THE INCORPORATION OF [^{14}C] D-GALACTOSE AND [^{3}H] D-MANNOSE INTO GOLGI FRACTIONS OF RAT LIVER AND INTO SERUM

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1. Introduction

Plasma glycoproteins are known to contain four principal sugars, L-fucose, D-galactose, D-mannose and D-glucosamine as part of the carbohydrate chain. Whereas the polypeptide portion is synthesized in the rough endoplasmic reticulum, the sugar units are believed to be added mainly in the Golgi complex. The evidence that the Golgi complex is the main subcellular site of attachment of carbohydrate rests on two types of observations. Firstly in autoradiographic studies, the early appearance of silver grains over the Golgi complex has implicated these membranes as the site of assembly of certain carbohydrates such as fucose and galactose [1–4], whereas mannose [3] and glucosamine [5, 6] were reported added in the rough endoplasmic reticulum. Secondly, the presence of glycosyl transferases in isolated Golgi fractions [13], implicates these membranes in glycoprotein synthesis.

Both autoradiographic and biochemical data in this laboratory have confirmed that fucose is added to glycoprotein mainly in the Golgi membrane system in rat liver [7]. Good precursor–product relationships were obtained between perchloric acid-soluble material of the Golgi apparatus and serum, indicating a flow of material from the Golgi membrane system to the plasma. Although autoradiographic studies are not available for galactose incorporation, the biochemical data reported here for galactose was similar to that of fucose. With mannose, the findings could not be interpreted in a simple fashion. In an earlier study we reported that radioactivity could be recovered from all major sugar components following a single intravenous injection of [^{3}H]D-mannose [8]. The incorporation of mannose can be explained on the basis of rather extensive conversion of mannose to sialic acid, fucose, galactose and glucosamine.

2. Materials and methods

Male Wistar rats weighing 200–220 g, fasted 15 hr, were used to study the incorporation of the two sugars: [1-^{14}C]D-galactose (specific activity 55.7 mCi/mM) and D-mannose-2-T (specific activity 1.0 Ci/mM) which were obtained from Amersham-Searle. A single injection of 5 μCi of each radioactive material was given to each rat via the jugular vein. At intervals of 5, 10, 15, 30, 60, 90 and 120 min after injection, rats were anaesthetized with ether, exsanguinated and the liver removed. The incorporation of each sugar was studied in at least 3 rats at each time interval.

Golgi membranes were isolated separately from each rat liver using a modification of the method described by Morré et al. [9]. The details of the procedure are described elsewhere [10].

Rat serum and Golgi membranes isolated from each liver were separated into perchloric acid-soluble (PCA) and -insoluble fractions. The pelleted Golgi preparations were extracted with cold 0.6 N PCA, and the serum with an equal volume of 1.2 N PCA. After addition of acid, each sample was centrifuged at 1800 rpm for 20 min at 4°. The perchloric acid insoluble material was extracted with chloroform/methanol/ether (2:1:1), centrifuged, the supernatant discarded and the pellet dried and dissolved in 0.5 N sodium hydroxide.

The perchloric acid-soluble portion (seromucoid) was precipitated with 1/5 volume of 5% phosphotungstic acid in 2 N hydrochloric acid. After standing in ice

