THE INCORPORATION OF L-[^{14}C]FUCOSE INTO GLYCOPROTEIN FRACTIONS OF LIVER PLASMA MEMBRANES

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1. Introduction

It is generally held that in the liver [1], as well as other secretory organs [2], proteins are synthesized on membrane-bound ribosomes, pass through the endoplasmic reticulum to the Golgi apparatus where they are glycosylated and are secreted from the cells. Hence, the study of the biosynthesis of plasma membrane glycoproteins of such cells is complicated by the simultaneous synthesis and liberation from the cell surface of secretory glycoprotein. In an attempt to differentiate the two, we have focused upon that stage in the biosynthesis between the Golgi apparatus and the plasma membrane. L-[^{14}C]fucose was chosen as precursor since it is first incorporated in the Golgi and is not converted to other sugars in the rat liver [3]. We have made use of antiserum to the secreted glycoprotein to show that this sugar is incorporated into both membrane glycoprotein and membrane-associated secretory glycoprotein but at very different rates.

2. Materials and methods

Male Wistar rats (approximately 200 g) were fasted for about 15 hr, injected intravenously with L-[^{14}C]-fucose (5 μCi/rat for experiments reported in fig. 1 and 10 μCi/rat for those in fig. 3). After different periods of time, 2 rats were anaesthetized with ether, exsanguinated, and the livers removed. One half of each liver was used for isolation of plasma membranes by the method of Ray [4]; the other half for isolation of Golgi according to Sturgess and et al. [5].

The isolated membranous fractions were either treated with perchloric acid or solubilized in 2.5% sodium deoxycholate containing 0.25 M sucrose. When perchloric acid was used, the membranes were extracted with 0.6 N acid and the high molecular weight glycoprotein fraction was prepared by precipitation with phosphotungstic acid [5]. Protein was measured according to Lowry et al. [6] and radioactivity as described earlier [5]. The specific activity of each fraction was expressed as dpm/mg protein.

Rabbit anti-rat serum was raised by subcutaneous injection of 1 ml of rat serum in 1 ml complete Freund's adjuvant. A second injection of half the amount in complete Freund's was given 5 weeks after the first. Rabbits were bled after 4 weeks and the serum separated. To determine the proportion of plasma membrane precipitated by various amounts of this serum, membranes labelled with ^{125}I using lactoperoxidase [7], were solubilized in sodium deoxycholate (2.5% in 0.25 M sucrose). Non-specific precipitates were removed by adding human albumin and rabbit anti-human serum (GIBCO) to the DOC soluble material and incubating at 37°C for 1 hr followed by 4°C for 24 hr. After centrifugation to remove the precipitated material, anti-rat serum was added to the supernatant, incubated for 1 hr at 37°C and then 24 hr at 4°C. The precipitate formed was removed by centrifuging, washed twice with 0.9% NaCl and dissolved in 0.5 NaOH. Protein and radioactivity were determined on aliquots of the supernatants and dissolved precipitates [5,6]. L-[^{14}C] fucose labelled membranes (fig. 3) were treated with antiserum in the same way.

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3. Results

3.1. *L-[^{14}C] fucose incorporation into Golgi, plasma membrane and serum*

The time course of incorporation of L-[^{14}C]fucose into high molecular weight perchloric acid soluble and insoluble fractions of isolated Golgi apparatus, plasma membranes and serum is shown in fig. 1. The specific activity of the perchloric acid insoluble fraction of the Golgi increased rapidly to a maximum value of 1.75×10^4 dpm/mg protein between 10 and 15 min after fucose injection and then decreased to approximately 4.2×10^3 dpm/mg at 1 hr. The specific activity of the perchloric acid soluble glycoprotein fraction rose even more rapidly to a peak of 5.5×10^4 dpm/mg protein at 15 min and then declined to 1.0×10^4 dpm/mg protein at 1 hr.

The corresponding serum fractions are also shown in fig. 1. The specific activity of the perchloric acid-soluble glycoprotein fraction (seromucoid) rose slowly for the first 30 min and leveled off at about 1 hr, implying that glycoprotein had moved from the Golgi perchloric acid soluble fraction to the corresponding serum fraction. Little incorporation was found in the perchloric acid insoluble fraction.

