Characterization of the membrane proteins of rat liver lysosomes

Composition, enzyme activities and turnover

Joan BURNSIDE and Donald L. SCHNEIDER

Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755, U.S.A.

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Lysosomes prepared from the livers of untreated rats and from the livers of rats injected with either Triton WR-1339 or dextran yielded membranes that were similar in both polypeptide composition and activities of ATPase and acid 5'-nucleotidase. The administration of Triton WR-1339 (and dextran) resulted in an increase in ATPase activity of liver homogenates that was associated with a parallel increase in the ATPase activity of the lysosomal membrane. On the other hand, plasma membranes appear to be different from lysosomal membranes with respect to polypeptide composition and enzyme activities. The ATPase activity of lysosomal membranes is not affected by ouabain and suramin, inhibitors of the plasma-membrane ATPase. The plasma-membrane alkaline 5'-nucleotidase has little activity at acid pH. Pulse-labelling of lysosomal membranes with [³H]fucose and with [³H]- and [¹⁴C]-leucine occurred rapidly, faster than labelling of plasma membranes. The labelling kinetics indicate that lysosomal membranes may be assembled independently of plasma membranes. These data suggest that, in liver, little bulk transport of plasma membrane to lysosomes takes place, and lysosomal-membrane proteins may not be derived from those of plasma membranes.

Endocytosis is extensive, especially in liver, where 10% of the serum albumin and circulating proteins are internalized each day (Waterlow *et al.*, 1978). A concomitant transport of plasma-membrane proteins to lysosomes and mixing with lysosomal-membrane proteins might be expected to occur. These transport processes are thought to be bidirectional, inasmuch as soluble markers (Schneider *et al.*, 1979) and membrane markers (Muller *et al.*, 1980*a,b*) can re-appear at the cell surface after internalization. The transfer of membrane components into and out of lysosomes is central to many current studies in cell biology, including organelle biogenesis and regulation of surface-receptor concentrations. Thus it is of interest to determine the properties of lysosomal membranes and the extent to which plasma-membrane components are incorporated into lysosomes during endocytosis.

We have previously reported on the properties and topographical organization of membrane proteins of lysosomes isolated from the livers of rats treated with Triton WR-1339 (Schneider *et al.*, 1978). In the present study we examined the polypeptides of lysosomes prepared by three different techniques. Triton WR-1339-loaded lysosomes

are formed in liver by endocytosis of serum proteins carrying the Triton. Similarly, lysosomes can be loaded, *in vivo*, with the non-digestible polysaccharide dextran; the incorporation of this also requires endocytosis (Thinès-Sempoux *et al.*, 1966; Thinès-Sempoux, 1973). In the third technique, lysosomes from the livers of untreated rats are purified by flotation gradient centrifugation through metrizamide (Wattiaux *et al.*, 1978).

By studying labelling kinetics, we have shown that lysosomal-membrane proteins are rapidly metabolized, more rapidly than the average liver protein and more rapidly than those of plasma membranes. On the basis of these comparisons of the lysosomal-membrane and plasma-membrane proteins, it appears that extensive mixing of the proteins of the two membranes does not occur, and lysosomal membranes have a unique and characteristic composition.

Experimental

Animals

Male rats (125–175 g body wt.) were obtained from Charles River (Wilmington, MA, U.S.A.).

Animals were maintained on a 12h-light/12h-dark cycle, and were fed *ad libitum*. Radioisotopically labelled compounds, Triton WR-1339 and dextran were administered intraperitoneally as saline (0.9% NaCl) solutions. Rats were killed by decapitation, and exsanguinated before removal of the liver.

