THE CONVERSION OF MANNOSE-^{3}H TO PROTEIN BOUND SIALIC ACID, FUCOSE, GALACTOSE AND HEXOSAMINES IN RAT SERUM

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SUMMARY

After a single intravenous injection of mannose-^{3}H, radioactivity was recovered in all major sugar components released by acid hydrolysis of rat serum glycoproteins including sialic acid, fucose, mannose, galactose and hexosamine. The specific activity(DPM/μmol) of fucose and sialic acid was much higher than mannose within the first 5-10 minutes. After 5 minutes most of the label was recovered as sialic acid (83%), an appreciable amount in fucose, galactose and hexosamine, and only 3.2% in mannose. The amount of radioactivity was never less than 64% in sialic acid and never more than 14% in mannose.

Mannose is one of the major sugars released by acid hydrolysis of glycoproteins. The others are fucose, galactose and glucosamine. Most biochemical studies of glycoprotein biosynthesis have used D-glucosamine as precursor (1,2,3) while autoradiographic studies, fucose and galactose have been selected as the best markers (4,5,12,13). The incorporation of mannose was followed in one study of the rat thyroid gland where, by autoradiography, it was reported to be assembled into thyroglobulin mainly as mannose at the rough endoplasmic reticulum (4).

Recently the incorporation of four major sugars, fucose, galactose, glucosamin and mannose was investigated in glycoprotein in the Golgi complex isolated from rat liver and in serum (6). Whereas the overall pattern of incorporation of fucose galactose and glucosamine were similar, mannose presented a complex incorporation with early maximum specific activity in glycoprotein of the Golgi complex followed by a level of approximately two-thirds maximum activity maintained for long periods after injection. Since the early incorporation occurred at a similar time interval

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to that of fucose, it seemed likely that mannose was converted to other sugars such as sialic acid or fucose; these would be assembled rapidly into the glycoprotein without delay before addition of the other sugars. In this study, the incorporation of mannose has been investigated further in rat serum glycoprotein to demonstrate its conversion to other sugars including sialic acid, fucose, galactose and hexosamine.

MATERIALS AND METHODS

Male Wistar rats weighing 200-220 g, fasted 15 h, were used to study the incorporation of D-mannose-2-T (specific activity 1.0 C/μmol). A single intravenous injection of 5 μC radioactive material was given to each rat via the jugular vein. At different time intervals, 5,10,15,30,60,90 and 120 minutes after injection, each rat was anesthetized with ether, exsanguinated and a sample of blood collected. The incorporation of mannose was studied separately in at least 3 rats at each time interval.

Each sample of serum was precipitated by adding 0.5 ml of 10% TCA in the cold to 0.25 ml of serum diluted with 0.25 ml water. The precipitate was centrifuged, washed twice with cold chloroform/methanol/ether (2:1:1), centrifuged and the supernatants discarded. The pellet was hydrolysed with 4 ml of 0.1 N H_2SO_4 at 80° for 60 minutes to remove sialic acid. After centrifuging, the supernatant was removed (supernatant I).

The remaining pellet was hydrolysed with 3 ml of 2 N H_2SO_4 in a glass stoppered tube at 100° for 5 h. After centrifuging,the supernatant was removed (supernatant II) Both supernatants I and II were neutralized by the addition of 0.3 N $Ba(OH)_2$. Insoluble barium sulfate was removed by centrifuging and the supernatants were concentrated by drying in a rotary evaporator.

A portion of supernatant I was used to measure sialic acid by the method of Svennerholm (9) and a second portion was used for counting in a Nuclear Chicago Mark I scintillation counter.

Supernatant II was separated on Dowex-50 into hexosamine and neutral sugar fractions by the method of Boas (10). Hexosamine was measured by the method of Boas as modified by Johnson (7). The neutral sugar fraction which did not adsorb to the

resin was evaporated to dryness and the components were separated by paper chromatography on Whatman #4 paper in n-butanol/pyridine/water (50:30:20) for 17 h at room temperature. Individual sugars were located by comparison with appropriate markers which were run down both sides of each chromatogram; these showed the order of migration from the origin was galactose, mannose and then fucose. The spots corresponding to galactose, mannose and fucose, were cut from the paper and eluted with water. Aliquots of each eluate were used to assay reducing sugar by the method of Guinn (8) and for radioactive counting.

RESULTS

After injection of D-mannose-^{3}H, radioactive label was recovered in each of the major carbohydrate components of serum glycoprotein, including sialic acid, fucose, galactose hexosamine, as well as mannose. The variation in specific activit

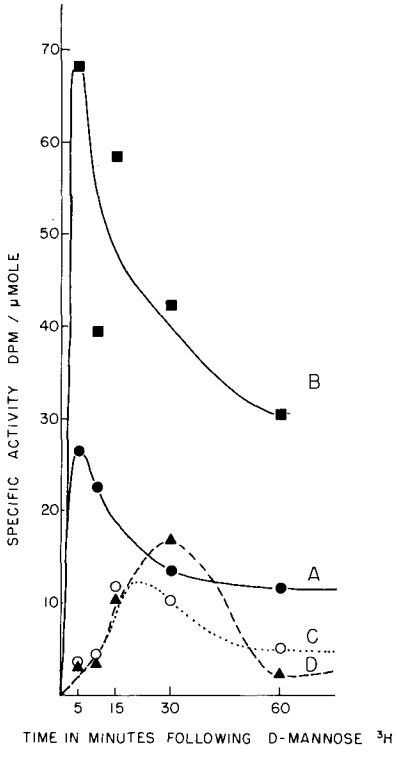

FIGURE 1

The change in specific activity (DPM/µmol sugar) various times from 5-60 minutes following a single intravenous injection of D-mannose-^{3}H. Curve A, sialic acid; B, fucose; C, mannose and D, galactose.

was calculated as DPM/μmol for each sugar and the findings are illustrated in Fig.1.

