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Biochimica et Biophysica Acta 1327 (1997) 162–170

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

A membrane protein primarily associated with the lysosomal compartment

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Received 21 November 1996; revised 17 February 1997; accepted 27 February 1997

Abstract

A monoclonal antibody designated MBR 39 has been generated against a membrane associated protein found selectively on lysosomes. MBR 39 reacts with the cytosolic face of the lysosome and was used to develop an organelle binding assay which reacted with high density organelles characteristic of lysosomes. These organelles contained lysosomal enzyme markers which included the integral membrane protein acetyl-CoA:α-glucosaminide *N*-acetyltransferase and the soluble lysosomal enzyme markers acid phosphatase (mature form), β-hexosaminidase, arylsulfatase, and α-L-iduronidase. Under conditions which disrupt lysosomes the release of the latter soluble lysosomal enzymes was demonstrated from MBR 39 bound organelles. Immunoblots of MBR 39 with purified fibroblast lysosomal membrane, demonstrated reactivity with polypeptides of molecular mass 63 kDa (major species) and 73 kDa (minor species). © 1997 Elsevier Science B.V.

Keywords: Lysosome; Biogenesis ; Membrane protein; Monoclonal antibody; Antibody; Organelle purification

1. Introduction

The molecular composition of the vacuolar network, which includes the endoplasmic reticulum, Golgi, endosomes, carrier vesicles and lysosomes, and the control of protein targeting in these organelles, have yet to be fully defined. However, some aspects of the biogenesis of lysosomes and the intracellular transport of constituent lysosomal proteins have already been described [1–5]. In general, newly synthesized secretory, plasma membrane, lysosomal membrane and soluble lysosomal proteins follow a common pathway of synthesis in the rough endoplasmic reticulum and transport to the trans-Golgi network [6]. Selective lysosomal transport is mediated through mannose-6-phosphate receptors [4,7], although other mechanisms of lysosomal targeting exist, suggesting further complexity in this transport process, particularly for integral membrane proteins [3,4,8].

The identification of human integral lysosomal membrane proteins or lysosomal associated membrane proteins (LAMPs) have been reported [9–12], and include hLAMP-1 and hLAMP-2 [13–15]. The major proportion of both hLAMP-1 and hLAMP-2 molecules reside on the lumenal side of the lysosomal membrane, and may function to protect the lysosomal membrane against degradation [16]. The pathway of intracellular traffic of hLAMP-1 and

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PII S0005-2736(97)00053-9

hLAMP-2 molecules to the lysosome is similar to that for many soluble lysosomal hydrolases which interact with the mannose-6-phosphate receptor, but targeting is mediated by specific cytoplasmic tail sequences. Other integral or membrane bound enzyme markers such as β-glucocerebrosidase and acetyl-CoA:α-glucosaminide *N*-acetyltransferase (*N*-acetyltransferase) have been described, however like hLAMP-1 and h-LAMP-2, show a cellular distribution which is not restricted to the lysosome [2,3,17].

Some lysosomal proteins show a more restricted organelle distribution, frequently showing either no detectable expression on the cell surface or in endosomal membranes [4]. The pathways for the intracellular transport of lysosomal membrane proteins require further definition, particularly with regard to the added complication that lysosomes and related acidic organelles appear to be heterogeneous in function [18,19]. To address these problems, lysosomal organelles have been partially purified by density sedimentation to allow separation from mitochondria, peroxisomes and plasma membrane. However, the diversity of acidic and other membrane bound organelles has made it difficult to obtain pure preparations. To resolve the question of contaminating organelles in purified preparations, markers have been sought which assess organelle purity and some have been partially characterized [4].

In this paper a membrane associated protein is described which appears to show a restricted distribution on lysosomal organelles. A monoclonal antibody to this protein appears to react with the cytosolic side of the lysosome and has been utilized to purify lysosomal organelles on an analytical scale, from subcellular organelle preparations.

2. Materials and methods

The monoclonal antibody MBR 39 and the polyclonal antibody against human LAMP-1 (hLAMP-1) were generated as previously described [20,21]. For MBR 39, placenta was used as an antigen source as this allowed the purification of preparative quantities of lysosomal membrane. The lysosomal membrane used for immunization was from Percoll-enriched lysosomal organelles, which were lysed and salt washed to remove soluble proteins and weakly associated membrane proteins [20]. MBR 39 (IgM subclass antibody) was produced as a culture supernatant from the hybridoma cell line. The monoclonal antibody 4-S 4.1 (also designated ASB 4.1) was produced and characterized as previously described [22]. Sheep anti-mouse immunoglobulin antibodies (peroxidase-labelled, un-conjugated, or FITC-labelled) were purchased from Silenus Laboratories, Melbourne, Australia. Digitonin (saponin), Percoll (colloidal polyvinylpyrolidone coated silica) was from Sigma, St. Louis, MO, USA. Colloidal Indian ink was Pelican drawing ink A (17 Black) from Pelican AG, Hannover, Germany.