Chemicals

Radioisotopically labelled compounds purchased from New England Nuclear (Boston, MA, U.S.A.) were L-[4,5-³H]leucine (5 Ci/mmol), L-[U-¹⁴C]-leucine (335 mCi/mmol), L-[6-³H]fucose (12 Ci/mmol) and NaB³H₄ (100 mCi/mmol); [G-³H]-ouabain (49 Ci/mmol) was from Amersham/Searle (Arlington Heights, IL, U.S.A.). Suramin was a gift from Bayer (Leverkusen, Germany); other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Lysosome preparations

Three different methods were used for the purification of rat liver lysosomes. Triton-filled lysosomes were prepared 3.5 days after injection of Triton WR-1339 (0.85 g/kg body wt.) essentially as described by Trouet (1964) and modified by Leighton *et al.* (1968), except that rats were not deprived of food. When livers from non-starved rats are fractionated, it is important that the large-granule fraction, ML (de Duve *et al.*, 1955), pelleted from the extract by differential centrifugation, is decanted incompletely. The loose material associated with the large-granule pellet contains lysosomes and some microsomal contamination. However, the microsomal contamination is largely removed during flotation gradient centrifugation. Resuspension of the large-granule fraction in a small volume of 0.25 M-sucrose before the addition of denser sucrose solution results in less breakage and higher yields. As previously reported (Schneider, 1977; Schneider *et al.*, 1978), yields of 28–33% were obtained.

Dextran-filled lysosomes were prepared essentially as described by Beaufay (1972). Rats were injected intraperitoneally with dextran, daily, for 5 days. The large-granule fraction was resuspended in 33% (w/w) sucrose, and overlaid on a discontinuous gradient of 47% (w/w), 57% (w/w) and 67% (w/w) sucrose and centrifuged at 24 000 rev./min ($g_{\max}$ = 100 000) for 2 h in a Beckman SW27 rotor. The dextran-filled lysosomes were collected as a pellet beneath the 67%-sucrose layer.

Lysosomes were purified from normal rat liver by the method of Wattiaux *et al.* (1978), in which the light-mitochondrial fraction, L (de Duve *et al.*, 1955), was mixed in 57% (w/v) metrizamide, overlaid with a metrizamide gradient and centrifuged in the Beckman SW27 rotor at 24 000 rev./min

for 2 h. Normal lysosomes were collected from the upper portions of the gradient.

Membrane preparations

Plasma membranes were prepared as described by Ray (1970). This preparation involves pelleting by low-speed centrifugation and sucrose-density-gradient flotation to a density of 1.16 g/ml, and is thus enriched in the contiguous face of the liver plasma membrane (Wisher & Evans, 1975). However, the membranes were enriched at least 20-fold in alkaline 5'-nucleotidase activity, indicative of the sinusoidal and bile-canicular faces. Since liver contains 4–5 times more sinusoidal face than bile-canicular face (Evans, 1976), the membrane preparations most probably contain substantial amounts of the sinusoidal face. Contamination by endoplasmic reticulum, mitochondria and Golgi apparatus was low.

Membranes were prepared from intact lysosomes after freeze-thawing in 200 mM-NaCl/50 mM-Tris/H₂SO₄/1 mM-EDTA medium, pH 8, as described previously (Schneider *et al.*, 1978).

In labelling experiments performed *in vivo*, lysosomes and plasma membranes were prepared from the same Triton WR-1339-treated rat; 30% (wet wt.) of the liver was used in the preparation of plasma membranes. Plasma membranes isolated from Triton-treated rats appear to be identical with those from untreated rats, in terms of activities of 5'-nucleotidase and ATPase, and in terms of polypeptide composition.

Labelling procedures

The galactose oxidase/NaB³H₄ technique of Gahmberg & Hakomori (1976) was used for the labelling of the carbohydrate portions of membrane glycoproteins. Membranes suspended in 20% (w/w) sucrose buffered with 10 mM-sodium phosphate, pH 7.4, were pretreated with freshly prepared 1 mM-NaBH₄ for 5 min at room temperature. The lysosomes were then treated with neuraminidase (Sigma type VIII) (0.05 unit/mg of lysosomal protein) for 15 min at 37°C. Galactose and galactosamine residues were then oxidized by incubation with galactose oxidase (Sigma type IV) (0.2–0.3 mg of protein/mg of lysosomal protein) at 37°C for 30 min. The resulting aldehydes were then reduced by incubation with NaB³H₄, freshly prepared in 10 mM-NaOH.