In the case of the acid insoluble fraction of the plasma membrane, the specific activity showed only a slight increase during the 1 hr of incorporation resembling the behaviour of the acid insoluble fraction of serum. On the other hand, curves representing the acid soluble portions of the plasma membrane and serum differed markedly. For the plasma membrane, the curve was biphasic, exhibited an early rapid rise in specific activity, peaked at 10 min, declined at 1/2 hr and rose slowly between 1/2 and 1 hr. When the specific activity in the plasma membrane rose rapidly, that of serum rose slowly followed by a more rapid increase between 15 and 30 min at which time the specific activity of the plasma membrane decreased.

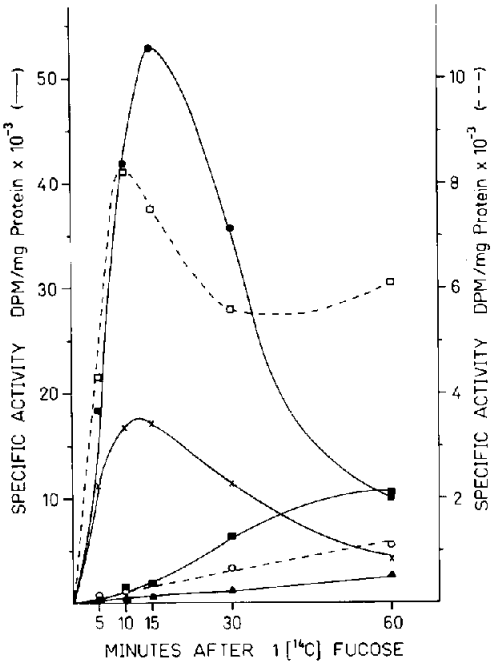

Fig. 1. Kinetics of incorporation of L-[^{14}C]fucose into Golgi (● perchloric acid soluble; X perchloric acid insoluble), plasma membranes (□ perchloric acid soluble; ○ perchloric acid insoluble) and serum (■ perchloric acid soluble; ▲ perchloric acid insoluble). The scale on the left ordinate applies to the solid curves (Golgi and serum); that on the right to the broken curves (plasma membranes).

3.2. *Immune Precipitation of Plasma Membrane*

When anti-rat serum was added to the DOC solubilized plasma membrane fraction, the curve shown in fig. 2 was obtained. A maximum of 28 000 dpm representing about 9% of ^{125}I-labelled plasma membrane was precipitated. Maximum precipitation was obtained with a ratio of antiserum protein to DOC soluble plasma

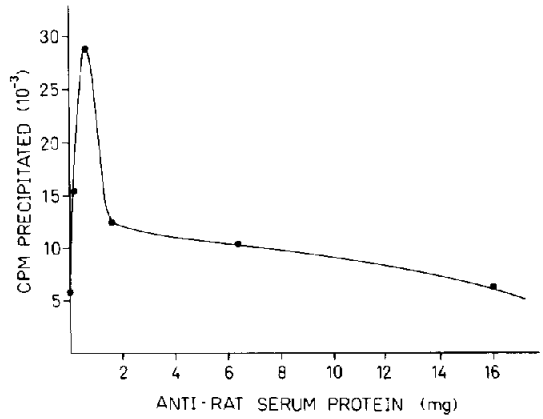

Fig. 2. Precipitation of ^{125}I-labelled plasma membrane (100 μg protein at each point) with increasing amounts of anti-rat serum in the presence of sodium deoxycholate. At the peak of the curve, about 9% of the membrane-bound radioactivity was precipitated.