2.1. Fibroblast culture and harvesting

Human diploid fibroblasts were cultured as previously described [20], and were used to characterize the intracellular distribution of the antigen detected by MBR 39. For organelle fractionation experiments fibroblasts were harvested by removing the cell culture medium, washing the cell layer twice with phosphate-buffered saline (PBS) before detaching the cells by trypsin digestion and vigorous agitation as previously described [23]. 5 ml of PBS containing 1% (v/v) fetal calf-serum was used to suspend the cells which were then centrifuged at $200 \times g$ for 10 min and washed twice by resuspension in 0.25 M sucrose. Cells from ten to fifteen 75 cm^2 flasks were suspended in 2.5 ml of 0.25 M sucrose, disrupted by repeated (10–20-times) hypobaric shock [24], and the homogenate centrifuged at $200 \times g$ for 10 min at 4°C to remove cellular debris and unbroken cells. The post-nuclear supernatant was centrifuged at $6000 \times g$ for 15 min at 4°C and the pellet resuspended in 0.25 M sucrose, 10 mM Hepes buffer (pH 7.0) for fractionation.

2.2. Fibroblast organelle fractionation

Granular fractions were subfractionated on self generating Percoll gradients in 0.25 M sucrose, 10 mM Hepes buffer (pH 7.0). Gradients were centrifuged and fractionated as previously described [25]. Fractions were isolated from the top of the gradient, then either washed in 0.25 M sucrose (pH 7.0) (by centrifugation at $6000 \times g$ for 15 min at 4°C), for binding assays, or washed then freeze-thawed six

times to disrupt the organelles for lysosomal enzyme analysis.

2.3. Organelle immunobinding assay

Fibroblast organelles from granular fractionations were bound to MBR 39 as previously described [20]. Briefly, sheep anti-mouse Ig antibody was bound to a polyvinyl chloride plate and used to capture MBR 39 monoclonal antibody from culture supernatant (approximately 10 μg/ml Ig). Separate subcellular fractions from Percoll fractionated fibroblast organelles (in 0.25 M sucrose, 10 mM Hepes buffer (pH 7.0)), were then incubated on individual wells coated with monoclonal antibody for 2–4 h at 20°C. Unbound organelles were removed by aspiration and each well washed three times with 200 μl of 0.25 M sucrose, 10 mM Hepes buffer (pH 7.0). Wells were then assayed for lysosomal enzyme activities or used for antibody analysis.

2.4. Lysosomal enzyme activities

Acid phosphatase activities were determined using 4-methylumbelliferyl phosphate [26]. β-Hexosaminidase (β-*N*-acetylglucosaminidase) activity was determined by the method of Leaback and Walker [27]. α-L-Iduronidase activity was determined using methylumbelliferyl α-L-iduronide [28] and arylsulfatase activity using 4-methylumbelliferyl sulfate [22]. *N*-Acetylgalactosamine-4-sulfatase (4-sulfatase) activity was determined by the method of Brooks et al. [29]. *N*-Acetyltransferase activity was determined by the method of Hopwood and Elliott [30]. Glucose-6-phosphatase and alkaline phosphatase activities were determined as previously described [31,32].

2.5. Western blotting

Purified lysosomal membrane from placenta [20], was resuspended in electrophoresis sample buffer (0.02 M Tris-HCl buffer (pH 7.0), 5% (v/v) mercaptoethanol, 1% (w/v) SDS, 10% (v/v) glycerol) and 50–100 μg of membrane protein (in 100 μl of sample buffer) electrophoresed and immunoblotted as previously described [33].

