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Short Communications

Sialyltransferase: Regulation of α-Foetoprotein Microheterogeneity during Development

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The temporal accumulation of the electrophoretic components of mouse α-foetoprotein in foetal plasma and amniotic fluid is reported. To explain the progressive appearance of the sialylated α-foetoproteins, the activity of sialyltransferase in foetal liver and yolk sac was measured. These results indicate that the increase in sialyltransferase activity in these tissues is responsible for the increased sialylation of α-foetoprotein.

Several glycoproteins have been shown to exhibit microheterogeneity due to variations in the type and amount of sialic acid residues attached to the glycoprotein (Beckman & Beckman, 1967; Robinson & Stirling, 1968; Oshiro & Eylar, 1968; Albert *et al.*, 1972). In order to understand regulation of glycoprotein synthesis, we have been studying the temporal changes in microheterogeneity of mouse α-foetoprotein during development. α-Foetoprotein is present in both foetal plasma and amniotic fluid and exhibits up to five electrophoretic components when subjected to polyacrylamide-gel electrophoresis (Gustine & Zimmerman, 1972, 1973). These components have been designated Fp1, Fp2, Fp3, Fp4 and Fp5 on the basis of their increasing anodic mobility at pH 8.4. On day 12.5 of gestation only components Fp1, Fp2 and Fp3 are present in amniotic fluid. Subsequently there is a progressive appearance of the faster-moving components in both amniotic fluid and foetal plasma. By day 15.5 all five electrophoretic forms are present and by day 18.5 only component Fp5 is found in foetal plasma. The difference between these electrophoretic forms is attributed to both a variation in the amount as well as the type of linkage of sialic acid attached to the glycoprotein. Thus component Fp1 contains no sialic acid, components Fp2, Fp3 and Fp4 contain one residue each that could differ from the others by positional isomerism, and component Fp5 contains two residues (E. F. Zimmerman, D. Bowen, J. Wilson & M. Madappally, unpublished work).

Since the microheterogeneity of α-foetoprotein results, in part, from differences in the amount of sialic acid present, it is possible that during early development the embryo has a limited ability to sialylate α-foetoprotein due to a low activity of sialyltransferase. If this assumption is correct one would expect a marked increase in the activity of sialyltransferase with time of development in tissues where synthesis of α-foetoprotein occurs.

Materials and methods

Foetal plasma and amniotic fluid were collected from C3H/An mice and α-foetoproteins were separated by polyacrylamide-gel electrophoresis as previously described (Gustine & Zimmerman, 1973), except for the following modifications. Samples (150 μg of protein each) were subjected to electrophoresis at 4°C on 5 mm × 160 mm gels. The gels were run for 2 h at 3.5 mA/gel and then an additional 2 h at 2.6 mA/gel. The stained gels were measured densitometrically at 600 nm and the proportion of each α-foetoprotein on a gel was determined by integration of the peaks.

For the assay of sialyltransferase activity, the following procedures were employed. Pregnant mice from day 12.5 to day 18.5 of gestation were killed and each uterus was removed. Foetal liver and yolk sac were separated from several embryos and pooled to make a 10% (w/v) homogenate in 20 mM-Tris–HCl buffer, pH 7, containing 1 mM-GSH and 0.25 M-sucrose. The tissue homogenates were successively fractionated by centrifugation at 600 *g* for 10 min and at 30000 *g* for 30 min at 4°C. The pellet fractions were resuspended with one stroke in a Dounce homogenizer and sialyltransferase activity was determined in each of these fractions.

Fetuin was purified from calf serum by the method of Spiro (1960). The purified calf fetuin was desialylated by incubation with *Vibrio cholerae* neuraminidase for 24 h (Spiro, 1962) and separated on a Bio-Gel P-30 column (1.4 cm × 6 cm). The desialylated calf fetuin in the void volume was precipitated with ethanol (80%, v/v) at −20°C overnight and the precipitate was dissolved in 50 mM-sodium acetate buffer, pH 5.6.