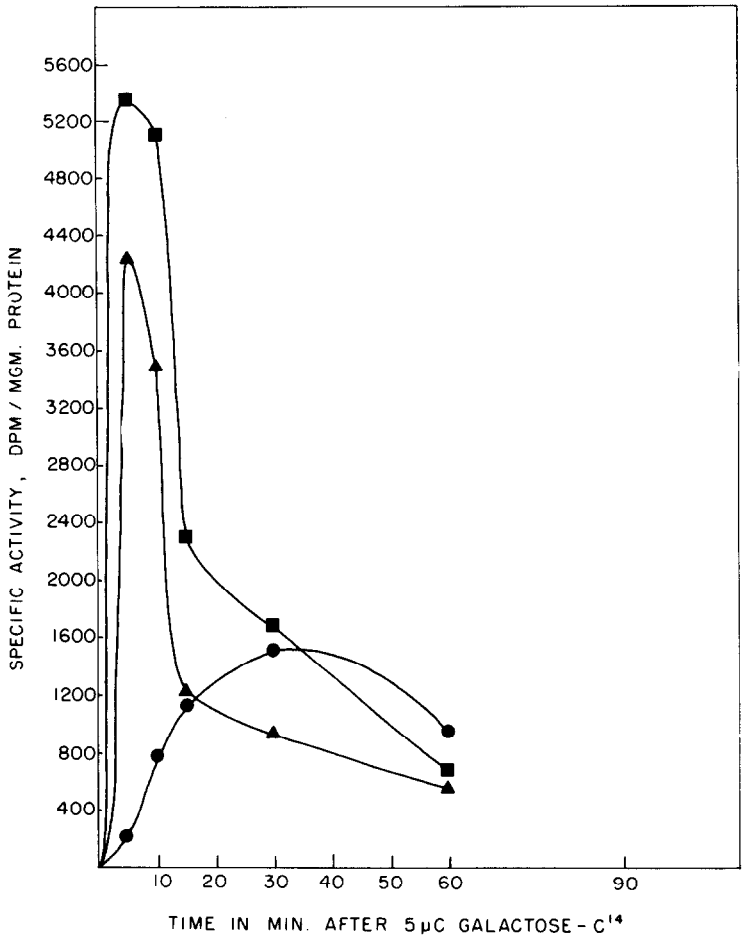

Fig. 1. The change in specific activity in the perchloric acid-soluble (■——■——■) and perchloric acid-insoluble (▲——▲——▲)fractions of Golgi isolated from rat liver and in the perchloric acid-soluble fraction of serum (●——●——●) after a single intravenous injection of [^{14}C] D-galactose.

for 20 min, the precipitate was centrifuged, washed with 5% TCA and centrifuged again. The pellet was extracted with chloroform/methanol/ether, the soluble fraction removed and the pellet was dried and dissolved in 0.5 N sodium hydroxide.

For each fraction, protein was determined in one aliquot by the method of Lowry [11]; another aliquot was used for counting in a Nuclear Chicago Mark I scintillation counter. For counting, 100 μl fraction was dissolved in 1.3 ml of hyamine hydroxide and 10 ml of toluene scintillation fluid (0.4% PPO and 0.03% POPOP) was added. The results were expressed as specific activity: dpm/mg protein.

3. Results

The incorporation of [^{14}C] D-galactose and D-mannose-2-T was followed separately in serum and Golgi fractions from each rat. The changes in specific activity (dpm/mg protein) with time for the 2 sugars, is shown in figs. 1 and 2. The three curves represent the perchloric acid-insoluble and perchloric acid-soluble fraction of serum (serum seromucoid). The points on each curve represent the mean for 3 animals.

3.1. *D-Galactose*

The change in specific activity in Golgi and in serum

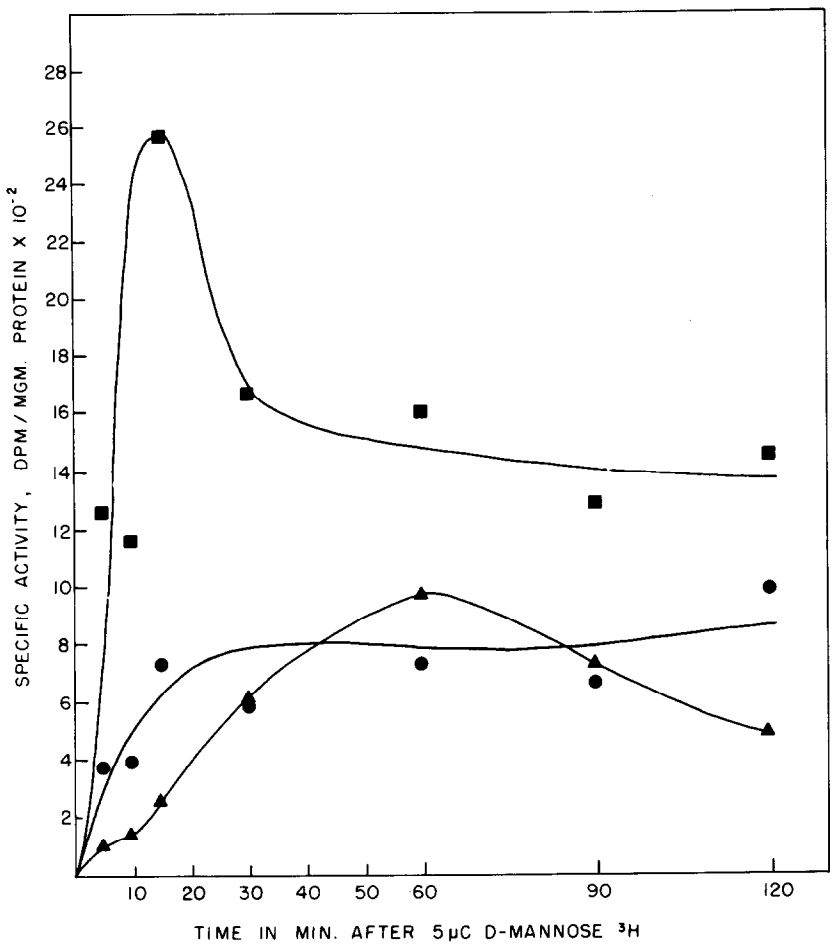

Fig. 2. The change in specific activity in the perchloric acid-soluble (■——■——■) and perchloric acid-insoluble (▲——▲——▲) fractions of Golgi isolated from rat liver and in the perchloric acid-soluble fraction of serum (●——●——●) after a single intravenous injection of D-mannose-2-T.

