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THE RELATIVE RATES OF DEGRADATION OF THE PLASMA MEMBRANE GLYCOPROTEINS FROM NORMAL RAT LIVER

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SUMMARY

Rat liver plasma membranes, as fractionated by sodium dodecylsulfate/polyacrylamide gel electrophoresis, have been examined for the incorporation in their subunits of radioactive leucine, glucosamine and fucose. Specific spectra were obtained. In contrast to leucine, where the activity is distributed in many peaks all over the fractions, the glucosamine and fucose activities are found principally in the high molecular weight region.

The relative rates of degradation of the glycoprotein components of the plasma membrane have been measured in normal liver using the double isotope technique. A marked heterogeneity of degradation was observed among the different subunits and a correlation between the rate of degradation and the size of the labelled subunits was found with glucosamine and fucose as well as with leucine. This suggests a similar mode for the degradation of these membrane components.

INTRODUCTION

Several studies have shown that the plasma membrane constituents experience a relatively rapid turnover. Windell and Siekevitz [1] using [^{3}H]leucine have found a half-life of 85 h for the total proteins. Arias et al. [2] have performed similar studies with [^{14}C]guanido-arginine and obtained a value of 43 h. Using [^{14}C]glucosamine, Kawasaki and Yamashina [3] found a half-life of 37 h for the total plasma membrane hexoamine and sialic acid. They concluded from these experiments that on the average the polypeptide and carbohydrate components of the membrane turnover in a concerted manner. More detailed studies have been performed by Dehlinger and Schimke [4] on the relative rates of degradation of each individual protein species. They found that membrane proteins exibit differential turnover and further that the degradation rate of a subunit is related to its molecular weight. No similar studies have been carried out for the carbohydrate-containing species of the membrane.

In the present study the relative rates of degradation of individual plasma membrane glycoproteins are measured in normal rat liver.

EXPERIMENTAL PROCEDURE

Animals. Male Wistar rats weighing 175–250 g were used in all experiments. The animal had access to food and water ad libitum.

Labelled precursors. L-[4, 5-$^3H_2$] Leucine (51 Ci/mmol), D-[6-^{3}H]glucosamine (12.6 Ci/mmol), L-[1-^{14}C]leucine (60 Ci/mol), D-[1-^{14}C]glucosamine (58 Ci/mol) and L-[1-^{14}C]fucose (57 Ci/mol) were obtained from Amersham Searle Co. L-[1, 5, 6-$^3H_3$]fucose (5 Ci/mmol) was from New England Nuclear Corp.

Double isotope technique. All labelled precursors were administered through the jugular vein. Following the double isotope technique first described by Arias et al. [2], one labelled precursor (^{14}C) was administered and allowed to decay for 3 days. Then, the second isotopic form (^{3}H) of the same precursor was injected. The animal was sacrificed 3.5 h later, the liver plasma membrane isolated and the different protein subunits separated by electrophoresis on sodium dodecylsulfate/acrylamide gels. The ratios of ^{3}H: ^{14}C activities along the gels yielded a measure of the relative rates of degradation of the separated subunits. Control experiments were performed where both isotopic forms of the same precursor were injected simultaneously.

Preparation of plasma membranes. Plasma membranes were isolated by the modified version [5] of Neville's method [6] except that the liver was perfused in situ through the portal vein with ice-cold saline. Protein concentration was determined by the method of Lowry et al. [7] using bovine serum albumin as standard. The yield of plasma membranes varies from 1 to 1.5 mg protein per g wet weight of liver. The degree of purity of the isolated membranes was checked by electron microscopy and enzymatic markers. The 5'-nucleotidase was assayed according to the procedure of Emmelot and Bos [8] and glucose-6-phosphatase as described by Ray [5]. The 5'-nucleotidase specific activity expressed as the amount of μmol of phosphate liberated per h per mg of protein was 50–70, a 15- to 20-fold increase over the whole homogenate while a relative specific activity of 0.8–1.0 was found for the glucose-6-phosphatase.

