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METABOLIC STUDIES OF RAT LIVER PLASMA MEMBRANES USING D-[1-^{14}C]GLUCOSAMINE

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SUMMARY

The metabolism of plasma membranes of rat liver cells was studied using D-[1-^{14}C]glucosamine. The labelling of plasma membranes occurred more slowly than that of microsomes, reaching a maximum at about 3 h after injection compared to 1.5 h for microsomes, and the radioactivity decayed with a half-life of 37 h which is close to the value obtained using [*guanidino*-^{14}C]arginine to label proteins. Hexosamine and sialic acid of plasma membranes were found to metabolize at practically equal rates.

These findings suggest that complete glycoproteins are built into plasma membranes after being synthesized in the endoplasmic reticulum, and the polypeptide and carbohydrate moieties then turn over in a concerted manner.

INTRODUCTION

The metabolism of plasma membranes of rat liver cells has been studied using amino acids to label proteins, and the results appear to suggest that proteins are built up into plasma membranes after being synthesized in the endoplasmic reticulum[1]. However, the observed turnover rates of plasma membrane proteins varied considerably depending on the amino acids used to label proteins[2,3].

In connection with our recent work on the characterization of glycoproteins existing in plasma membranes of rat liver cells[4-6], we have investigated the rate of synthesis and degradation of carbohydrate moiety of the plasma membranes using D-1-^{14}C]glucosamine.

MATERIALS AND METHODS

Female rats, Wistar strain, weighing 110–130 g, were used. The animals were fasted overnight prior to death. A dose of 3 μC or 6 μC of [^{14}C]glucosamine (D-[1-^{14}C]-glucosamine hydrochloride (11.5 mC/mmole) (New England Nuclear Corp.)) in 0.5 ml of saline was injected intraperitoneally. At varying time intervals after injection, groups of three to four animals were exsanguinated from the carotid artery and the jugular vein to collect blood, and the livers were then immediately perfused *in situ*. The blood was allowed to clot, and the serum recovered by centrifugation. Plasma

membranes were isolated from the livers according to the method of NEVILLE[7], and microsomes by the method of ERNSTER *et al.*[8].

The yield of plasma membranes was about 1 mg protein per 10 g wet wt. of liver. The purity of the isolated plasma membranes was checked by electron microscopy and by assaying the marker enzymes, *i.e.* 5'-nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) for plasma membranes and glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) for endoplasmic reticulum. The former was assayed according to the procedure of SONG AND BODANSKY[9], and the latter according to the method of NORDLIE AND ARION[10].

The specific activities of 5'-nucleotidase and glucose-6-phosphatase of the isolated plasma membranes expressed as the amounts of P_i in μmoles liberated per min per mg protein were 0.55–0.81, a 10 to 17-fold increase over the whole homogenate, and $23 \cdot 10^{-3}$–$39 \cdot 10^{-3}$, respectively. From the latter value, contamination by endoplasmic reticulum in the plasma membrane preparation was estimated to be 9–15%.

To determine protein and radioactivity, plasma membranes and microsomes were dissolved in barbital buffer, $I = 0.01$ and pH 8.6, containing 0.1% sodium dodecyl sulphate and 1 M urea. Trichloroacetic acid precipitates of the liver homogenate were dissolved in 1 M NaOH. These solutions were used for protein determination according to the method of LOWRY *et al.*[11] and for the radioactivity determination. To determine specific radioactivity of the sugars, sialic acid was released from the membrane preparations by hydrolysis in 2.5% trichloroacetic acid at 80° for 1 h, and after centrifugation the precipitates were heated in 2 M HCl at 100° for 16 h to release hexosamine. The liberated sugars were adsorbed on ion exchangers and eluted as described previously[5]. Aliquots from each eluate were used for colorimetric determinations according to the procedure of WARREN[12] for sialic acid, and by the Elson–Morgan reaction for hexosamine[13]. Radioactivity was determined in the scintillation mixtures of MADSEN[14] using a liquid scintillation counter.

