ISOLATION AND IDENTIFICATION OF TWO NOVEL URIDINE NUCLEOTIDE OLIGOSACCHARIDE CONJUGATES FROM HUMAN MILK

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During the ion exchange chromatographic study of the nucleotides of human milk and colostrum, two novel uridine nucleotides (UX1 and UX3) were detected (Kobata et al. 1962). A perchloric acid extract prepared from 20 litres of human milk was fractionated by gradient elution chromatography (Formic-Acid System of Hurlbert et al. 1954). UX1 was eluted just after GDP-mannose while UX3 a little before GDP-mannose. These nucleotides were subjected to a series of purifying procedure including rechromatography (C-2 System of Kobata et al. 1962), paper chromatography with ethanol-1M ammonium acetate, pH 7.5 (75:30). These steps resulted in the isolation for each nucleotides of a single nucleotide sugar conjugate free from ultraviolet-absorbing, carbohydrate-containing or amino acid-containing contaminants. The overall yield was about 12 μ moles of UX1 and 32 μ moles of UX3. There was no remarkable difference in their quantity between human milk and colostrum.

UX1 and UX3 had the characteristic uridine spectra both in pH 2.0 and in pH 12.0. The ratio of uridine to acid-labile phosphate and to total phosphate was approximately 1;1;2 for both nucleotide (Table 3). Their electrophoretic mobilities at pH 4.2 were less than UDPAG but greater than UMP (Table 1), while they had higher Rf values than UMP when chromatographed with ethanol-1M ammonium acetate, pH 7.5 (75:30).

When heated in 0.01N HCl for 20 min. at 100°, UX1 decomposed liberating approximately 1 mole each of UDP and a disaccharide, X1. UX3 liberated on

Table 1

Paper electrophoresis of UX1, UX3 and their fragments

Compounds*	Electrophoretic mobilities (cm)					
	pH 4.2**			pH 7.4***		
	from UX1	UX3	authentic compounds	from UX1	UX3	authentic compounds
UX1	+25.5	-	-	+12.4	-	-
UX3	-	+23.0	-	-	+11.2	-
UDP	+37.8	+37.8	+37.8	+19.0	+19.0	+19.1
UMP	+20.9	+20.9	+20.9	+16.9	+16.8	+16.9
X1-P	+16.3	-	-	-	-	-
X3-P	-	+14.2	-	-	-	-
UDPAG	-	-	+31.9	-	-	+13.6

* UV absorbing spots were located by irradiation with a 253.6 mμ mineral lamp. Phosphorylated compounds were detected with the molybdate reagent of Hanes et al. (1949).

** Estimated on Toyo Filter Paper No. 51A (54 cm long) in 0.1M acetate buffer at 65V/cm for 60 min.

*** Estimated on the same paper in 0.05M phosphate buffer at 40V/cm for 60 min.

addition to them, 1 mole of fucose by the same treatment. X1 was recovered by elution with water following paper chromatography with ethylacetate-pyridine-water (2:1:2). When heated in 1N HCl for 1 hour at 100°, the X1 was further decomposed into hexosamine and galactose. Degradation of the former with ninhydrin (Strominger et al. 1959) lead to the formation of arabinose as a sole product. When subjected to a milder treatment (in 0.05N HCl for 2 hours at 100°), the great part of the hexosamine was isolated as a N-acetyl derivative. This N-acetylhexosamine was subjected to modified Morgan-Elson color reaction (Reissig et al. 1955), and gave a color yield of $\epsilon 584 = 21400$ calculated on the basis of the reducing power (Park et al. 1949). Intact X1 was almost negative to the Morgan-Elson reaction. After treatment with $NaBH_4$, X1 was heated in 1N HCl for 2 hours at 100°, and the hydrolysate was examined paper chromatographically. Galactose was still detected as a reducing spot, but glucosamine completely dis-

Table 2

Paper chromatography of the sugar moieties and their fragments

Compounds*	R glucose values					
	Solvent 1**			Solvent 2***		
	from UX1	UX3	authentic compounds	from UX1	UX3	authentic compounds
Galactose	0.87	0.86	0.87	0.89	0.89	0.89
N-acetylglucosamine	1.86	1.86	1.88	1.20	1.19	1.21
Glucosamine	0.51	0.50	0.50	0.67	0.66	0.65
Fucose	-	2.22	2.22	-	1.20	1.20
X1	0.42	0.42	0.42****	0.90	0.90	0.89****