2.6. Immunofluorescence

Fibroblasts cultured in microscope slide chambers were washed twice with 1 ml of PBS per chamber, the wash buffer removed and cells fixed in 0.5 ml of 2% (v/v) formaldehyde in PBS, after 10 min at 20°C the slide chambers were emptied and chambers washed with 1 ml of methanol and followed by two separate incubations with 1 ml of 10% (v/v) fetal calf serum in PBS for 15 min. Each chamber was incubated with monoclonal antibody culture supernatant (1 ml) containing 0.15% (w/v) digitonin (saponin) for 2 h at 20°C. The culture supernatant was aspirated and each chamber subjected to three 5 min washes with 1 ml of PBS containing 10% (v/v) fetal calf serum. Each chamber was incubated with 0.5 ml of a 1:15 dilution of FITC-conjugated sheep anti-mouse Ig antibody in PBS containing 10% (v/v) fetal calf serum for 1 h at 20°C, and following three washes with 1 ml of PBS each, the chamber mount was removed (from the slides) and 20 μl of 50% (v/v) glycerol in PBS added to the slide before addition of a coverslip. Cells were inspected by epifluorescence microscopy.

3. Results

3.1. Preliminary characterization of MBR 39

In preliminary experiments a monoclonal antibody designated MBR 39 was shown to react by ELISA with lysosomal membrane and in an organelle binding assay reacted with vesicles which contained β-hexosaminidase, acid phosphatase and arylsulfatase enzyme activities [20].

3.2. Immunofluorescence

To visualize the intracellular distribution of the MBR 39 antigen, human fibroblasts were reacted with the monoclonal antibody MBR 39 and an FITC-labeled second antibody. Non-permeabilized cells were not reactive with MBR 39 demonstrating little or no detectable cell surface antigen expression. However, saponin-treated cells revealed immunofluorescent organelles with a characteristic perinuclear distribution (Fig. 1A). MBR 39 appeared to consis-

tently react with at most a subset of the organelles detected by a polyclonal antibody against hLAMP-1, when tested on different samples of the same fibroblast cell lines (see representative example Fig. 1A for MBR 39 and Fig. 1B for hLAMP-1).

3.3. Interaction of organelles with MBR 39

The lysosomal nature of the organelles bound by the MBR 39 monoclonal antibody was substantiated in an organelle immunobinding assay using fibroblast post-nuclear supernatant, granular fraction and lysed granular fraction (Fig. 2). MBR 39 bound organelles contained acid phosphatase, β-hexosaminidase, α-L-iduronidase and arylsulfatase activities, from post-nuclear supernatant and from granular fraction but not from either freeze-thawed granules (i.e., non-intact organelles), or from post-nuclear supernatant which had been centrifuged to remove the organelle fraction (Fig. 2a and Fig. 2b). Thus MBR 39 only interacted with soluble lysosomal enzymes where they were contained within intact organelles. Soluble

