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Hepatic endosome fractions contain an ATP-driven proton pump

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Endosome fractions were isolated from rat liver homogenates on the basis of the subcellular distribution of circulating ligands, e.g. ^{125}I-asialotransferrin internalized by hepatocytes by a receptor-mediated process. The distribution of endocytosed ^{125}I-asialotransferrin 1–2 min and 15 min after uptake by liver and a monensin-activated Mg^{2+}-dependent ATPase activity coincided on linear gradients of sucrose and Nycodenz. The monensin-activated Mg^{2+}-ATPase was enriched relative to the liver homogenates up to 60-fold in specific activity in the endosome fractions. Contamination of the endosome fractions by lysosomes, endoplasmic reticulum, mitochondria, plasma membranes and Golgi-apparatus components was low. By use of 9-aminoacridine, a probe for pH gradients, the endosome vesicles were shown to acidify on addition of ATP. Acidification was reversed by addition of monensin. The results indicate that endosome fractions contain an ATP-driven proton pump. The ionophore-activated Mg^{2+}-ATPase in combination with the presence of undegraded ligands in the endosome fractions emerge as linked markers for this new subcellular organelle.

Cell surfaces are endowed with a variety of receptors that have the capacity to bind and transport circulating ligands to intracellular compartments where the receptor–ligand complexes dissociate and are then processed independently. A variety of ligands have been shown to be transferred from the plasma membrane to complex membrane networks located beneath the cell surface and in the Golgi–lysosome axis (Pastan & Willingham, 1981; Brown *et al.*, 1983; Geuze *et al.*, 1983; van Deurs & Christensen, 1984). Studies using pH-sensitive fluorescent ligands administered to various cells have indicated that the intracellular compartments where the ligands are concentrated are able to acidify their interiors rapidly (Tycko & Maxfield, 1982; van Renswoude *et al.*, 1982; Geisow & Evans, 1984). The probable mechanism of acidification is believed to involve an ATP-dependent proton pump (Yamashiro *et al.*, 1983) and a pH of below 6 generated inside these vesicles facilitates the dissociation of receptor–ligand complexes.

ATP-dependent proton pumps have been studied in lysosomes (Schneider, 1981; Okhuma *et al.*, 1982; Harikumar & Reeves, 1983), clathrin-coated vesicles (Forgac *et al.*, 1983; Stone *et al.*, 1983), chromaffin granules (Cidon & Nelson, 1983), and the Golgi apparatus (Glickman *et al.*, 1983; Zhang & Schneider, 1983). In the present paper we investigate whether hepatic endosome fractions show properties compatible with the presence of an ATP-driven proton pump. The endosome fractions were isolated from liver homogenates by following the subcellular distribution of ^{125}I-asialotransferrin (Debanne *et al.*, 1982), a ligand that binds to the hepatocyte's asialoglycoprotein receptors, is endocytosed, and is then released into blood after partial resialylation (Regoeczi *et al.*, 1982*a*,*b*). We now show that ^{125}I-asialotransferrin and an ionophore-activated Mg^{2+}-ATPase show a similar subcellular distribution. This result, combined with the demonstration that the endosome vesicles acidified their interiors after addition of ATP, a process reversed by monensin, indicates that hepatic endosomes can regulate their internal pH, a property that is probably linked to their role in receptor–ligand dissociation and processing.

Abbreviations used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Mg^{2+}-ATPase, Mg^{2+}-dependent ATPase.

Methods

Preparation of subcellular fractions

The endosome fractions were prepared as described by Debanne *et al.* (1982), with the