Polyacrylamide gel electrophoresis. The membrane fraction was solubilized and dialysed according to the procedure of Glossman and Neville [9], except that urea was omitted in the dialysis buffer. Gel electrophoresis was performed using the buffer system developed by Neville [10]. Stacking gels (2.5 %) and separating gels (7.5 %) were prepared in 8-mm glass cylinders from stock solutions containing acrylamide and methylene-bis-acrylamide in proportion of 40 : 1. The gels contained 0.1 % sodium dodecylsulfate and were polymerized by adding 0.05 % tetramethylethylenediamine and 0.11 % $(NH_4)_2S_2O_8$. The buffers used were: upper reservoir, 0.04 M H_3BO_3/0.04 M Tris (pH 8.6)/0.1 % sodium dodecylsulfate; stacking gel, 0.026 M H_2SO_4/0.054 M Tris (pH 6.1); separating gel and lower reservoir, 0.03 M HCl/0.42 M Tris (pH 9.2). The dialysis sample was distributed on top of the gels in a volume of 125 μl per gel (up to 500 μg of membrane proteins). Acrylamide gels were calibrated with marker proteins of different molecular weight according to Neville [10].

Counting. Total plasma membrane activities were measured by counting 125 μl of solubilized membranes in 10 ml of Aquasol (New England Nuclear). For the evaluation of the ^{3}H and ^{14}C activities among the different plasma membrane species, acrylamide gels were sectioned in 100 1-mm thick slices. The slices were placed into scintillation vials and incubated overnight with 0.2 ml of 30 % H_2O_2 at 40 °C. 10 ml of Aquasol were added and the samples counted in a Mark II scintillation counter (Nuclear Chicago).

RESULTS

The double isotope technique was used to evaluate the relative rates of degradation of the leucine-, glucosamine- and fucose-containing species in the plasma membranes of normal rat liver. The pattern distribution on sodium dodecylsulfate/polyacrylamide gel of the radioactive leucine incorporated at 3.5 h (^{3}H) and 3 days (^{14}C) is shown in Fig. 1. The incorporation is relatively high in fractions 60–70, and the fact that an appreciable amount of activity is detected at both the origin and the dye position suggests that leucine is distributed in all species of the membrane that can be separated by electrophoresis. The ratio of the ^{3}H activity over the ^{14}C activity detected in each fraction is a measurement of the relative rate of degradation of a separate species. As shown in Fig. 2, a marked heterogeneity is obtained among the different fractions with a tendency for the fastest species to have the lowest ratios. Since sodium dodecylsulfate/polyacrylamide gel electrophoresis fractionates according to molecular size [10–12], a general correlation can be inferred between the subunit molecular size and the degradation rate constant. There are exceptions since a fraction of 25 000 molecular weight exhibits a ratio similar to those obtained in the 100 000 to 150 000 molecular weight region.

The same procedure was followed to determine the relative distribution of the glucosamine- and fucose-containing species and their relative rate of degradation. Fig. 3 represents the distribution of [^{3}H]glucosamine and [^{14}C]glucosamine incorporated at 3.5 h and 3 days, respectively. In contrast to leucine, the activity is concentrated into five main fractions located in the higher molecular weight region, particularly

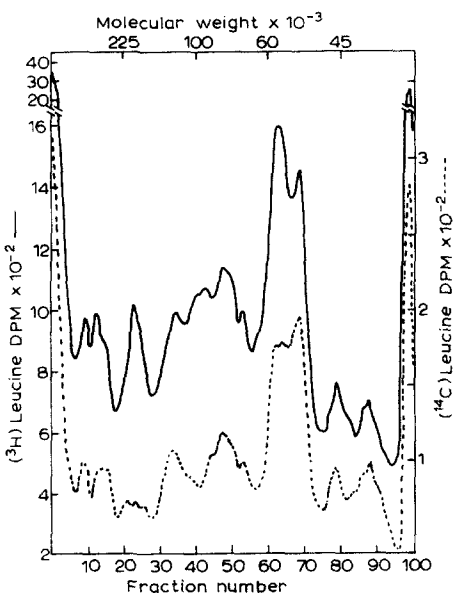

Fig. 1. Distribution of [^{14}C]leucine(- - -) and [^{3}H]leucine (—) activities in the rat liver plasma membrane as fractionated by sodium dodecylsulfate/polyacrylamide gel electrophoresis. The animal was given 250 μCi of [^{3}H]leucine 3 days after being injected with 60 μCi of [^{14}C]leucine. The direction of migration is from left to right. Molecular weights are given on top in a non-linear scale.