* Sugars were located with silver nitrate solution (Trevelyan et al. 1950) and with periodate-benzidine (Gordon et al. 1956).
** n-BuOH, water, EtOH (4:1:1).
*** Ethylacetate, pyridine, water (2:1:2). Toyo Filter Paper No. 51A (descending) was used.
**** Values of 4-β-O-D-galactopyranosyl-N-acetylglucosamine.

appeared. These results mentioned above demonstrated that X1 is identical with N-acetyllactosamine isolated from human milk by Kuhn et al. (1954). Then X1 was compared paper chromatographically with authentic galactopyranosyl-N-acetylglucosamines using n-BuOH, acetic acid, water (120:30:50) as a solvent. It was shown to be identical with 4-O-β-D-galactopyranosyl-N-acetylglucosamine. The spots corresponding to 6-O-β- and 3-O-β- derivatives were not detected.

Incubation of UX1 and UX3 with snake venom pyrophosphatase resulted in the liberation of UMP and a new phosphorylated disaccharide, X1-P and phosphorylated trisaccharide, X3-P respectively (Table 1). The UMP consumed one mole periodate, and by digestion with prostatic 5'-nucleotidase liberated uridine. UX1 and UX3 were analysed colorimetrically and found to contain approximately 1 mole each of N-acetylglucosamine and galactose, and N-acetylglucosamine, galactose and fucose per mole uridine respectively (Table 3). Both UX1 and UX3 were considerably stable to alkali treatment and quantitatively recovered without degradation after being heated in conc. NH_4OH for 5 min. at 100°. Intact UX1 and UX3 consumed 3.4

Table 3

The molar ratios for each of the constituents of the isolated compounds

Constituents	UX1	UX3	X1-P	X3-P	X1
Labile phosphate	0.96	1.01	1.00	1.00	-
Total phosphate	1.92	1.93	0.99	0.98	-
Glucosamine	1.03	0.93	1.01	1.04	0.90
Galactose	0.93	0.96	0.98	0.99	1.00
Fucose	0.00	0.83	0.00	0.79	-
Uridine	1.00	1.00	-	-	-

Each component was estimated by the method of the following authors. Phosphate; Chen et al. (1956), glucosamine; Rimington (1940), galactose; Dische et al. (1949), and fucose; Dische et al. (1948). Uridine was estimated on the basis of ultraviolet absorption ($\epsilon_{260} = 10.0 \times 10^3$).

moles and 4.4 moles periodate respectively, while after mild acid hydrolysis (in 0.01N HCl for 20 min. at 100°), 4.4 and 6.4 moles of periodate were consumed respectively.

Summarizing all of the data mentioned above, it is concluded that the chemical structure of UX1 is UDP-N-acetyllactosamine and of UX3 is UDP-N-acetyllactosamine-fucoside (Fig. 1).

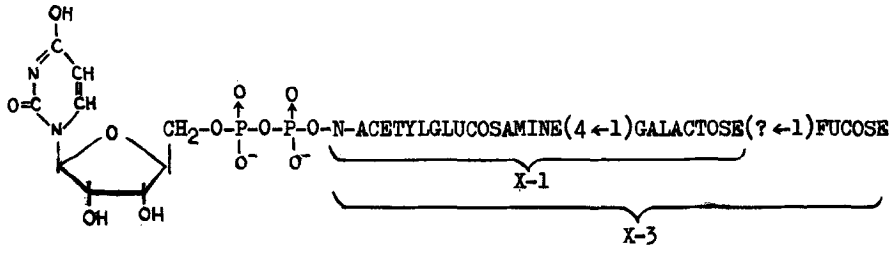

Fig. 1

The chemical composition of UX3.

Jourdian et al. recently isolated UDP-N-acetyllactosamine-sialic acid from goat colostrum (Jourdian et al. 196la, b). They also obtained UDP-N-acetyllactosamine as one of the sialidase digest of their UDP-trisaccharide. According to them, the disaccharide moiety is a mixture of 4-O-β-D-galactopyranosyl-N-acetylglucosamine and 6-O-β-D-galactopyranosyl-N-acetylglucosamine.

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