following modifications. Livers were perfused *in situ* with ^{125}I-asialotransferrin, Type 3 (1 μg/100 g rat wt.) for 1–2 or 15 min. Livers were removed and homogenized in 0.25 M-sucrose (3 ml/g wet wt.) with a Dounce homogenizer (clearance 0.072 mm), filtered, and a nuclear pellet was obtained by centrifugation at 1000 g for 10 min and washed a further two times. The combined nuclear supernatant was centrifuged at 33000 $g_{av.}$ for 8 min at speed (Beckman type 30 rotor), and the supernatant was collected, ensuring that the fluffy layer on the pellet was not disturbed. The supernatant (50 ml/rat liver) was layered on sucrose gradients in 38 ml tubes containing 1 ml of 70% (w/v) sucrose, 5 ml of 43% sucrose and a continuous gradient made by mixing 7.5 ml of 40% and 7.5 ml of 15% sucrose. After centrifugation for 3½ h at 28000 rev./min. (Beckman SW.28 rotor), 1 ml fractions were collected from the gradients. Fractions D (density 1.095–1.117 g/ml at 4°C), E (density 1.125–1.140 g/ml) and P (density 1.140–1.160 g/ml) were collected. Fraction D was diluted with an equal volume of water (final volume 3–4 ml) and fractionated further on continuous Nycodenz gradients constructed by mixing 6 ml of 27.6% (w/v) Nycodenz and 6 ml of 13.8% Nycodenz dissolved in 8% sucrose on a 1 ml cushion of 70% sucrose. After centrifugation for 18 h at 25000 rev./min in the SW.28 rotor, 20 0.8 ml fractions were collected and analysed. Membranes in the pooled peaks from the sucrose and Nycodenz gradients were pelleted and resuspended in 0.25 M-sucrose for transport studies. Fraction density was determined by refractometry.

Golgi-apparatus fractions (Golgi-light, -intermediate and -heavy) were prepared from rat liver homogenates as described by Bergeron (1979). A Golgi 'intact' fraction was also prepared by the rapid procedure of Bergeron *et al.* (1982). Endosome-depleted Golgi-apparatus fractions were prepared as follows. The fluffy layer on the multilaminate pellet remaining after removal of the supernatant used for preparation of fractions D, E and P (see above) was collected, adjusted to density 1.15 g/ml by addition of 70% sucrose, and Golgi subfractions were prepared by flotation to the 8%/20.5%, 20.5%/29.5% and 29.5%/39.5% (w/v) interfaces, as described by Bergeron (1979). Lysosome fractions were prepared from rat liver homogenates as described by Wattiaux *et al.* (1978), and liver plasma-membrane subfractions as described by Wisher & Evans (1975).

Enzyme and ligand-binding assays

Mg^{2+}-ATPase in the presence of 600 μM-ouabain, 1.3 μM-monensin or 1 μM-FCCP and 5′-nucleotidase activities were measured as described by Saermark & Vilhardt (1979), by using Malachite Green to estimate phosphate release. Galactosyltransferase was measured as described by Vischer & Reutter (1978), with ovalbumin as acceptor, and alkaline phosphodiesterase as described by Razzel (1963). Succinate dehydrogenase was measured as described by Saermark *et al.* (1984). Acid phosphatase was measured at pH 5 with *p*-nitrophenyl phosphate as substrate (Engstrom, 1961). Adenylate cyclase activity was determined by using a cyclic AMP assay kit (Amersham International). Protein was measured by the method of Lowry *et al.* (1951); protein in the Nycodenz gradients was measured by dye-binding, by the Bio-Rad protein assay. Asialo-orosomucoid binding was determined as described by Pricer & Ashwell (1976).

9-Aminoacridine-uptake studies

Membrane fractions (150–300 μg) were incubated at 25°C for 30 min in 200 μl-capacity cuvettes containing 20 mM-Tes, 0.5 mM-EGTA, 1 mM-$MgCl_2$, 0.5 mM-ouabain and 250 mM-sucrose, pH 7.0. 9-Aminoacridine (1 μM) was added (Schuldiner *et al.*, 1972) and the fluorometric tracings were recorded by using a computer display. ATP, monensin and oligomycin were added, to final concentrations of 0.2 mM, 1.0 μM and 10 μg/ml respectively.

Materials

Human asialotransferrin Type 3 was a gift from Dr. E. Regoeczi, McMaster University, Hamilton, Ontario, Canada. Monensin, 9-aminoacridine and other reagents were purchased from Sigma. Nycodenz [5-(*N*-2,3-dihydroxypropylacetamido)-2,4,6-tri-iodo-*NN′*-bis-(2,3-dihydroxypropyl)isophthalamide] was obtained from Nyegaard and Co., Oslo, Norway, and densities were calculated from refractive indices by reference to tables provided by the manufacturer.