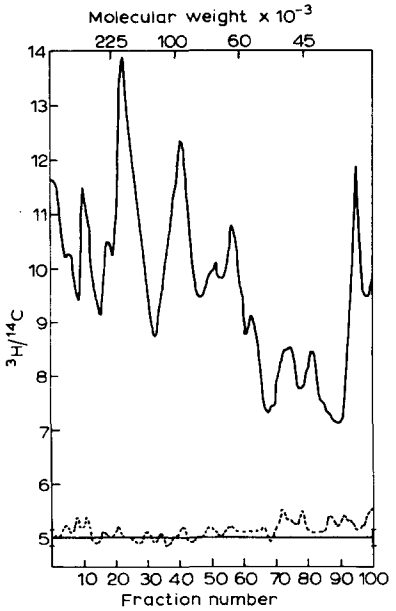

Fig. 2. Relative degradation of the leucine-containing species of the liver plasma membrane from data of Fig. 1. The lower curve represents the results from a control experiment where 75 μCi of [^{14}C]leucine and 375 μCi [^{3}H]leucine were given to a rat 3.5 h before sacrifice. The mean and the standard deviation are indicated.

in species of about 250 000 and above. The distribution of the ^3H : ^{14}C ratios over all the fractionated species is given in Fig. 4. For the glucosamine-containing species of molecular weight larger than 60000 a correlation similar to that obtained in the leucine experiment is observed between the ratio and the molecular size. For the remaining fractions, this correlation is much less apparent. Fig. 5 shows the relative distribution

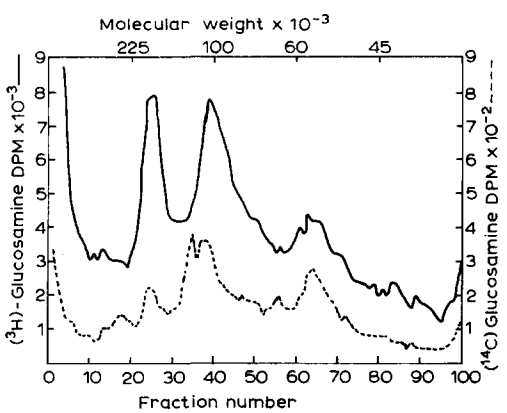

Fig. 3. Distribution of [^{14}C]glucosamine (- - -) and [^{3}H]glucosamine (—) activities in the plasma membrane as fractionated by sodium dodecylsulfate/polyacrylamide gel electrophoresis. The animal was given 200 μCi of [^{3}H]glucosamine 3 days after the administration of 40 μCi of [^{14}C]glucosamine.

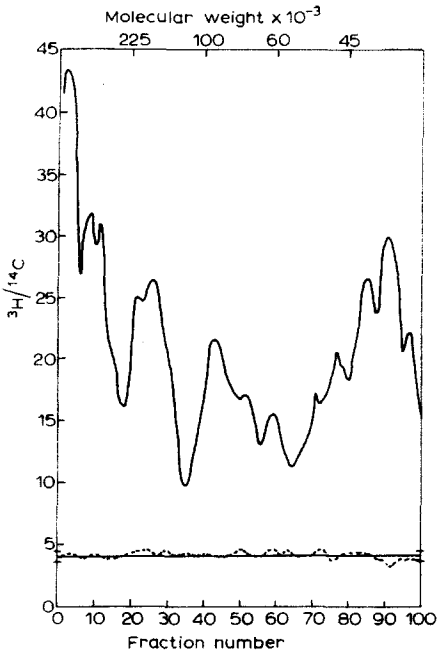

Fig. 4. Relative degradation of the glucosamine-containing species of the liver plasma membrane from data of Fig. 3. The broken line indicates the results of a control experiment, where [^{14}C]glucosamine (50 μCi) and [^{3}H]glucosamine (240 μCi) were given as described in Fig. 2.

of radioactive fucose at the time chosen for the two previous experiments. The precursor seems incorporated only in those species that also contain glucosamine. A large portion of the radioactivity is detected in the high molecular weight fractions. The ratio distribution, presented in Fig. 6, shows a correlation between the degradation

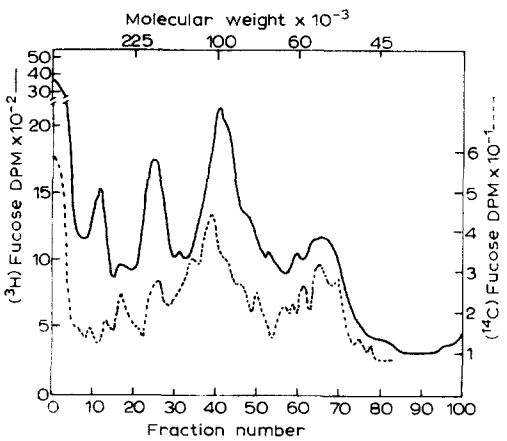

Fig. 5. Distribution of [^{14}C]fucose (- - -) and [^{3}H]fucose (—) activities in the plasma membrane as fractionated by sodium dodecylsulfate/polyacrylamide gel electrophoresis. [^{3}H]Fucose (250 μCi) was injected 3 days after administration of [^{14}C]fucose (50 μCi).

