OCCURRENCE OF SOLUBLE GLYCOSYLTRANSFERASES IN HUMAN AMNIOTIC FLUID[1]

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Received October 1,1973

SUMMARY.

Human amniotic fluid obtained by amniocentesis during the third trimester of pregnancy was found to contain glycosyltransferases for the transfer of galactose, N-acetylgalactosamine, N-acetylglucosamine and sialic acid from their nucleotide derivatives to various exogenous protein and small molecular weight acceptors. The specific activity of the galactosyl- and N-acetylgalactosaminyl transferases was found to be 30 to 40 times higher in amniotic fluid as compared to serum. The specific activity of N-acetylglucosaminyl- and sialyl transferases was only 3 to 6 fold higher in amniotic fluid.

Coupled with our earlier findings on the occurrence of soluble glycosyltransferases in human and animal sera (1,2,3) and on the activity of galactosyltransferase during development (4,5), we now present evidence for the occurrence of four glycosyltransferases (for the transfer of galactose, N-acetylgalactosamine, N-acetylglucosamine and sialic acid to appropriate acceptors) in human amniotic fluid. It has been postulated that the presence of these glycosyltransferases in extra-cellular media may be important for cell-cell interactions during growth and differentiation (1,6). It is possible that the glycosyltransferases in amniotic fluid play a role in the normal development of the fetus. A preliminary account of this work has been presented (7).

METHODS.

Amniotic fluid and serum: Human amniotic fluid was a gift from Drs. P.G.R. Harding and F. Possmayer, Department of Obstetrics

[1]Supported by research grants from the Medical Research Council of Canada and the Ontario Heart Foundation.

and Gynaecology, St. Joseph's Hospital, London, Ontario. It was obtained by amniocentesis during the third trimester of pregnancy, then stored at -20°C until assayed. In rats the fluid was withdrawn from the amniotic sac with a syringe. Before assaying, the samples were spun at 1000 g for 5 minutes to remove cellular debris. Blood was obtained by venopuncture from humans and from the abdominal aorta of rats. The clotted blood was centrifuged for 15 minutes at 1000 g to obtain the serum.

Assay for glycosyltransferases: All enzyme assays were performed with 25 µl of amniotic fluid (25-150 µg protein) or 25 µl of serum (approximately 1 mg protein). The final reaction volume was 50 µl. Preliminary experiments established the linearity of the reaction with respect to time and protein concentration. Triton was not required in any of the enzyme assays.

A. Unless otherwise specified, the assay mixture for UDP-galactose: glycoprotein galactosyltransferase contained UDP-U-^{14}C galactose (0.01 µCi, specific activity, 4 mCi/mM) 2.5 nanomoles; 2-(N-morpholino) ethane sulfonic acid (MES-buffer), pH 6.8, 6.25 µmoles; $MnCl_2$, 0.625 µmoles and desialized degalactosylated fetuin, [DSG-fetuin (8)], as acceptor protein. After incubation for 1 hr at 37°C, the reaction was terminated and the radioactivity incorporated into acid-insoluble acceptor protein was counted as described previously (9). The modulatory effect of lactalbumin on the transfer of galactose to the monosaccharide acceptors N-acetylglucosamine and glucose by galactosyltransferase was also studied (10).

B. UDP-N-acetylgalactosamine: glycoprotein N-acetylgalactosaminyl transferase was assayed essentially according to Kim et al. (11). The incubation mixture contained 0.32 nanomoles of UDP-N-acetylgalactosamine-1-^{14}C (0.017 µCi; specific activity 51.5

mCi/mM). Desialized mucin (DS-mucin) was used as acceptor (12) and the concentration of buffer, divalent cation and the incubation procedure and the processing of the reaction products was the same as for the galactosyltransferase assay.

C. UDP-N-acetylglucosamine: glycoprotein N-acetylglucosaminyl transferase activity was measured as described by Mookerjea et al. (1). The incubation mixture contained 3 nanomoles (0.0125 µCi) of UDP-N-acetylglucosamine-1-^{14}C (specific activity 4.1 mCi/mM) with α-1-acid glycoprotein depleted of sialic acid, galactose and N-acetylglucosamine (DSGG-protein) as acceptor protein. The concentration of buffer and divalent cation and the incubation procedure and the processing of the reaction products was the same as for the galactosyltransferase assay.

D. CMP-N-acetylneuraminic acid: glycoprotein sialyltransferase was assayed essentially as described previously (3,13). The reaction mixture contained 15 nanomoles (0.01 µCi) of CMP-N-acetylneuraminic acid-4,5,6,7,8,9-^{14}C (specific activity, 6.56 mCi/mM). Desialized fetuin (DS-fetuin) was used as acceptor (13). Divalent cation was omitted but the same buffer and incubation conditions were used as for the above assays. The reaction was terminated with 20 µl of 0.5 M EDTA in 1% sodium tetraborate and subjected to high voltage electrophoresis at 3000 volts for 90 minutes. Radioactivity transferred to DS-fetuin was determined by liquid scintillation after preliminary scanning of the electrophoretograms (13).

All the results are expressed as nanomoles ^{14}C-sugar transferred to acceptor per mg protein of tissue fluid in 60 minutes. Protein was determined by the method of Lowry et al. (14) with bovine serum albumin as standard. All reagents were of commercial origin and the radioactive sugar-nucleotides were purchased from New England Nuclear, Dorval, Quebec.

Table 1. Glycosyltransferase activities in human amniotic fluid.