fractions at different times after injection of [1-^{14}C] D-galactose is shown in fig. 1. Five minutes after injection of the label, maximum specific activity was observed in both perchloric acid-soluble and-insoluble fractions of Golgi. The curves rose and fell sharply. The specific activity of the serum seromucoid increased to a maximum at 30 min and then decreased steadily.

3.2. *D-Mannose*

The change in specific activity in Golgi and serum fractions at different times after the intravenous injection of [^{3}H]D-mannose is shown in fig.2. The maximum specific activity of the perchloric acid-soluble fraction occurred at 15 min after injection, fell to two-thirds the maximum level at 30 min, and then decreased only slightly even 120 min after injection of label. The specific activity of the perchloric acid-insoluble fraction rose slowly for the first 20 min, reached a maximum at 60 min and then decreased slowly. The specific activity of the serum seromucoid rose rapidly to a maximum at about 20 min and remained at this level between 20–120 min.

4. Discussion

The incorporation of radioactive label from [^{14}C]D-galactose and [^{3}H]D-mannose was considerably different in Golgi fractions and in serum glycoprotein. The time of appearance of maximum specif-

ic activity was earlier with galactose than with mannose and the pattern of the incorporation curves for each sugar was significantly different.

4.1. *D-Galactose*

The pattern of D-galactose incorporation into the Golgi fractions and serum was similar to that found for L-fucose [7]. The occurrence of maximum specific activity in the perchloric acid insoluble fraction, slightly before the maximum in the soluble fraction (15 min) suggested that D-galactose was first bound to Golgi membranes and then appeared in the glycoprotein (perchloric acid-soluble) fraction of Golgi before release into serum.

The incorporation into the Golgi complex of the liver cell occurred within a similar time interval to that described in autoradiographic studies for other cell types. In comparison with maximum incorporation into the Golgi fraction at 8–10 min, in this study D-galactose was reported to reach the Golgi apparatus within 5 min in the thyroid gland [4] and within 7–8 min in the chick embryo gonads [12].

With D-galactose, maximum specific activity in the Golgi fraction was seen at a similar time interval to that found with L-fucose. This finding reflects the close proximity of L-fucose and D-galactose in the glycoprotein molecule in which galactose is thought to occupy the subterminal and fucose the terminal position of the oligosaccharide side chain.

4.2. *D-Mannose*

The incorporation of D-mannose into the Golgi fractions was different to that found for galactose and for other sugars [6,7] since the specific activity rose first in the soluble fraction of Golgi and, after an initial peak, was maintained at a high level even 120 min after injection of the label. In the perchloric acid insoluble fraction, D-mannose was incorporated over a wide time interval. These findings resemble the pattern described for D-glucosamine in which the specific activity remained high for a long time due to incorporation early as sialic acid and later as D-glucosamine.

Previous autoradiographic studies have implicated the rough endoplasmic reticulum as the site of mannose incorporation in the thyroid gland [4], which was in agreement with the location of D-mannose close to the peptide core of the glycoprotein. However, in this study, the early appearance of radioactive label in the Golgi fractions suggested some assembly on the Golgi membranes. This evidence was supported by the early rise in specific activity of serum glycoprotein. In a recent report [8] we have shown that high levels of radioactivity can be recovered from serum glycoprotein in sialic acid, fucose, galactose, glucosamine and mannose after a single intravenous injection of [^{3}H]D-mannose. This implied that mannose may be converted into other sugars which are assembled in the Golgi apparatus at different sites in the glycoprotein molecule. The early appearance of radioactivity in the Golgi fractions probably represents incorporation of label at more terminal sites in the oligosaccharide chain as sialic acid, fucose and galactose, after conversion from [^{3}H]mannose. The high specific activity of the Golgi fractions and, in parallel, in serum glycoprotein seromucoid even 120 min after injection, showed that incorporation of radioactive label continued at a different site to that of the earlier incorporation. Since D-mannose has been reported to occupy a position close to the peptide core, the delayed appearance of radioactive label in the seromucoid fractions of Golgi and serum would reflect incorporation as D-mannose and also by conversion of D-glucosamine. Therefore it seemed that the different pattern for mannose probably reflected incorporation curves for more than one sugar at more than one membrane site.

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