ISOLATION AND IDENTIFICATION OF A LACTOSE PHOSPHATE ESTER FROM COW COLOSTRUM

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A new lactose phosphate ester has been found in cow colostrum, which, from the results reported below, has been tentatively identified as O-β-D-galacto-pyranosyl 3-O-phosphate-(1-4)-D-glucopyranose. As a trivial name for this ester we propose lactose-3'-phosphate (L3'P)

The new ester was isolated by chromatography on a column of Dowex 1-X8, 200-400 mesh, formate form, prepared according to Carubelli et al. (1961). The colostrum was deproteinized by leaving overnight with 1.3 vol. of 95 % ethanol and filtered. The clear fluid was passed through the column and this washed; the elution was carried out with 0.05, 0.1, 0.2, 0.3, and 0.4 M formic acid. L3'P is eluted with 0.3 M formic acid; its peak appears in front of the peak containing galactose-1-P, glucose-1-P and glucose-6-P. The ester was determined by running it on paper chromatography simultaneously with its products of hydrolysis after 15 min and after 24 h in boiling 1N HCl. Butanol-pyridine-water (solvent system (a) of Marinetti et al., 1960) was used as solvent. The intact ester remains in the origen; the products of hydrolysis after 15 min show that most of the glucose but practically no galactose

had been liberated, while, after 24 h of hydrolysis the amounts of free glucose and galactose are equal.

The ester eluted from the column was dried in the vacuum on NaOH, dissolved in small amount of water and run on paper with 75 % ethanol as solvent (R_F = 0.60). The eluted ester was passed through a column of Dowex 50 and dried; the powder was extracted with pyridine, dried, and the combined pyridine eliminated by passing through Dowex 50. Determination of the hexoses (by anthrone method) plus the phosphate group (by Fiske and SubbaRow method) accounted for 91 % of the total dry weight. The acid titration curve showed the two dissociation groups of phosphate; the equivalent weight was 456. Treatment with kidney phosphatase gave lactose which was identified by electrophoresis in borate buffer and by paper chromatography with three different solvents. The ester reduces the reagent of Somogyi (1937) to an extent equivalent to 75 % of lactose. The study of the products of acid hydrolysis after NaIO oxidation showed that the reducing group of glucose is free. The time of half hydrolysis in 1N H_2SO_4 at 100° was 5 h 22 min while that of galactose-6-P was 10 h 52 min.

The experiments mentioned above with the products of hydrolysis of the new ester after 15 min and 24 h in 1N HCl showed that the phosphate group is attached to the galactose moiety of lactose. Of the various positions in this hexose in which the phosphate can be attached, that on C6 was discarded because L3'P is definitely more labile to acid hydrolysis than galactose-6-P and because the products of 3 hours acid hydrolysis failed to produce glycolyl aldehyde (Dische and Borenfreund, 1944) after periodation in conditions in which galactose-6-P produced it. Furthermore, in the

oxidation with Na periodate at pH 4.65, the intact ester consumed 2 moles of periodate and its galactose was not destroyed while lactose consumed 4 moles of periodate and its galactose was destroyed. These results are best explained by assuming that the phosphate group is attached to the C3 of galactose.

The amounts of L3'P found in cow colostrum are between 15 and 20 umoles per liter. Since we don't know of any other cell component in which the position 3 of galactose is esterified by a phosphoric group, no speculations can be made at the present time on the possible functions of L3'P. In relation to the trivial name of lactose-3'-phosphate, this was adopted because we can not find a simpler way to number the carbons in lactose than to number the C in glucose from 1 to 6 and those of galactose from 1' to 6'.

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