Results

Co-fractionation in density gradients of ^{125}I-asialotransferrin and monensin-activated Mg^{2+}-ATPase

The subcellular localization of Mg^{2+}-ATPase activity and endocytosed ^{125}I-asialotransferrin Type 3 was compared by fractionation in sucrose gradients of a supernatant of rat liver homogenates from which nuclei, mitochondria, lysosomes, plasma membranes and major Golgi-apparatus components had first been removed by differential pelleting. The position in the sucrose gradient of the endocytosed ^{125}I-asialotransferrin depended on the time elapsed after injection of the ligand into the portal vein. Thus, 1–2 min after injection into the portal vein, ^{125}I-asialotransferrin was recovered in fraction E (density 1.125–1.140 g/ml),

and 15 min later the intact ligand was recovered mainly in fraction D (density 1.095–1.117 g/ml) (Fig. 1*a*). A similar time-dependent transfer of other ligands [including prolactin, insulin and asialo-(alkaline phosphatase)] endocytosed by liver and recovered in fractions of low density also occurred (Evans *et al.*, 1983; W. H. Evans, unpublished work). The position in the sucrose gradient of ^{125}I-asialotransferrin at these times coincided very closely with two peaks of monensin-activated Mg^{2+}-ATPase activity (Fig. 1*b*). Also present in components sedimenting in these density ranges were asialo-glycoprotein-binding sites (Fig. 1*c*). Relatively little monensin-activated Mg^{2+}-ATPase activity was present in fraction P (density 1.14–1.16 g/ml). The ^{125}I-asialotransferrin and Mg^{2+}-ATPase specific activities also showed a similar distribution when the fractions in the sucrose gradients containing the highest amount of the ligand 15 min after uptake (fraction D) were centrifuged in Nycodenz gradients. Fig. 2 shows that endosome fraction D yielded two subfractions, designated DN1 (peak density 1.090 g/ml) and DN2 (density 1.115 g/ml), and that there was a coincidence between the density positions of ^{125}I-asialotransferrin and Mg^{2+}-ATPase activity.

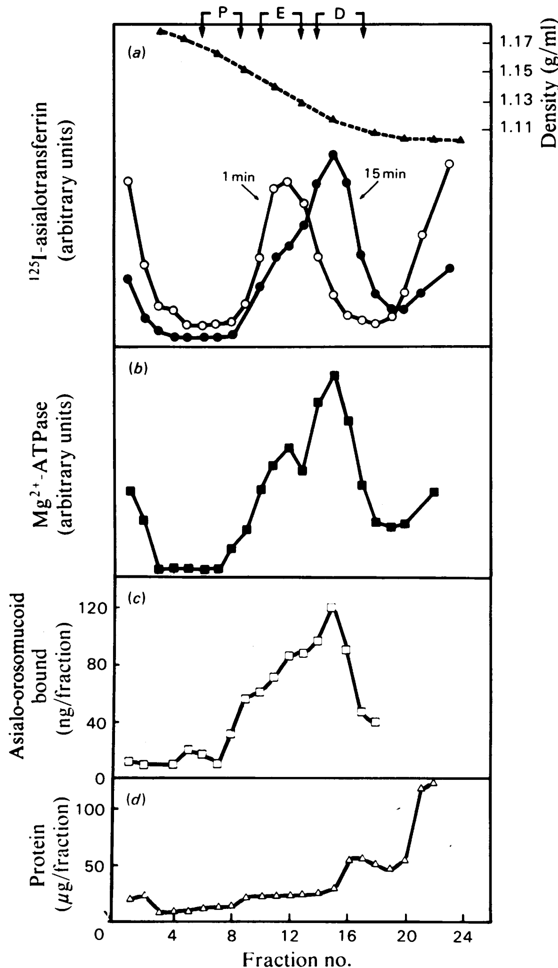

Fig. 1. *Distribution of (a) ^{125}I-asialotransferrin, (b) monensin-activated Mg^{2+}-ATPase, (c) asialo-orosomucoid binding and (d) protein on sucrose gradients*

Livers were homogenized in 0.25 M-sucrose 1 min and 15 min after administration via the portal vein of ^{125}I-asialotransferrin, and then fractionated as described in the Methods section. The density ranges for collection of fractions P, E and D are indicated. Fractions E and D corresponded to the position in the gradient of the ^{125}I-asialotransferrin at 1 min and 15 min respectively.