It should be noted that the radioactivity of plasma membranes was located entirely in hexosamine and sialic acid.

RESULTS AND DISCUSSION

The rate of labelling with [^{14}C]glucosamine of plasma membranes is shown in Fig. 1 compared with those of serum proteins, whole liver homogenate (homogenized with 1 mM $NaHCO_3$), and its 5% trichloroacetic acid precipitate and microsomes. The radioactivity of plasma membranes increased for 3 h and then decreased gradually. This pattern, resembling that of serum glycoproteins, was also similar to that found when [^{3}H]leucine was used to label proteins, as reported by RAY *et al.*[1], but was markedly different from that of microsomes which acquired radioactivity rapidly, the activity reaching its peak about 1.5 h after the isotope injection. Radioactivities of the trichloroacetic acid-soluble fraction of the whole liver homogenate calculated from the values of the whole homogenate and its trichloroacetic acid precipitate, representing the activities of low-molecular intermediates, were decreasing sharply.

These data appear to be consistent with the idea that microsomes contain precursors of plasma membrane glycoproteins. This possible relationship was further investigated by measuring the specific radioactivities of hexosamine and sialic acid of plasma membranes at various times after the isotope injection.

The results shown in Table I indicate that the activities of the microsomal sugars were always higher than those of the sugars in plasma membranes. Thus, a single precursor–product relationship could not be deduced between glycoproteins of these two fractions. However, it is likely that some of the microsomal glycoproteins may be precursors of plasma membrane glycoproteins since the former is metabolically heterogeneous[5].

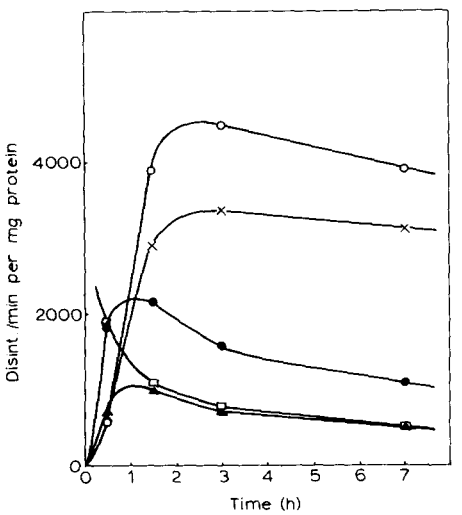

Fig. 1. The kinetics of the incorporation of D-[1-^{14}C]glucosamine into liver subfractions and serum proteins of rat. Each rat received 3 μC of [^{14}C]glucosamine intraperitoneally and was killed at the indicated times. ○—○, serum proteins (serum dialyzed against saline); ×—×, plasma membranes □--□, whole liver homogenate; ▲--▲, trichloroacetic acid precipitate of whole liver homogenate ●—●, microsomes.

TABLE I

INCORPORATION OF D-[1-^{14}C]GLUCOSAMINE INTO HEXOSAMINE AND SIALIC ACID OF PLASMA MEMBRANES AND MICROSOMES

Each rat received 3 μC of [^{14}C]glucosamine intraperitoneally and was killed at the indicated times. The values are mean values with about 10% accuracy from duplicate samples in one representative experiment.

Time (h)	*Disint./min per nmole*			
	Plasma membranes			*Microsomes*
	Hexosamine	*Sialic acid*	*Hexosamine plus sialic acid**	*Hexosamine plus sialic acid***
0.5	5.92	2.62	4.40	42.0
1.5	18.0	19.7	18.8	48.8
3.0	20.9	21.2	21.1	35.5
7.0	18.4	21.3	19.8	24.2

* Values were calculated from the specific activities of hexosamine and sialic acid and the contents of these sugars in plasma membranes, *i.e.* 51.7 μmoles/g protein and 44.4 μmoles/g protein, respectively, determined according to the methods described previously[4].