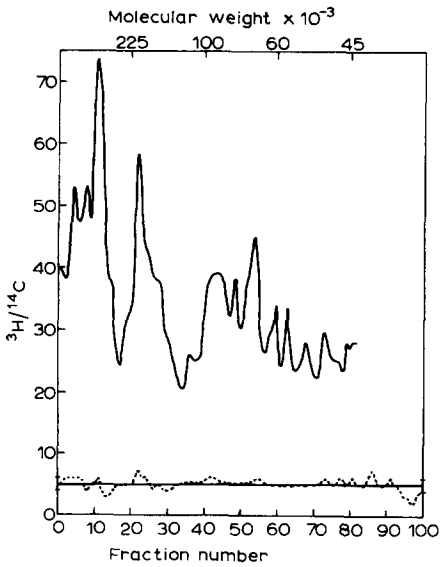

Fig. 6. Relative degradation of the fucose-containing species in liver plasma membrane from data of Fig. 5. The broken line represents the results of a control experiment, where [^{14}C]fucose (50 μCi) and [^{3}H]fucose (250 μCi) were administered as described in Fig. 2.

and the molecular weight similar to that observed with glucosamine.

In order to determine the variability due to experimental design in the calculations of the ratios, the two isotopic forms of the same precursor (^{3}H and ^{14}C) were injected simultaneously and 3.5 h later, the plasma membrane species were separated as above. The results obtained for the ratio distribution of leucine is shown in the lower part of Fig. 2, that of glucosamine and fucose in the Figs 4 and 6, respectively. No systematic variations in ^{3}H : ^{14}C ratios are detected along the gels which indicate that the peaks seen in the previous spectra are not due to errors inherent to the label method as applied to gel electrophoresis. This further confirms the results that in plasma membrane from normal rat liver the relative rates of degradation of the glycoprotein species is heterogeneous.

DISCUSSION

The main objectives of the present studies were to determine the relative distribution of the leucine-, glucosamine- and fucose-containing species in rat liver plasma membrane and to evaluate their relative rate of degradation. As found by Dehlinger and Schimke [4], leucine is incorporated in all the fractions that can be resolved by sodium dodecylsulfate/polyacrylamide gel electrophoresis. The incorporation profile for glucosamine and fucose shows 4 to 5 well resolved peaks located in the high-apparent molecular weight region. From the work reported by Glossman and Neville [9] and Evans [13], periodic acid /Schiff staining of sodium dodecylsulfate gels demonstrated the presence of a least five carbohydrate components in the same range of molecular weight. Our results suggest very strongly that all the periodic acid/Schiff stain-positive components contain both glucosamine and fucose. The relatively high

incorporation of both precursors in the fractions above 100 000 molecular weight, further suggests that a large amount of carbohydrates is associated with these peptide species.

The double isotope studies show that the glycoprotein subunits are degraded in a heterogeneous manner and suggest that the degradation rate is related to the apparent molecular size. Previous measurements of the degradation rate constants of the proteins associated subunits in the membranes [4, 14] and the soluble fraction [15] have yielded similar results. Our studies demonstrated that, at least in plasma membranes, this mode of degradation is not specific to the amino acid-containing subunits. Proposed models for the location of glycoproteins in the lipid bilayer represent the glucosidic termini as ligands exposed on the outer surface of the membrane [16, 17]. Evidences for the presence of glycosyltransferases involved in the synthesis of carbohydrate components on cell surface have been reported Roth et al. [18]. As for the mode of degradation of surface glycoproteins, the results presented here suggest that the glucosidic moities of glycoproteins are degraded in a concerted manner with the peptide chain at which they are attached or at least, they are degraded at a rate predicted by the apparent molecular weight of the species. The fate of these species upon degradation is ill defined. Studies in cell culture have demonstrated the presence in the surrounding medium of glycoproteins released apparently from the surface [19–21]. Mild treatment of the surface with proteases liberates glycopeptides very similar to those found after incubation in serum-free medium [21]. Their possible roles remain an open question.

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