Biochemical characterization of the endosome fractions

Table 1 shows a 20–63-fold increase in specific activities of ^{125}I-asialotransferrin and monensin-activated Mg^{2+}-ATPase in endosomal fractions D and E, and fractions DN1 and DN2 derived from fraction D when it was centrifuged in Nycodenz gradients (Fig. 2). Table 1 also records the specific

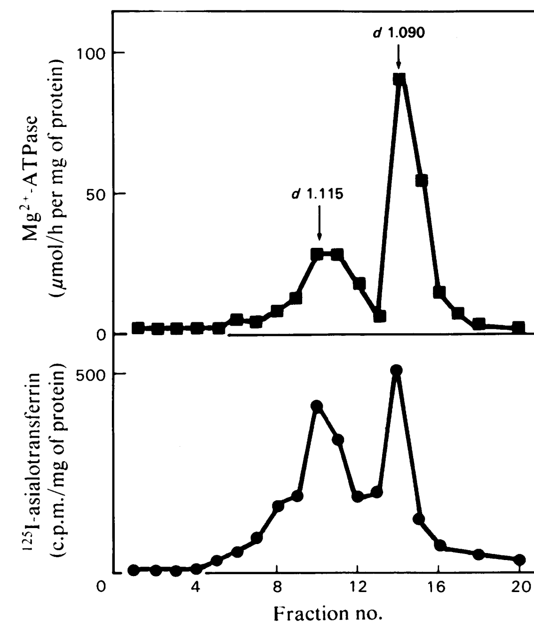

Fig. 2. *Distribution of (a) monensin-activated Mg^{2+}-ATPase and (b) ^{125}I-asialotransferrin when endosome fraction D prepared 15 min after administration of label (see Fig. 1) was fractionated on Nycodenz gradients*

The positions in the density gradients of the peak tubes of enzyme specific activity and radioactivity are indicated.

Table 1. *Distribution of ^{125}I-asialotransferrin and various enzymic markers in rat liver endosome and Golgi fractions*

^{125}I-Asialotransferrin distribution is expressed as specific activity in the respective fractions relative to that of liver homogenates prepared 15 min after injection into the portal vein. Enzyme activities are expressed as μmol/h per mg of protein, except for galactosyltransferase, which is expressed as nmol/h per mg of protein. Values represent mean of two or three ($\pm$S.D.) separate preparations. The recovery of protein in fractions D and E was 0.6–1.2 mg/g rat liver wet wt. and in fractions DN1 and DN2 0.10–0.25 mg/g liver wet wt. The recovery of ^{125}I-asialotransferrin from liver homogenates prepared 15 min after administration of the ligand in endosome fraction D was 17.9 $\pm$ 1.9% of the radioactivity in homogenate particulate material; the recoveries at the same time interval in endosome-depleted (ED, prepared as described in the Methods section) Golgi fractions was 4.1 $\pm$ 0.7%, and in a Golgi fraction prepared as described by Bergeron *et al.* (1982) was 9.7%. The recoveries from homogenates of the enzymes in the endosome fractions were: adenylate cyclase, D and E <1%; 5′-nucleotidase, D, 6%, E 3.7%; alkaline phosphodiesterase, D 2.5%, E 2.7%; galactosyltransferase, D 25%, E 6%; acid phosphatase, D 0.3%, E 0.8%; NADPH–cytochrome *c* reductase, D 1.75%, E 4.41%. The recovery of galactosyltransferase in the Golgi fractions was 35–40%. Owing to the multiplicity of ATPase activities in the homogenate, recovery of monensin-activated Mg^{2+}-ATPase was not determined.