Assays

N-Acetyl- β -D-glucosaminidase was used as a marker for lysosomes, and was assayed as described by Findlay *et al.* (1958). A unit of activity is defined as 1 μ mol of *p*-nitrophenyl N-acetyl- β -D-glucosaminide hydrolysed/min. 5'-Nucleotidase (AMPase), at alkaline pH, was used as a marker for

plasma membrane and was assayed as reported by Widnell (1972). 5'-Nucleotidase was also measured at pH 5, in 0.1 M-sodium acetate buffer. ATPase was assayed at pH 7, as previously reported (Schneider *et al.*, 1978). Phosphate liberated by these activities was determined with ascorbate/molybdate (Chen *et al.*, 1956), and a unit of activity is defined as 1 μ mol of phosphate released/min. Protein was measured by a modification of the Folin method (Lowry *et al.*, 1951), with bovine serum albumin as standard. Interference by sucrose was eliminated by precipitation of protein with 5% (w/v) trichloroacetic acid in the presence of 0.1% sodium deoxycholate. Protein-bound radioactivity was determined, after trichloroacetic acid precipitation followed by solubilization in 0.2 M-NaOH/2% sodium deoxycholate, by scintillation counting in Biofluor (New England Nuclear).

Sodium dodecyl sulphate / polyacrylamide - gel electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed by the procedure of Weber & Osborn (1969). Acrylamide gels (7.5% or 9.5%) were cast in glass tubes with an internal diameter of 5 mm. Samples were prepared and electrophoresed as described previously (Schneider *et al.*, 1978). After fixation in 10% trichloroacetic acid, gels were stained either for protein, with Coomassie Blue, or for carbohydrate, with periodate/Schiff staining reagent (Zacharius *et al.*, 1969), and scanned on a Gilford recording spectrophotometer. Gels containing radiolabelled protein were sliced into 1 mm slices with a Bio-Rad model 190 Gel Slicer, and digested with 10% (v/v) H₂O₂ before addition of scintillant. *Escherichia coli* β -galactosidase (mol.wt. 135 000), bovine serum albumin (mol.wt. 68 500), hen ovalbumin (mol.wt. 45 000) and bovine trypsin (mol.wt. 23 500) were used as molecular-weight standards.

Results

Polypeptide composition of the lysosomal membrane

The three different techniques used to purify lysosomes from rat liver yielded preparations of comparable purity, at least 60-fold enriched for the soluble enzyme *N*-acetyl- β -D-glucosaminidase. Membranes prepared from each of the lysosomal preparations were compared by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The electrophoretic profiles of each of these three preparations were similar (Fig. 1a). There were apparent quantitative differences; for example, membranes from normal lysosomes always indicated more protein in the 125 000-mol.wt. region just

before the major band in membranes from Triton-filled lysosomes, which was at about 95 000 mol.wt. (Fig. 1b). The membranes contain a number of distinct high-molecular-weight polypeptides that are noteworthy. If the membranes contained a significant amount of degradation products, then a myriad of low-molecular-weight polypeptides would be expected. Since this was not observed, the pool of membranous degradation products appears to be small. The membranes of dextran-filled and Triton-filled lysosomes might be expected to show similarities, not only between themselves, but also with the plasma membrane, because dextran and Triton WR-1339 treatments are accompanied by endocytosis of these materials, and perhaps plasma membrane, into liver. On the other hand, lysosomes purified by centrifugation through metrizamide represent a normal population of lysosomes. Although endocytosis is occurring at a significant rate at all times in liver, it is possible that dextran and Triton lead to enhanced rates of endocytosis. At least with Triton, the lysosomal membranes appear to be altered, because they equilibrate on sucrose gradients at densities of 1.11–1.14 g/ml, in contrast with 1.19–1.23 g/ml for normal lysosomal membranes. Nonetheless the profiles of the membrane polypeptides of the three lysosomal-membrane preparations appear to be very similar to one another, and distinctly different from that of plasma membranes.