Sialyltransferase activity was assayed essentially by the method of Kim *et al.* (1971). The reaction mixture contained 66 μmol of cacodylate–acetate buffer, pH 6.8, 0.5 mg of Tergitol, 200 μg of desialylated calf fetuin as acceptor protein, 110 pmol (5×10^4 d.p.m.) of CMP-[^{14}C]sialic acid and 50 μl of enzyme preparation

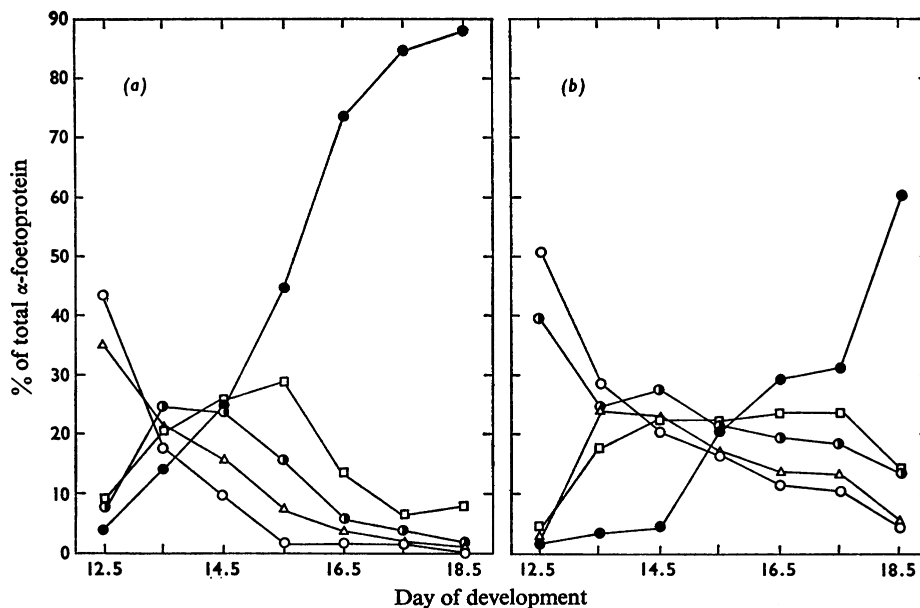

Fig. 1. *Proportion of α-foetoproteins in mouse foetal plasma (a) and amniotic fluid (b) with time of development*

Determination of the percentage of each α-foetoprotein (○, Fp1; △, Fp2; ◑, Fp3; □, Fp4; ●, Fp5) by polyacrylamide-gel electrophoresis is described in the text under 'Materials and methods'. Foetal plasma could not be obtained at day 12.5 of gestation. Since the densitometric pattern of the 30000*g* supernatant (30min) of the total embryo (minus liver) was similar to that of foetal plasma on days 13.5 and 14.5, the proportions of the α-foetoproteins on day 12.5 were estimated from total embryo (minus liver). The concentration of total α-foetoprotein did not markedly change in foetal plasma and amniotic fluid during mouse development (Gustine & Zimmerman, 1972, 1973; E. F. Zimmerman, A. Neuer & D. Bowen, unpublished work). Therefore the relative proportion of each α-foetoprotein reflects the relative concentration of each.

(approx. 290 μg of protein) in a final volume of 0.33 ml. Duplicates were incubated at 37°C for 2h and the reaction was stopped with 5 ml of 1% (w/v) phosphotungstic acid in 0.5 M-HCl. The precipitate was washed three times with 1% phosphotungstic acid and dissolved in 1 ml of Soluene, and the radioactivity was determined with the use of 10 ml of liquid scintillator [4g of 2,5-diphenyloxazole and 100mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene/l of toluene].

Results and discussion

Since the sialylation of α-foetoproteins in amniotic fluid lags behind that in foetal plasma (Gustine & Zimmerman, 1972), the proportion of each α-foetoprotein component was measured in both compartments during the latter stages of gestation. Fig. 1 shows the proportions of components Fp1, Fp2, Fp3, Fp4 and Fp5 in foetal plasma and in amniotic fluid with time of development. In foetal plasma a progressive decrease in the relative concentrations of components Fp1 and Fp2 is seen from day 12.5, whereas the proportions of components Fp3 and Fp4 first rise and then fall. Finally, a striking increase in the fully sialylated component Fp5 is observed throughout the period examined, until at day 81.5 it represents virtually all of the plasma α-foetoprotein. Amniotic fluid showed similar types of fluctuations in the relative proportions of the five components of α-foetoprotein: the non-sialylated component Fp1 decreased with time, the monosialylated components Fp2, Fp3 and Fp4 decreased after day 13.5 and the disialylated component Fp5 increased continuously through to day 18.5. However, as previously observed (Gustine & Zimmerman, 1972), the sialylation of component Fp5 in amniotic fluid lags behind that in foetal plasma. For example, component Fp5 comprises 45 and 85% of the α-foetoprotein in plasma and only 22 and 32% in amniotic fluid at days 15.5 and 17.5 respectively.

Sialyltransferase activity was measured in foetal tissue during development to explain the temporal sialylation of α-foetoprotein. Enzyme activities in liver and yolk sac were measured since both tissues synthesize α-foetoprotein (Abelev, 1971; E. F.