The change of specific activity of sialic acid (DPM/μmol N-acetylneuraminic acid) at various intervals after injection of D-mannose is shown in Fig. 1 (curve A). The specific activity increased rapidly and reached the maximum level after 5 minutes and then fell to approximately half the maximum by 30 minutes. In comparison with other sugars, the specific activity was higher than that of either galactose or mannose but lower than that of fucose.

The change of specific activity for the neutral sugars, fucose, mannose and galactose, (DPM/μmol of hexose) with time after a single intravenous injection of 5 μC of mannose is shown in Fig. 1 (curves B,C and D respectively). The specific activity of fucose (curve B) rose sharply to a maximum within 5 minutes of D-mannose injection and then fell rapidly. This followed a similar pattern of incorporation as sialic acid except that the specific activity was mugh higher for fucose. The specific activity of mannose (curve C) increased slowly and reached the maximum at 15-20 minutes. It was lower than any sugar, the maximum being only one-tenth of that in fucose. The specific activity of galactose (curve D) was higher than mannose and occurred later at 30 minutes.

Since a single injection of D-mannose resulted in the appearance of radioactivity in all other major sugar fractions, the extent of conversion of D-mannose was determined by calculating the radioactivity of each sugar as a percentage of the total radioactivity recovered as carbohydrate. The findings are summarized in Table I. At any time after mannose injection, the radioactivity recovered as protein-bound mannose represented a small amount of the total radioactivity (<14%). After 5 minutes most of the label was found in sialic acid (83%) indicating a very rapid conversion of mannose. A significant amount of radioactivity was recovered as fucose (4-16%) with slightly less in the hexosamine fraction (3-11%) and galactose (2-10%).

DISCUSSION

After a single intravenous injection of D-mannose-^{3}H, isotope was distributed among the major sugars in serum glycoproteins: mannose, galactose, fucose, hexo-

TABLE I

Time after 5 μC mannose-^{3}H	TOTAL DPM	% OF TOTAL DPM				
	Mannose+Galactose+Fucose +Hexosamine+Sialic acid	Mannose	Galactose	Fucose	Hexosamine	Sialic acid
5	10,649	3.2	1.8	8.0	3.1	83
10	9,372	5.4	3.5	3.6	2.8	84
15	8,435	5.3	5.3	7.3	4.6	78
30	6,876	13.6	10.2	6.0	3.9	66
60	6,595	13.4	7.8	6.7	6.6	66
90	6,106	7.6	5.3	16.0	6.9	64
120	5,459	5.8	6.2	11.3	11.0	65

samine and sialic acid. Of these, by far the largest amount was recovered as sialic acid, released after mild acid hydrolysis of the serum proteins.

D-mannose is readily converted to D-mannose-6-phosphate by nonspecific hexokinases. In the presence of phosphomannosiomerase, D-mannose-6-phosphate is converted to D-fructose-6-phosphate, which represents the metabolic origin of most of the hexosamines. Sialic acid can then be formed through a series of metabolic reactions, involving the condensation of N-acetylmannosamine-6-phosphate with phosphoenolpyruvate.

The conversion of D-glucosamine-1-^{14}C to a variety of acid-soluble products has been studied in rat liver by DelGuiacco and Mahley (11) who detected labeled sialic acid in the acid-soluble pool within 15 seconds. On the basis of specific activity data they concluded that the sialic acid was derived from UDP-N-acetylglucosamine.

In our experiments the majority of the radioactivity was recovered as sialic acid but the other terminal sugar, fucose, also contained an appreciable amount of radioactive label. A mechanism for the conversion of mannose to fucose is well established via GDP-mannose, in a sequence of three reactions to GDP-fucose which would facilitate rapid conversion of radioactive label to this carbohydrate.

The radioactivity found in galactose probably arose by conversion of mannose to glucose. From glucose the label would be expected to be dissipated in a number of reactions, one of which would terminate as galactose.

The distribution of radioactivity reported here clearly showed that 14% or less of the radioactivity injected as mannose could be recovered as mannose from rat serum glycoprotein. A different situation was reported by Whur et al (4) for rat thyroglobulin where, in autoradiographic studies, they failed to detect silver grains over the Golgi apparatus of rat thyroid gland early after injection of mannose. They concluded that mannose was added to the glycoprotein in the rough ER, an observation consistent with the proximity of this sugar to the peptide core of the molecule. Furthermore, the same authors reported that 75% of the radioactivity in thyroid proteins was present as mannose although no data was given. However, the high background in mannose autoradiographs by these authors may be attributed at

least in part, to the various sites of incorporation of metabolic products of mannose. The findings presented here emphasize the importance of understanding the interconversion of sugars and their assembly into glycoprotein of each cell type.

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