Membrane glycoproteins were identified either by prior labelling with galactose oxidase/NaB³H₄ or by staining with periodate/Schiff reagent. Sodium dodecyl sulphate / polyacrylamide - gel electrophoretic analysis of glycoproteins labelled in disrupted lysosomal membranes revealed a number of radioactive peaks (Fig. 2a). The profile was similar to that obtained after staining with periodate/Schiff reagent (Fig. 2b). Heavy labelling of the lysosomal membrane was noted in the 110 000–90 000-mol.wt. range, where major glycoproteins of the lysosomal membrane are found. One band, with apparent mol.wt. 30 000 (slice 24), was labelled with NaB³H₄ but did not stain with periodate/Schiff reagent.

By comparison, plasma membrane showed a diffuse labelling pattern with possible major peaks at 102 000 and 84 000 mol.wt. (slices 9 and 13). Also, plasma-membrane glycoproteins stained only weakly with periodate/Schiff reagent; twice as much plasma-membrane protein as lysosomal-membrane protein was applied to obtain the same intensity of staining (Fig. 2d). These results suggest that lysosomal membranes are relatively enriched in glycoproteins that are not present in plasma membrane. It is noteworthy that the major lysosomal peak (Fig. 2b) was consistently about 5000 daltons larger than the corresponding peak obtained with plasma membrane (Fig. 2d).

Fig. 1. Polypeptide profiles of lysosomal membranes prepared in three different ways and of plasma membranes after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Enzymes associated with lysosomal membranes

The presence of 5'-nucleotidase (AMPase) and ATPase activities in the lysosomal membrane has been previously reported (Colbeau *et al.*, 1971; Schneider, 1977). The specific activities of these enzymes in the membranes from the different lysosome preparations and in the plasma membrane were measured, as shown in Table 1. All three lysosomal-membrane preparations exhibited ATPase activity at pH 7 and 5'-nucleotidase activity at pH 5, although the ATPase activity in the normal lysosomal membrane was lower than in the other lysosomal-membrane preparations. In contrast, plasma-membrane preparations had very little acid 5'-nucleotidase activity, but had high 5'-nucleotidase activity at pH 8 (Table 1). In addition, the 5'-nucleotidase activities responded differently with respect to activation by the detergents Triton X-100 and sodium deoxycholate; the activity of the plasma membrane was stimulated by detergents at acid and alkaline pH by 30% and 48% respectively. The activity of membranes from Triton-filled lysosomes was slightly enhanced, by 8.3% and 7.5% respectively. Furthermore, ouabain and suramin, compounds that are known to inhibit plasma-membrane ATPase activity, were not inhibitors of lysosomal ATPase activity (Table 2). Also, the ATPase activity of the plasma membrane was somewhat more sensitive to the thiol-blocking reagent *N*-ethylmaleimide and less sensitive to inhibition by Nikkol [octyl(ethylene glycol) dodecyl ether] than was that of lysosomal membrane (Table 2). Thus plasma-membrane and lysosomal-membrane ATPase and 5'-nucleotidase activities behave differently and may be due to separate proteins. However, additional comment on the lysosomal ATPase activity is required.

The total ATPase activities of liver homogenates prepared from normal and Triton WR-1339-treated rats were compared. Table 3 shows that the homogenates from treated rats had a greater absolute amount of ATPase activity. Furthermore, this increase was specific for lysosomal-membrane ATPase activity, as the amount of plasma-mem-

Membranes were isolated from rat liver as described in the Experimental section. Photographic (a) and densitometric (b) recordings of gels obtained after electrophoresis of 30 μ g of each sample through 7.5% acrylamide are shown. Key: DM, membranes from dextran-filled lysosomes; MM, membranes from normal lysosomes after purification through metrizamide; LM, membranes from Triton-filled lysosomes; PM, plasma membranes. The gels were stained with Coomassie Blue. Arrows indicate the mobility of molecular-weight markers: β -galactosidase, bovine serum albumin, ovalbumin and trypsin.