Fraction	^{125}I-asialo-transferrin	Monensin-activated Mg^{2+}-ATPase	Adenylate cyclase	5′-Nucleo-tidase	Alkaline phospho-diesterase	Galactosyl-transferase	Acid phosphatase	NADPH-cytochrome *c* reductase	Succinate dehydrogenase
Homogenate	1	0.77	0.069	2.52	1.96	2.23	3.6	1.3	1.41
Endosome D	18.5	15.1	0.115	16.5	6.5	34.5	0.75	1.74	<0.05
Endosome E	–	12.6	0.136	–	6.9	9.4	2.0	3.59	<0.05
Endosome DN1	32	49.0 ± 15.9	–	10.5	10.4	<1	<0.1	0.98	–
Endosome DN2	29	21.7 ± 9.0	–	5.3	6.6	12.5	<0.1	2.41	–
Golgi (ED)	–	–	–	–	–	197	–	–	–
Golgi	–	–	–	–	–	220	–	–	–

activities and recoveries of marker enzymes for other subcellular components. Mitochondria (succinate dehydrogenase) were absent and lysosomes (acid phosphatase) were recovered in very low amounts in these fractions. Contamination by endoplasmic reticulum (NADP–cytochrome *c* reductase) was also low, with the highest recovery being recorded in fraction P pooled from the sucrose gradient (Fig. 1). Galactosyltransferase was increased in specific activity in endosome fractions D and E, but on the Nycodenz gradients activity was confined mainly to subfraction DN2. The specific activities and recoveries of galactosyltransferase in the endosome fractions D, E, DN1 and DN2 were lower than those obtained in the Golgi preparations used in the studies. In the Golgi fractions prepared by a modification of the Bergeron (1979) method designed to minimize the contribution of endosome components (see the Methods section), recovery of ^{125}I-asialotransferrin administered 15 min before preparation of liver homogenates was only 5%, compared with the 18% recovered in endosome fraction D (Table 1). When an 'intact' Golgi fraction (Bergeron *et al.*, 1982), which probably also contained appreciable amounts of endosomal components, was prepared, the recovery of ^{125}I-asialotransferrin was 9%. These results extend previous work (Debanne *et al.*, 1982) showing that the fractions used in the present work differed biochemically from Golgi fractions. At least one of the endosome fractions now described (DN1) was largely free of galactosyltransferase activity. The endosome fractions showed low activities and recoveries of adenylate cyclase, a plasma-membrane marker. However, higher recoveries and specific activities of 5′-nucleotidase and alkaline phosphodiesterase were obtained, especially in fraction DN1, in agreement with the results presented for an endosomal fraction prepared from rat hepatocytes by alternative methods (Luzio & Stanley, 1983). Taken together, the results suggest that these two plasma-membrane markers are also located in intracellular, probably endosomal, membranes, recovered in the present work in subfraction DN1. Finally, morphological studies of the fractions (Debanne *et al.*, 1982; Evans *et al.*, 1983) indicated they comprised smooth vesicles, and that no coated vesicles were present.

Ionophore activation of Mg^{2+}-ATPase activity

The ionophore activation of the Mg^{2+}-ATPase activities in endosome fractions D and E, fraction P and the Golgi fractions was investigated. Table 2 shows that the monensin- and FCCP-activated Mg^{2+}-ATPase was present in fractions D and E, but not in fraction P. The activation was concentration-dependent within the range 0.1–10 μg/ml

Table 2. *Effects of ionophores on Mg^{2+}-ATPase activity in hepatic subcellular fractions*
Monensin and FCCP were included in incubation mixtures at 1 μg/ml. Fractions were prepared as described in the Methods section. The Golgi fractions tested were endosome-depleted, prepared as described in the Methods section. Results are means ± S.D. for the numbers of experiments given in parentheses. Abbreviation: N.D., not determined.

