The uridyl transferase of mammary gland

MUNCH-PETERSEN, KALCKAR, CUTOLO AND SMITH[1] have shown that brewers yeast contains an enzyme catalysing the reversible reaction UTP + α-glucose-1-phosphate $\rightleftharpoons$ UDPG + pyrophosphate, the enzyme responsible being classified as a uridyl transferase. The existence of this enzyme has also been demonstrated in galactose-adapted *Saccharomyces fragilis*[2] and in isolated liver nuclei[3,4]. In view of the occurrence of UDPG in the lactating mammary gland[5,6,7] it was considered probable that this organ might also contain the enzyme. Studies indicate that the actively lactating gland is, in fact, a rich source of uridyl transferase and the following method has been used to obtain a partially purified preparation of this enzyme.

Mammary glands were excised from a lactating rat, 12 days post partum, washed rapidly in ice-cold water, and chopped finely with a McIlwain tissue chopper. The tissue was extracted for 10 min with 10 vols. ice-cold 0.2 *M* tris (hydroxymethyl) amino methane (TRIS) buffer pH 7.4, the extract centrifuged at 0° and filtered through glass wool. Solid $(NH_4)_2SO_4$ was added to the supernatant to a final concentration of 3 *M* and the solution allowed to equilibrate at 0° for several hours.

2.0 g Standard Super-Cel (Celite 519 A, Johns-Manville Co.) were added per 100 ml solution. After standing at 0° for 30 min, the material was filtered and the filter cake extracted with 4 × 15 ml volumes of 2.5 *M* $(NH_4)_2SO_4$ solution, filtering at each elution. The process was then repeated with 2.0, 1.5 and 1.0 *M* $(NH_4)_2SO_4$ solutions respectively. The enzyme in the resulting eluates was precipitated with $(NH_4)_2SO_4$ at a final concentration of 3 *M*, centrifuged at 0°, dissolved in the minimum volume of water and dialysed overnight at 0°. Such fractions are referred to as the 2.5, 2.0, 1.5 and 1.0 *M* fractions respectively.

On dialysis, varying amounts of precipitate formed in all fractions. Spectrophotometric assay of the supernatants by pyrophosphorolysis of UDPG[1] indicated little or no uridyl transferase activity in any fraction. On elution of the centrifuged precipitates with suitable volumes of 0.1 *M* TRIS buffer pH 7.8, uridyl transferase activity was found to be localised in the 1.5 *M* fraction. 5 g initial wet mammary gland tissue yielded 2 ml solution at this stage which, on assay by the method of MUNCH-PETERSEN *et al.*[1] showed an initial ΔE_{340} of 0.08 per minute per 100 μl enzyme solution. Activity was proportional to the enzyme concentration and the solution was free from organic pyrophosphatase. A synthesis of UDPG from UTP and α-glucose-1-phosphate was obtained with this enzyme preparation.

It is possible that the uridyl transferase of mammary gland may be functional in lactose synthesis in view of the observation of KITTINGER AND REITHEL[8] that the galactose moiety of lactose arises from glucose-1-phosphate and the glucose moiety from glycogen. A possible reaction mechanism would involve a synthesis of UDPG from α-glucose-1-phosphate in the above manner, with subsequent inversion to UDP galactose. In this respect it has been claimed by CAPUTTO AND TRUCCO[5] that mammary gland contains an active galactowaldenase.

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The abbreviations UTP for uridine triphosphate and UDPG for uridine diphosphate glucose are used throughout.

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