Acceptor	nmoles ^{14}C/mg protein/hr
A. Galactosyltransferase activity.	
None	0.26
+ Fetuin, 0.25 mg	1.56
0.50 mg	3.38
+ DSG-fetuin, 0.25 mg	12.36
0.50 mg	12.60
+ ovalbumin, 0.25 mg	4.40
0.50 mg	7.68
+ N-acetylglucosamine, 8 mM	7.80
+ N-acetylglucosamine(8 mM) + α-lactalbumin (0.4 mg)	4.84
+ Glucose, 8 mM	0.48
+ Glucose(8 mM) + α-lactalbumin (0.4 mg)	17.00
B. N-acetylgalactosaminyltransferase activity.	
None	0.008
+ Fetuin, 0.25 mg	0.059
0.50 mg	0.044
+ DSG-fetuin, 0.25 mg	0.034
0.50 mg	0.041
+ Sub-maxillary mucin, bovine, 0.25 mg	0.014
0.50 mg	0.060
+ DS-mucin, 0.25 mg	0.080
0.50 mg	0.084
C. N-acetylglucosaminyltransferase activity.	
None	0.19
+ DSGG-protein, 0.75 mg	1.39
1.50 mg	1.38
+ Fetuin, 0.25 mg	0.14
0.50 mg	0.36
+ Ovalbumin, 0.25 mg	0.66
0.50 mg	0.76
D. Sialyltransferase activity.	
None	0.11
+ Sub-maxillary mucin, bovine, 0.25 mg	0.23
0.50 mg	0.28
+ DS-mucin, 0.25 mg	1.54
0.50 mg	1.97
+ Fetuin, 0.25 mg	1.54
0.50 mg	3.95
+DS-fetuin, 0.25 mg	2.50
0.50 mg	2.80

Table 2.

The specific activity of glycosyltransferases in amniotic fluid compared to serum*

Sugar	galactose	galactosamine	glucosamine	sialic acid
Acceptor (0.25 mg)	DSG-fetuin	DS-mucin	DSGG-protein	DS-fetuin
	nanomoles ^{14}C-sugar transferred/mg protein/hr.			
Human				
Serum, adult	0.45	0.004	0.48	0.465
Amniotic fluid	15.0	0.111	1.39	2.94
Rat				
Serum, adult	0.71	0.003	-	0.57
Serum, mother	1.00	0.005	-	0.41
Serum, newborn	1.40	0.011	-	0.51
Amniotic fluid	29.8	0.097	-	2.93

* Values are average from 2 to 12 samples, except for galactose- and N-acetylgalactosaminyltransferase in human amniotic fluid which are average of 40-60 samples.

RESULTS.

Results presented in Table 1 clearly establish the occurrence of soluble glycosyltransferases for the transfer of galactose, N-acetylgalactosamine, N-acetylglucosamine and sialic acid in human amniotic fluid. The enzymes from amniotic fluid were stable to storage at -20°C for at least two months. The table also compares the activity of the different enzymes towards various native and deglycosylated protein and non-protein acceptors. Native ovalbumin which has only mannose and N-acetylglucosamine as its oligosaccharide components is an active acceptor for galactosyltransferase. The addition of α-lactalbumin in the

assay caused the appearance of lactose synthetase activity and inhibition of N-acetyllactosamine synthetase activity, a pattern which is obtained for the galactosyltransferase enzyme in other tissues and serum (10). For N-acetylgalactosaminyltransferase, bovine sub-maxillary mucin, both native and sialic acid depleted, was most active for this sugar (15). DSGG-protein was an excellent acceptor for N-acetylglucosaminyltransferase, as it is for the serum and membrane-bound enzyme (1,2). Sialyltransferase was most active towards desialylated fetuin (13). Its almost equal activity towards native fetuin may be due to the fact that the commercial preparation of fetuin was shown upon analysis (16) to be already 50-70% depleted of its sialic acid. Results in Table 2 show that the specific activity of galactosyl- and N-acetylgalactosaminyl transferases of both human and rat amniotic fluid was 30 to 40-fold higher as compared to adult serum. Similarly, sialyl- and N-acetylglucosaminyl transferase activities were found to be 3 to 6-fold higher in amniotic fluid. In the rat it was possible to assay the serum of the newborn as well as that of the mother. Galactosyl- and N-acetylgalactosaminyltransferases were only slightly higher in these sera.

DISCUSSION.

Since the discovery of soluble N-acetylglucosaminyltransferase in human sera (1), a number of publications have appeared showing the presence of various other glycosyltransferases in human as well as animal sera. As in serum, the physiological significance of the occurrence of the glycosyltransferases in soluble form in amniotic fluid is unknown. The occurrence of soluble glycosyltransferases in fluid surrounding embryonic chicken brain and the variations in the level of some of these enzymes with gestational age has raised the possibility of a role

for these enzymes in the development of the brain (6). It is possible that the glycosyltransferases in amniotic fluid play a similar role in the maturation of the fetus.

It is striking that the specific activities of both galactosyl- and N-acetylgalactosaminyltransferases are very high in human and rat amniotic fluid. The difference in protein concentration between serum and amniotic fluid is not enough to account for this high specific activity. Mother's serum does not immediately indicate itself as the source of the amniotic fluid enzyme either, but this does not rule out a specific concentrating effect by the fetal-placental unit. The only other enzyme shown so far to be elevated in human amniotic fluid is alkaline phosphatase (17). Its specific activity increases 35-fold by the seventh month of pregnancy, then remains constant until term. It is believed that this activity is due to an isoenzyme of alkaline phosphatase produced by the fetus (18). Similarly serum proteins specific to the fetus have been detected in mouse amniotic fluid (19). There is a possibility therefore that glycosyltransferases in amniotic fluid are produced by the fetus. However only further research will determine the significance and the source of these enzymes in amniotic fluid.

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