Fraction	Mg^{2+}-ATPase (μmol/h per mg of protein)	Activation (%)	
		Monensin	FCCP
Endosome D	9.6 (2)	162 ± 19 (3)	153 ± 10 (3)
Endosome E	8.5 (2)	125 ± 12 (3)	145 ± 15 (2)
P	0.8 (2)	92 ± 5 (3)	90 ± 10 (3)
Golgi-light	0.2 (2)	98 (2)	N.D.
Golgi-intermediate	0.9 (2)	104 (2)	N.D.
Golgi-heavy	2.0 (2)	105 (2)	N.D.

with both ionophores. Golgi-apparatus fractions prepared from the post-nuclear pellet by conventional methodology after removal of the supernatant used to prepare fractions D, E and P (endosome-depleted Golgi fractions) contained Mg^{2+}-ATPase activity that was not activated by monensin. Furthermore, Mg^{2+}-ATPase activity in a plasma-membrane fraction was not activated by monensin.

The Mg^{2+}-ATPase activity measured in the endosome fractions was specific. Inclusion of ouabain in the reaction mixtures made a contribution by $Na^{+}+K^{+}$-activated ATPases to overall activity unlikely. Furthermore, a contribution to ATP-hydrolysing activity by nucleotide pyrophosphatases was eliminated, for in some measurements a coupled enzyme assay (Saermark & Vilhardt, 1979) was used. Finally, the negligible succinate dehydrogenase activity in the fractions (Table 1) made it unlikely that mitochondria contributed to the ATPase activity.

ATP-dependent 9-aminoacridine uptake

When the endosome fractions D and E were incubated with 1 μM-9-aminoacridine, a fluorescent amine shown to be a probe for pH gradients (Schuldiner *et al.*, 1972), the addition of ATP produced a decrease in its fluorescence (Fig. 3). This change is interpreted to be caused by an ATP-dependent protonation of the 9-aminoacridine taken up by the vesicles. Addition of monensin, shown to make membranes permeable to protons (Kinsky, 1970), resulted in a gradual increase in fluorescence in endosome fractions D and E (Fig. 3). In parallel experiments, lysosomal fractions, shown previously to contain an ATP-dependent acidification mechanism (Okhuma *et al.*, 1982; Schneider, 1981) behaved in a similar way, with the decrease in 9-aminoacridine fluorescence after addition of ATP reflecting a lowering of intravesicular pH; addition of monensin resulted in a very rapid increase in fluorescence (Fig. 3). The two endosome subfractions collected from the Nycodenz gradient, DN1 and DN2, were also shown by monitoring the fluorescence of 9-aminoacridine to acidify after addition of ATP (Fig. 3).

The quenching of 9-aminoacridine fluorescence by other hepatic subcellular fractions was also examined. A liver plasma-membrane fraction did not quench 9-aminoacridine fluorescence in the presence of ATP (results not shown). Ca^{2+} uptake was demonstrated in the plasma-membrane fraction (3.2 nmol/15 min per mg of protein), indicating that the fraction contained non-leaky membrane vesicles. Liver Golgi-apparatus fractions, prepared as described by Bergeron (1979), were able to quench 9-aminoacridine fluorescence (results not shown), in agreement with the results presented by Glickman *et al.* (1983) and Zhang & Schneider (1983). However, when endosome-depleted Golgi fractions were examined, they did not quench 9-aminoacridine fluorescence in the presence of ATP (results not shown). Fraction P, collected from the sucrose gradient (Fig. 1), gave a similar result, for it also had no effect on the fluorescence intensity of 9-aminoacridine in the presence of ATP (Fig. 3).

Discussion

The present work shows that a series of low-density subcellular fractions, where endocytosed ^{125}I-asialotransferrin was concentrated relative to the amounts in the liver homogenate, contained vesicles that acidified their content when ATP was added. The acidification, monitored by fluorimetry by using the pH-sensitive dye 9-aminoacridine, was reversed by addition of monensin. These observations, made *in vitro*, parallel the behaviour shown in morphological studies of endocytic vesicles containing fluorescein-labelled α_2-macroglobulin in fibroblasts (Yamashiro *et al.*, 1983). Low-density fractions containing endocytosed fluorescein-labelled dextran prepared directly on Percoll gradients from macrophages, fibroblasts and BHK cells also contained vesicles that