Fig. 2. Glycoprotein profiles of lysosomal membranes and of plasma membranes after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Triton-filled lysosomes (a) and plasma membranes (c) were labelled with NaB^3H_4 after enzyme treatment with neuraminidase and galactose oxidase (●). Controls (○) were not subjected to enzyme treatment. A $47\text{ }\mu\text{g}$ portion of lysosomal-membrane protein and $60\text{ }\mu\text{g}$ of plasma-membrane protein were applied to 7.5% acrylamide gels. Periodate/Schiff-stained 9.5% gels of lysosomal membrane (b, $80\text{ }\mu\text{g}$) and plasma membrane (d, $150\text{ }\mu\text{g}$) are also shown. Arrows indicate the mobility of molecular-weight markers: β -galactosidase, bovine serum albumin, ovalbumin and trypsin.

Table 1. Enzyme activities of rat liver lysosomal-membrane and plasma-membrane preparations
Membranes were prepared and assayed as described in the Experimental section.

Preparation	Activity ($\mu\text{mol}/\text{min}$ per mg of protein)		
	ATPase	5'-Nucleotidase (pH 5)	5'-Nucleotidase (pH 8)
Triton-filled lysosomes	0.84	2.3	0.28
Dextran-filled lysosomes	0.63	2.9	0.34
Normal lysosomes	0.33	1.9	0.23
Plasma membranes	0.53	0.03	1.0

brane ATPase activity was unchanged (Table 3) and the total amount of *N*-acetyl- β -D-glucosaminidase was not significantly changed (normal, $4.02 \pm 0.88\text{ }\mu\text{mol}/\text{min}$ per g of liver; Triton-treated, $3.43 \pm 0.52\text{ }\mu\text{mol}/\text{min}$ per g). Therefore the greater ATPase activity of membranes from Triton-filled

lysosomes as compared with that of normal lysosomal membranes can be tentatively attributed to enzyme induction. In order to explore further the properties of lysosomal membranes and their possible biogenic relationship to plasma membranes, we next studied pulse-labelling *in vivo*.

Table 2. *Effect of inhibitors on ATPase activities of lysosomal and plasma membranes*

Rats were treated with Triton WR-1339, membranes were prepared, and activities were assayed as described in the Experimental section. Ouabain- and suramin-sensitive ATPase activities were measured in the presence of 100 mM-NaCl and 5 mM-KCl. The data are the means $\pm$ S.D. for three determinations.

Inhibitor	Inhibition of ATPase activity (%)	
	Lysosomal membrane	Plasma membrane
Ouabain (1 mM)	0.0 $\pm$ 2.7	19.3 $\pm$ 6.5
Suramin (200 μ g/ml)	1.5 $\pm$ 4.0	47.2 $\pm$ 8.1
<i>N</i> -Ethylmaleimide (1 mM)	0.4 $\pm$ 2.1	5.4 $\pm$ 2.9
Octyl(ethylene glycol) dodecyl ether (0.5%)	83.6 $\pm$ 3.2	64.0 $\pm$ 17.3

Table 3. *ATPase activities of liver before and after treatment of rats with Triton WR-1339*

Liver fractions were obtained as described in the Experimental section. ATPase assays were in 0.25 M-sucrose and the medium included 1 μ g of atractyloside/ml to inhibit mitochondrial ATPase activity, which was about 25% of the total homogenate values. The data are the means $\pm$ S.D. for three determinations.

Treatment	ATPase activity (μ mol/min per g of liver)		
	Homogenate	Lysosomal	Plasma membrane
None	3.80 $\pm$ 0.23	0.063 $\pm$ 0.006	3.71 $\pm$ 0.49
Triton WR-1339	4.32 $\pm$ 0.67	0.348 $\pm$ 0.059	3.67 $\pm$ 0.44

Table 4. *Radioactivity in lysosomes, lysosomal membranes and plasma membranes of rat liver after labelling with [14 C]leucine 36 h and with [3 H]leucine 3 h before the animal was killed*

A rat was treated with Triton WR-1339, 250 μ Ci of [14 C]leucine was injected, and 33 h later 1 mCi of [3 H]leucine was administered. The rat was killed 3 h later, and cell fractions were prepared as described in the Experimental section. Trichloroacetic acid-precipitable radioactivity was measured and corrected for spill-over and counting efficiency, and was then normalized for 1 mCi per injection.