** Values were calculated from disint./min per mg protein of microsomes and the contents of hexosamine and sialic acid in microsomes, *i.e.* 29.8 μmoles/g protein and 12.6 μmoles/g protein, respectively[3].

Glycoprotein metabolism of plasma membranes differed from that of microsomes in that the ratio of specific radioactivities of hexosamine to sialic acid in plasma membranes was practically constant from 1.5 h after the isotope injection onward, whereas in microsomes the ratio had been shown to decrease from 1.45 at 1.5 h to 0.70 at 7 h, reaching 0.4 after 24 h (ref. 5). The values at 0.5 h are exceptional, which might indicate that a minor fraction of plasma membrane glycoproteins metabolizes differently from the total glycoproteins.

The decay of specific radioactivity of plasma membranes is shown in Fig. 2.

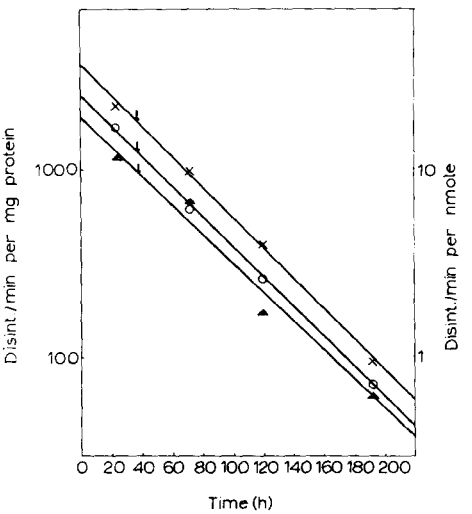

Fig. 2. Decay of specific radioactivities of plasma membranes, and hexosamine and sialic acid of plasma membranes. Each rat received 6 μC of [^{14}C]glucosamine intraperitoneally and was killed at the indicated times. Arrows denote half-life times determined graphically. ×—×, plasma membranes; ▲—▲, hexosamine of plasma membranes; ○—○, sialic acid of plasma membranes.

The regression lines were drawn by the least-squares method from four time points from 20 h to 200 h, showing a single phase decay. This differs for microsomal membranes which showed an inflection point between 1–3 days, indicating that microsomal glycoproteins turn over heterogeneously[5]. The calculated half-life of the carbohydrate moiety of plasma membranes was 37 h. This value is about half that of the carbohydrate moieties of microsomal membranes (80 h for the slower phase of decay after the inflection point) and is similar to that of a serum glycoprotein (26 h) reported previously[5].

Decay curves of hexosamine and sialic acid of plasma membranes determined separately are also shown in Fig. 2, the slope of the lines being in good agreement with that of the whole plasma membranes.

The turnover rate of total proteins of plasma membranes has been measured by other workers using amino acids to label proteins. By using [^{3}H]leucine and [$^{14}C_6$]arginine, the half-lives were estimated to be 85 h and 74 h, respectively[2,3]. However, when [*guanidino*-^{14}C]arginine was used to minimize the reutilization of isotope, a much shorter half-life, *i.e.* 43 h, was obtained[3] which is very close to the value reported in this paper. Thus, it may be assumed that the polypeptide and carbohydrate moieties of glycoproteins constituting plasma membranes turn over

in a concerted manner, although no study has been made as to how far the turnover rate of plasma membranes labelled with [^{14}C]glucosamine is affected by reutilization of isotope.

The plasma membrane glycoproteins may be synthesized in the endoplasmic reticulum and then built into plasma membranes. In this connection, it should be noted that glycosyltransferases (transferring sugars from nucleotide-sugars to acceptors) working in glycoprotein synthesis have been shown to be localized in the Golgi apparatus of rat liver, but not in plasma membranes[15-17]. This may also be relevant to our recent findings that plasma membranes possess as their constituents only acidic oligosaccharides that are considered to be completed carbohydrate chains containing neutral sugars, hexosamine and sialic acid, in contrast to the presence in endoplasmic reticulum of apparently incomplete carbohydrate chains containing only glucosamine and mannose[6].

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