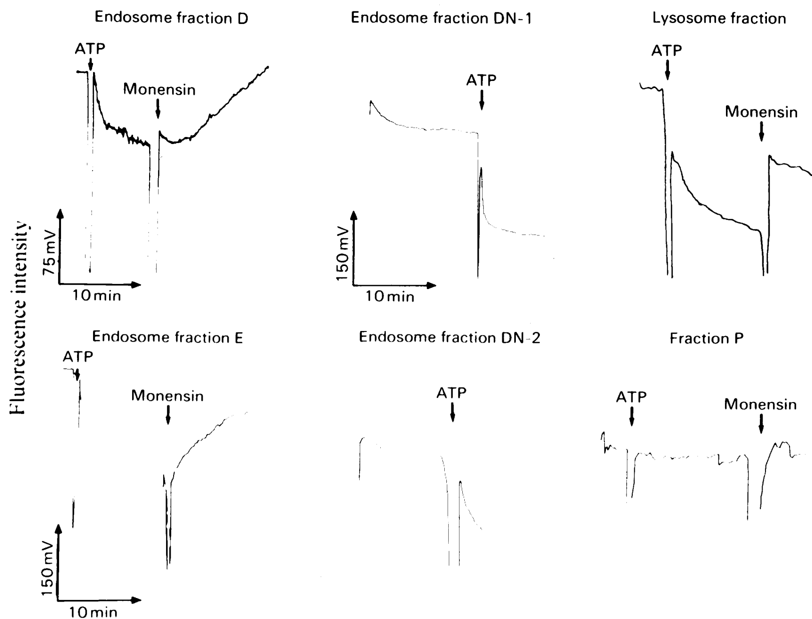

Fig. 3. *Time course of ATP-dependent acidification of endosomes, lysosomes and P-fraction*
Fractions (about 150–300 μg of protein) were incubated with 9-aminoacridine, and fluorescence intensity (excitation at 430 nm, emission at 475 nm) was measured. After establishment of a steady baseline, addition of ATP caused a decrease in fluorescence intensity (endosomes, lysosomes), indicative of a decrease in internal pH; this was reversed slowly (endosome fractions) or rapidly (lysosomes) by addition of monensin. Addition of ATP or monensin was without effect on fraction P and also on plasma membranes and endosome-depleted Golgi fraction (results not shown). For further details of preparation of subcellular fractions and assay conditions, see the Methods section.

acidified when ATP was added (Galloway *et al.*, 1983). The biochemically characterized hepatic endosome fractions now isolated contained a monensin-activated Mg^{2+}-ATPase of high specific activity relative to the homogenate. The enzyme activity and endocytosed ^{125}I-asialotransferrin co-distributed in the sucrose and Nycodenz density gradients used in the purification of the endosome fractions. This enzyme probably corresponds to the H^{+}-transporting ATPase responsible for the acidification of the vesicles in the endosome fractions.

Acidification has also been demonstrated by others in subcellular fractions containing lysosomes, secretory and clathrin-coated vesicles and Golgi apparatus. However, the endosome fractions used in the present work contained low amounts of these organelles. Contamination of the fractions, especially DN1 and DN2 isolated on the Nycodenz gradients, by lysosomes was negligible, and fraction DN1 contained little galactosyltransferase activity, a biochemical marker for the Golgi apparatus. Coated vesicles were absent from the fractions on the basis of morphology (Evans *et al.*, 1983) and polypeptide composition (Evans, 1985). Thus the acidification produced by an ATP-driven proton pump appears to be an intrinsic property of the endosome vesicles and is unlikely to be due to cross-contamination by other organelles shown to acidify *in vitro*. The results identify the monensin-activated component of Mg^{2+}-ATPase activity as a subcellular marker for endosome fractions, to be used in conjunction with the presence of undegraded ligands.