Fraction	Protein radioactivity (d.p.m./mg of protein)		$^3\text{H}/^{14}\text{C}$ ratio	Apparent t_1 (h)
	^3H	^{14}C		
Homogenate	162 000	104 000	1.56	52
Lysosomes	268 000	101 000	2.65	23
Soluble	218 000	84 000	2.60	24
Membranes	401 000	193 000	2.08	31
Plasma membranes	191 000	111 000	1.72	42

Turnover of lysosomal-membrane and plasma-membrane proteins and glycoproteins

In order to facilitate comparative metabolic studies, small-scale preparations of lysosomes and plasma membranes from the same Triton-WR-1339-treated rat were used. The plasma-membrane preparations were of high quality (20-fold enriched) and were apparently not affected by the Triton treatment. The lysosomes were more than 60-fold enriched in *N*-acetyl- β -D-glucosaminidase. Relatively high yields of both plasma membranes (22%) and lysosomes (38%) were obtained, even though single livers were used.

To determine the relative turnover rates of the components of lysosomal membranes, double-

labelling experiments were performed. A pulse of [14 C]leucine at 36 h before the animal was killed was followed by a pulse of [3 H]leucine at 3 h. After isolation of lysosomes and plasma membranes, the $^3\text{H}/^{14}\text{C}$ ratio, an index of turnover rates, was measured in the various cell fractions. Table 4 shows that the lysosomal fraction had a higher $^3\text{H}/^{14}\text{C}$ ratio than homogenate, 2.65 compared with 1.56. Thus lysosomal proteins are degraded more rapidly than the average liver protein. Although part of this higher ratio can be attributed to the high degree of labelling of soluble lysosomal proteins, the membranous fraction also had a high ratio, 2.08. Lysosomal membranes were labelled to a much higher specific radioactivity than was plasma membrane (401 000 compared with 191 000 d.p.m./mg) at 3 h,

Table 5. Radioactivity in lysosomes, lysosomal membranes and plasma membranes of rat liver after labelling with [^3H]fucose for 1, 3 and 14 h

After initial injections of Triton WR-1339, each rat received 250 μCi of [^3H]fucose and was killed 1, 3 or 14 h afterwards. Plasma membranes, lysosomes and lysosomal membranes were prepared as described in the Experimental section. Apparent half-lives, calculated from the 3 h and 14 h values, assume first-order kinetics.

Fraction	Radioactivity (d.p.m./mg of protein)			Apparent $t_{1/2}$ (h)
	1 h	3 h	14 h	
Homogenate	31 100	12 700	8500	19
Lysosomes	193 000	138 000	61 000	9
Soluble	195 500	105 000	51 300	11
Membranes	208 000	475 000	151 000	7
Plasma membranes	32 200	102 000	46 750	10

suggesting that the proteins of the lysosomal membrane are not originating at the cell surface. The ratio of 2.08 indicates an apparent average half-life for the proteins of the lysosomal membrane of about 1.3 days. Heterogeneous rates of turnover were detected for the individual polypeptides of the membrane (results not shown).

With [^3H]fucose as a precursor, the incorporation of radioactivity into the glycoproteins of the lysosomal and plasma membranes was measured after 1, 3 and 14 h. At each time, the specific radioactivity of the lysosomal membrane was higher than that of the plasma membrane (Table 5). The fucose contents of lysosomal and plasma membranes were estimated to be 7 and 25 $\mu\text{g}/\text{mg}$ of protein respectively, by using the cysteine/ H_2SO_4 method (Dische & Shettles, 1948). Thus the 3–6-fold greater specific radioactivity found in the lysosomal membrane as compared with the plasma membrane is most probably not due to a 2–3-fold greater fucose content, but due to more rapid synthesis of these lysosomal-membrane glycoproteins. Fucose labelling at 3 and 14 h indicates that the carbohydrate of lysosomal-membrane glycoproteins may be disappearing with a $t_{1/2}$ of about 7 h and that of plasma-membrane glycoproteins with a $t_{1/2}$ of about 10 h.

