with lysed than with 'intact' vesicles, although incubations with the former had half as much tissue equivalent and, presumably, half as much epimerase contaminant. We therefore discount this possible trivial explanation of our results.

Discussion

It appears from these results that the integrity of vesicles derived from the Golgi apparatus of mammary tissue protects the contained lactose synthase from inhibition by external UDP-glucose. By extension one may suppose that the UDP-glucose of mammary cytosol is also excluded from the Golgi lumen within the intact cell. Such a conclusion virtually implies the presence of a transport system in the Golgi membrane that is specific for UDP-galactose. Our experiments with UDP-glucuronate and UDP-*N*-acetylglucosamine illustrate further the degree of such specificity, although it should be noted that UDP-glucuronate is not itself a constituent of mammary tissue. In view of our failure to detect a galactosyl-lipid intermediate (Kuhn & White, 1975), and because UDP-galactose is the true substrate of the enzyme, a mechanism for the direct, facilitated, transport of UDP-galactose appears likely.

The significant inhibition of lactose synthesis that was seen even with preparations of 'intact' vesicles may imply that a proportion of these were not, in fact, wholly intact, or else that a sufficiently high concentration of other nucleotide sugars can eventually inhibit, or challenge the specificity of, the proposed transport system.

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