The presence in membrane fractions deriving from the endocytic compartments of an ATP-driven proton pump provides a mechanism to account for the pH-mediated dissociation of receptor-bound asialoglycoproteins (Harford *et al.*, 1983) and toxins and viruses (Meirion *et al.*, 1983). Transfer to lysosomes from the endocytic compartment results in rapid degradation of asialoglycoproteins (Geisow & Evans, 1984). In a control experiment, it was shown that lysosomes also acidified when Mg^{2+}–ATP was added, in agreement with the results of Okhuma *et al.* (1982) and Schneider (1981). The dissipation of the pH gradient caused by monensin was much more rapid with the lysosome fraction than with the endosome fractions, and this difference may relate to a

different susceptibility of the membranes of these organelles to ionophores. It is uncertain whether the ATPase responsible for proton pumping in endosomes and lysosomes is the same enzyme.

The Mg^{2+}-ATPase activity of liver plasma-membrane fractions was not activated by monensin, and the fractions did not quench 9-aminoacridine, although Ca^{2+} uptake was demonstrated in the present work, in agreement with others (Famulski & Carafoli, 1982). It is therefore of considerable interest that coated microvesicles contain a proton-transporting ATPase (Forgac *et al.*, 1983; Stone *et al.*, 1983). Coated vesicles showed a 10-fold higher specific activity of Mg^{2+}-ATPase than in plasma-membrane fractions prepared from brain homogenates (Blitz *et al.*, 1977; Saermark & Thorn, 1982). Since coated vesicles arise, at least in part, from coated pits involved in receptor retrieval at the plasma membrane (Pearse & Bretscher, 1981), it is possible that ATP-generated proton pumps, if present at the cell surface, may be restricted to those plasma-membrane domains where coated pits form and that these membrane fragments were not recovered in the plasma-membrane fractions used in the present work. The absence of $Na^{+}+K^{+}$-activated ATPase in bovine brain vesicles (Saermark & Thorn, 1982) also supports the possibility that they arise from restricted regions of the plasma membrane.

Golgi-apparatus subcellular fractions were able to acidify in an ATP-dependent fashion, as demonstrated by changes in the fluorescence of 9-aminoacridine, in agreement with results for rat liver Golgi fractions (Glickman *et al.*, 1983; Zhang & Schneider, 1983). However, it is well appreciated that Golgi-apparatus subcellular fractions are extremely heterogeneous, especially when prepared from secretory cells (Farquhar & Palade, 1981). In view of similarities in buoyant densities, it is likely that endosomal components contaminate Golgi fractions, and this may explain the recovery in these fractions of asialotransferrin (Table 1) and polypeptide hormones (Desbuquois *et al.*, 1979). The endosome-depleted Golgi fractions examined in the present wotk contained an Mg^{2+}-ATPase that was poorly activated by monensin, and we could not demonstrate acidification in the presence of ATP. These modified Golgi fractions were enriched, relative to tissue homogenates, in galactosyltransferase activity, but low amounts of ^{125}I-asialotransferrin were recovered in the fraction. However, in addition to the possibility that endosome vesicles recovered in the Golgi-apparatus fractions are the major repository of the ATP-activated proton-pumping activity, two other explanations cannot at present be discounted. First, it may be that galactosyltransferase and the proton-activated ATPase are differentially located in the Golgi apparatus complex. A second possibility that cannot be disregarded is that isolated Golgi vesicles were leaky and released the protonated 9-aminoacridine. For the present, we cannot distinguish between these possibilities.

In summary, the present work shows that endosomal vesicles purified from liver homogenates were able to acidify when ATP is present. A monensin- and FCCP-activated Mg^{2+}-ATPase emerges as a biochemical marker for the fractions, in addition to the presence inside the vesicles of intact ligands and their corresponding receptors. Studies on the uptake of fluorescent asialoglycoproteins by isolated hepatocytes indicate that the pH in the endosome compartments is 5.0–5.5 (Geisow & Evans, 1984). The endosome fractions now studied containing endocytosed undegraded asialoglycoproteins (Debanne & Regoeczi, 1981; Geisow & Evans, 1984) may originate from the peripheral and juxtanuclear compartments identified in morphological studies of the intracellular routing in animal cells of ligands and receptors (Hopkins, 1983).

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