After a 1 h pulse with [^3H]fucose, the lysosomal membrane was much more highly labelled than was the plasma membrane. Nevertheless the possibility that the labelled glycoproteins exist as precursors in plasma membranes was examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of fractions obtained from liver after labelling with [^3H]fucose for 1 h. Lysosomal membranes were extensively labelled in the high-molecular-weight region of the gel (Fig. 3), corresponding to the broadly periodate/Schiff-staining region (cf. Fig. 2*b*). The plasma-membrane proteins showed a diffuse labelling pattern, which lacked any obvious relationship to that of lysosomes (Fig. 3). Thus the labelling results fail to show a significant pool of lysosomal-membrane-protein precursor in the plasma membrane even at 1 h after labelling, and

Fig. 3. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of [^3H]fucose-labelled lysosomal and plasma membranes

Lysosomal membranes (a) and plasma membranes (b) were prepared from the same rat after a 1 h pulse with 250 μCi of [^3H]fucose. Preparations and electrophoresis were performed as described in Experimental section. Arrows indicate mobility of molecular-weight markers, as listed in the legend to Fig. 2. In each case 6000 c.p.m. was applied; recoveries were 96% for lysosomal membrane (a) and 74% for plasma membrane (b).

suggest that the glycoproteins of the lysosomal membrane do not originate in the plasma membrane.

Discussion

Rat liver lysosomal membranes appear to have a unique composition, as indicated by several kinds of

analyses. First, isolation of lysosomes by three very different techniques yielded membranes with strikingly similar polypeptide compositions, which were distinctly different from the polypeptides of plasma membrane. Two of the techniques used for isolation of lysosomes (Triton WR-1339 and dextran) depend on endocytic uptake of the exogenously supplied material. Yet the membranes isolated from normal lysosomes appeared to be similar with respect to polypeptide composition (Fig. 1). Secondly, it has been previously shown that both lysosomal and plasma membranes contain 5'-nucleotidase (Colbeau *et al.*, 1971) and ATPase activities (Schneider, 1977). Our findings suggest that these activities are not due to the same enzymes (Tables 1 and 2). In addition, we have been able to bind [³H]ouabain to plasma membrane in the presence of Na⁺ and MgATP. The lysosomal membrane, on the other hand, did not exhibit such binding (results not shown).

The kinetics of labelling of lysosomal membranes with leucine and fucose were surprisingly rapid, and may have significant implications for their metabolic activity and their function in the lysosomal system. In addition, the kinetics were quite different, the half-lives being about 1.3 days and 0.3 day for leucine and fucose respectively. The double-labelling study with leucine indicates that proteins in the lysosomal membrane are turning over somewhat faster than the average liver protein and faster than plasma-membrane proteins. The membranes of the lysosomes become labelled with [³H]fucose to a high specific radioactivity within 3 h. The plasma membrane is also maximally labelled within 3 h, but displays a lower turnover rate, the half-life being 10 h compared with 7 h for lysosomal membrane. This apparent lack of a precursor-product relationship suggests that the proteins and glycoproteins of the lysosomal membrane do not originate in the plasma membrane.

The rapid turnover of the lysosomal-membrane proteins indicates a high rate of membrane function, although the specific functions of this membrane are not known in detail. ATP-dependent acidification of lysosomes is perhaps carried out by the lysosomal membrane (Schneider, 1979), and the membrane protein involved is sensitive to proteolytic inactivation by endogenous proteinases (Schneider, 1981). Perhaps some proteins of this membrane are rapidly synthesized because of their susceptibility to the lysosomal hydrolases. A model for lysosomal-membrane degradation presented by Dean (1977) suggests that these membranes invaginate to transport intracellular material into lysosomes. The intralysosomal vesicles so formed would be subject to attack by the acid hydrolases. Thus the rate of degradation of the lysosomal-membrane proteins could be comparable with the rate of degradation of

the most rapidly turning-over components of the cell.

An alternative explanation is that the rapid labelling could be a result of a specific induction of the lysosomal system by Triton WR-1339 treatment. It has been reported that Triton WR-1339 leads to an induction of cathepsins B and D in rat liver (Dean, 1975). We have found that ATPase activity also increases after Triton WR-1339 treatment (Table 3); however, *N*-acetyl- β -D-glucosaminidase activity did not increase.