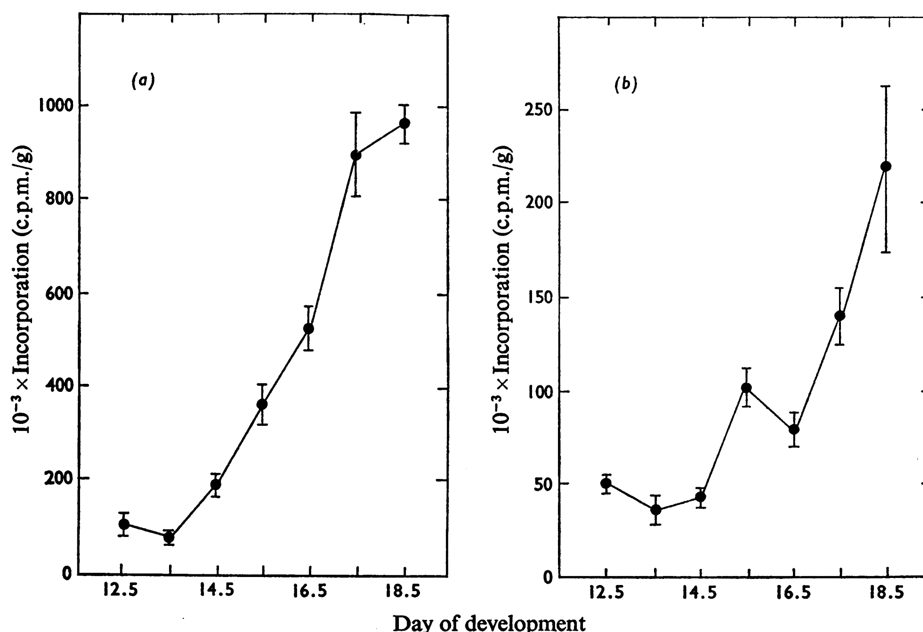

Fig. 2. *Sialyltransferase activity in mouse foetal liver (a) and yolk sac (b) with time of development*

Although the enzyme activity was primarily located in the 30000g pellet, the distribution of enzyme activity in each fraction varied slightly with time of development. Therefore the activities in all fractions were combined and the results are expressed as c.p.m. incorporated/g wet wt. of tissue during 2h incubation. Six to eight experiments were performed to obtain average ±S.E.M. values for sialyltransferase on any day of development. Sialyltransferase measured is essentially activity incorporating sialic acid into exogenous calf fetuin (M. Madappally & E. F. Zimmerman, unpublished work).

Zimmerman, A. Neuer & D. Bowen, unpublished work). Fig. 2 shows that the enzyme activity progressively increased in liver (12-fold) and yolk sac (6-fold) during the period examined. However, the increase in liver started earlier, increasing rapidly at day 13.5; the activity in yolk sac lagged behind that in liver, starting to increase significantly only at day 14.5. Further, the enzyme specific activity in liver was about 4-fold greater than that in yolk sac at day 18.5.

Comparison of the data in Figs. 1 and 2 reveals that the increase in the enzyme activity in liver is virtually identical with the proportion of component Fp5 in plasma, whereas that of yolk sac resembled that of amniotic fluid. Sialyltransferase activity was also measured in the total embryo (minus liver) and placenta at each time of gestation. Neither of these tissues synthesizes α-foetoprotein. Enzyme activity in the embryo (minus liver) did not significantly change. In contrast, placenta showed a 2.9-fold increase in sialyltransferase activity from day 13.5 to 17.5 (results not presented). The latter result suggests that the increased sialyltransferase activity may not necessarily be specific for regulating sialylation of α-foetoprotein. Changes in sialyltransferase activity with age have also been reported in other species (Hudgin & Schacter, 1972).

These results indicate that the increase in sialyltransferase in tissues that synthesize α-foetoprotein correlates well with the increased sialylation of α-foetoprotein. Conversely, in comparing transformed with normal 3T3 mouse cells, Grimes (1970) has suggested that the decrease in sialyltransferase was responsible for the decreased sialic acid content of the transformed cells. These observations are consistent with the postulate that there is a post-translational control of glycoprotein synthesis at the level of carbohydrate attachment. The developmental changes in activity of sialyltransferase reported in the present paper could represent an increase in synthesis or activation of the enzyme. Either effect could be mediated by hormonal changes during development.

In addition, the developmental microheterogeneity of α-foetoprotein in amniotic fluid lagged behind that in foetal plasma (Fig. 1). Fig. 2 indicates that the increase in sialyltransferase activity of yolk sac also lags behind that of foetal liver. This result could mean that

a major source of α-foetoprotein from foetal plasma is derived from foetal liver, whereas that in amniotic fluid comes from yolk sac.

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