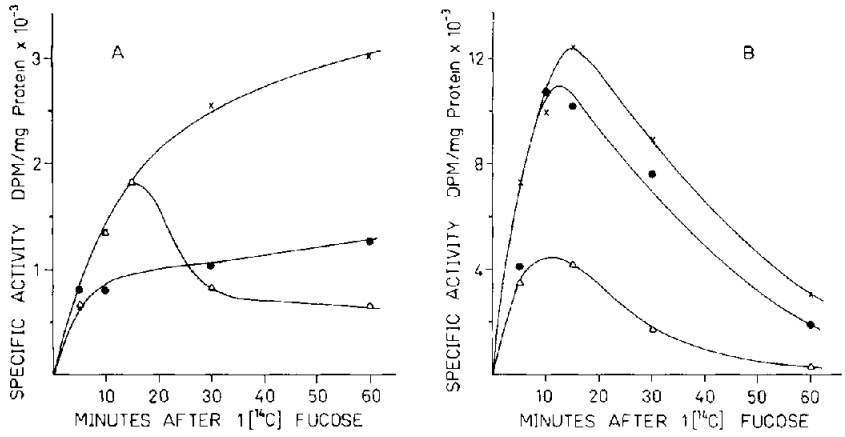

Fig. 3. A) Kinetics of incorporation of L-[^{14}C] fucose into the following fractions of plasma membranes: DOC insoluble (•), DOC soluble (×), and the portion of DOC soluble fraction precipitated by anti-rat serum (△). B) Kinetics of incorporation into the corresponding fractions of Golgi isolated from the same livers.

membrane protein of about 10:1. This ratio was used in all subsequent experiments.

The results of a series of experiments in which L-[^{14}C] fucose labelled Golgi and plasma membranes solubilized in DOC as above were reacted with anti-rat serum are shown in fig. 3. The specific activities of protein-bound L-[^{14}C] fucose in DOC soluble, insoluble and antiserum precipitated material were determined. In the case of plasma membrane (fig. 3a), the specific activity of the DOC soluble fraction exhibited a rapid increase which slowed after 15 min and was nearly constant by 1 hr. The antiserum precipitated a large portion of the material formed during the first 15 min but much less of that formed at later times. Therefore, from the point of view of immunochemical reactivity, the portion of the isolated plasma membrane exhibiting a rapid uptake of L-[^{14}C] fucose during the first 15 min of incorporation (fig. 1) was secretory glycoprotein destined for release into the serum. The later, slower phase of uptake is consistent with the turnover rates of plasma membrane components [8,9]. The specific activity in the DOC insoluble fraction of the membrane also increased at rapid rate during the first 5 min and the remained at about 1/3 of that of the soluble fraction.

Golgi was isolated from 1/2 of each of the livers from which the plasma membranes used above were isolated. The curves representing the incorporation of L-[^{14}C] fucose into the DOC soluble and insoluble fractions of Golgi are shown in fig. 3b. The DOC soluble protein precipitated by antiserum is also shown. All curves are similar but differ in maximum specific activities attained. A significant proportion of the DOC-soluble protein-bound fucose in the Golgi was precipitated by antiserum. However, the proportion of radioactivity precipitated by the antiserum at each time after injection of L-[^{14}C] fucose was fairly constant (0.26 to 0.44). This is in contrast to the experiments with plasma membrane where the proportion precipitated varied from 0.14 to 1.00.

4. Discussion

The kinetics of incorporation of L-[^{14}C] fucose into the isolated plasma membrane fraction revealed the presence of two distinctive subfractions exhibiting very different rates of turnover of this sugar. When labelled plasma membranes were solubilized in sodium deoxycholate the first rapidly turning-over subfraction was precipitated with anti-rat serum while the second slowly turning-over fraction was not. These findings provide an explanation for the biphasic curves relating specific activity and time of incorporation into liver plasma membranes also observed by Franke, Morré and co-workers [10] using L-[guanido-^{14}C] arginine. They showed two distinct phases which they interpreted as reflecting two separate subcellular pathways

by which the protein would move from the rough endoplasmic reticulum to the plasma membrane. They conjectured that the pathway for proteins labelled during the first phase of incorporation was from the rough endoplasmic reticulum to the smooth and then directly to plasma membrane while those responsible for the second slower phase travelled through the Golgi as well before reaching the plasma membrane. Although both their data and ours are not entirely consistent with movement of label from Golgi to the rapidly turning-over portion of the plasma membrane, it is important to note that consideration of either of the above pathways for membrane glycoproteins is entirely by way of analogy to the biosynthesis of secretory glycoproteins since there is as yet little if any conclusive evidence that plasma membrane proteins do reach the cell surface membrane by 'flow' of intracellular membrane. In fact, there is evidence that some soluble proteins (not associated with intracellular membrane) can be inserted into plasma membranes [11].

The findings which we have reported, however, do provide direct evidence that some secretory glycoprotein does become associated with the liver cell plasma membrane during secretion in such a way that this association persists after the isolation of these membranes. Furthermore, the possibility of preadsorbing this material from solubilized isolated membranes with antiserum enables one to begin to investigate the biosynthesis of integral plasma membrane glycoprotein.

Acknowledgements

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