Wang & Touster (1975) have also used the double-labelling technique to study the turnover of lysosomes from Triton-WR-1339-treated rats, and reported relatively long half-lives, 3.7 and 4 days for membranes and soluble lysosomal enzymes respectively. However, these values were slightly lower than that which they observed for the average cytoplasmic protein (4.7 days). In our double-labelling study, a shorter interval between administration of differently isotopically labelled leucine was used (1.4 days rather than 4 days). As discussed by Poole (1971), the interval between injections is a crucially important parameter; intervals such as those used by Wang & Touster (1975) may give results that primarily reflect the turnover of the free leucine pool. This interval may be even more critical in studying the turnover of lysosomes, as the longer time points may result in an increased labelling of degradation products with [¹⁴C]leucine, resulting in lower apparent turnover rates. Labelling with L-[*guanidino*-¹⁴C]arginine has been reported to indicate a half-life of 1.2 days for lysosomal membranes (Segal, 1975). Also, our value of 1.75 days for the half-life of plasma-membrane proteins (Table 5) is in agreement with the previously reported value of 1.8 days (Arias *et al.*, 1969). Thus the apparent variance with the data of Wang & Touster (1975) is probably a result of our use of more appropriate labelling times. In a recent study (Yamamoto *et al.*, 1980), the incorporation of [³H]glucosamine into lysosomes was measured, and maximum labelling of the membrane occurred within 3 h. Our [³H]fucose labelling study showed similar kinetics (Table 5).

Inasmuch as liver plasma membrane is known to have functional domains (Wisher & Evans, 1975), and as newly synthesized plasma membrane in hepatoma cells appears to enter heterogeneous surface and internal pools (Doyle *et al.*, 1978), we cannot rigorously rule out that individual plasma-membrane proteins may be transported to lysosomes, e.g. turnover of plasma membrane probably depends on transfer to lysosomes. On the other hand, plasma membrane conceivably contains precursor proteins destined for the lysosomal membrane. Although soluble proteins of lysosomes may exist as higher-molecular-weight precursors (Erickson & Blobel, 1979), precursors to lysosomal-membrane proteins have not yet been identified. The possible existence

of such precursors in plasma membrane seems unlikely in view of the more rapid labelling kinetics (Tables 4 and 5) and higher molecular weights of lysosomal proteins (Figs. 1–3).

Previous studies have shown that plasma-membrane proteins are transferred into and out of the vacuolar system without being degraded, e.g. low-density-lipoprotein receptor (Goldstein *et al.*, 1976) and asialoglycoprotein receptor (Stockert *et al.*, 1980; Steer & Ashwell, 1980). Other studies have demonstrated the presence of plasma-membrane proteins in lysosomes (Hubbard & Cohn, 1975; Muller *et al.*, 1980a,b) and have been interpreted to mean that a representative piece of plasma membrane is internalized during formation of endocytic vesicles. The latter systems may represent abnormal endocytosis, as large amounts of surface membrane are required to internalize large quantities of latex beads. Since we detect little, if any, plasma-membrane proteins in lysosomes, we suggest that normal endocytosis may occur with a shuttle system that facilitates extensive re-utilization of membrane, as previously suggested (Steinman *et al.*, 1976), and that minimizes extensive mixing of intrinsic proteins.

Our labelling data indicate that the carbohydrate moieties of the glycoproteins may be turning over faster than the peptide portions. Goldstein & Koenig (1974) have noted that the *N*-acetylneuraminic acid residues of the soluble proteins within lysosomes are turning over more rapidly than the protein backbones, and they suggest that this disparity may be the result of lysosomal glycohydrolase activity. Since the carbohydrate moieties of the membrane glycoproteins are presumably exposed to the soluble enzymes, they may be similarly 'processed'.

In conclusion, lysosomal membranes appear to have a unique and characteristic protein and glycoprotein composition. Further characterization and purification of the individual proteins may reveal their specific functions and explain their rapid rate of biosynthesis and degradation.

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