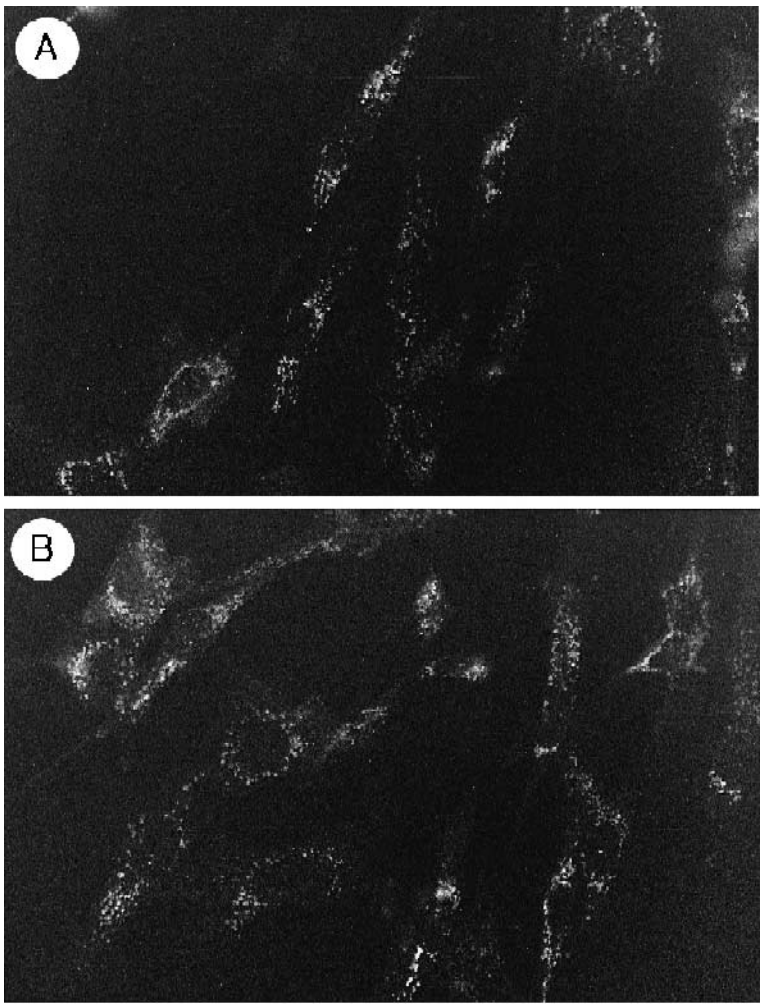


Fig. 1. The intracellular distribution of the MBR 39 protein as shown by immunofluorescence. In saponin-treated fibroblasts, MBR 39 reacted with organelles which showed a perinuclear distribution (Fig. 1A). No reactivity was observed on the cell surface either with or without saponin treatment (data not shown for without saponin treatment). MBR 39 only appeared to react with a subset of the organelles detected by a polyclonal anti-hLAMP-1 antibody, when tested on different samples of the same cell line (Fig. 1A for MBR 39 reactivity and Fig. 1B for hLAMP-1 reactivity). Fibroblasts stained with FITC-labeled second antibody only, showed little or no background reactivity (data not shown).

lysosomal enzyme activities could be released from the MBR 39 bound vesicles by freeze-thawing the organelles after attachment to the antibody (Fig. 3). The amount of lysosomal enzyme activities bound by MBR 39 were enriched in the granular fraction compared to the post-nuclear supernatant for all enzyme activities except acid phosphatase, suggesting that not all of the acid phosphatase activity is bound by MBR 39 (Fig. 2a). MBR 39 did not bind glucose-6-phosphatase activity, a microsomal marker, and did not bind alkaline phosphatase, a Golgi and plasma membrane marker, establishing specificity of this marker for lysosomal or endosomal organelles (Fig. 2).

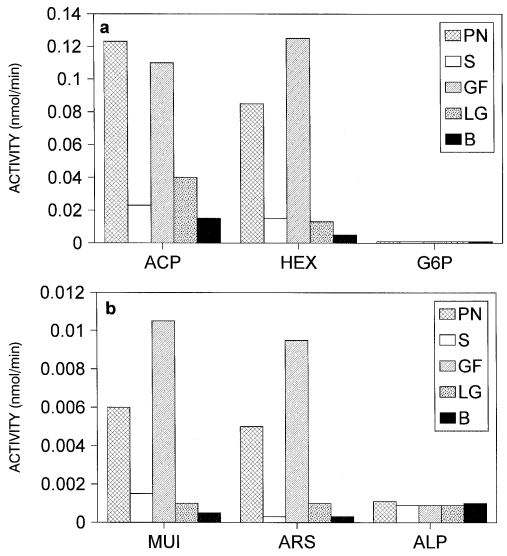


Fig. 2. An organelle binding assay was used to determine the nature of MBR 39 reactive vesicles. Organelles were from fibroblast preparations of either post-nuclear supernatant (PN), or post-nuclear supernatant minus granules (S), or granular fraction (GF), or lysed granular fraction (LG). Blank (B) represents substrate reactivity on MBR 39 antibody controls. Bound organelles (from 100 μl of sample) were assayed for either acid phosphatase (ACP), β-hexosaminidase (HEX), and glucose-6-phosphatase (G6P) shown in Fig. 2a, or α-L-iduronidase (MUI), arylsulfatase (ARS) and alkaline phosphatase (ALP) as shown in Fig. 2b. Results are a mean of duplicate determinants and are expressed as nmol min^{-1}. The total enzyme activities in 100 μl of fibroblast post-nuclear supernatant were: acid phosphatase (0.28 nmol min^{-1}), β-hexosaminidase (0.33 nmol min^{-1}), glucose-6-phosphatase (0.008 nmol min^{-1}), alkaline phosphatase (0.48 nmol min^{-1}), arylsulfatase (0.02 nmol min^{-1}) and α-L-iduronidase (0.03 nmol min^{-1}). The recovery of enzyme activities in MBR 39 bound organelles from the PNS starting material was up to 45%.

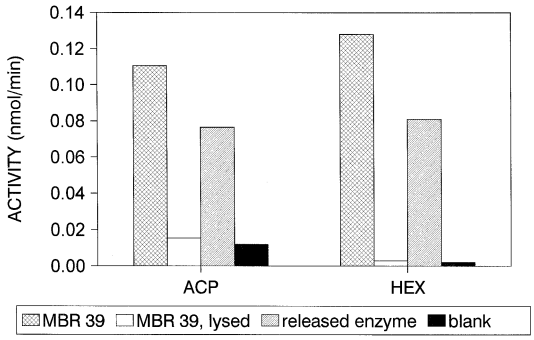
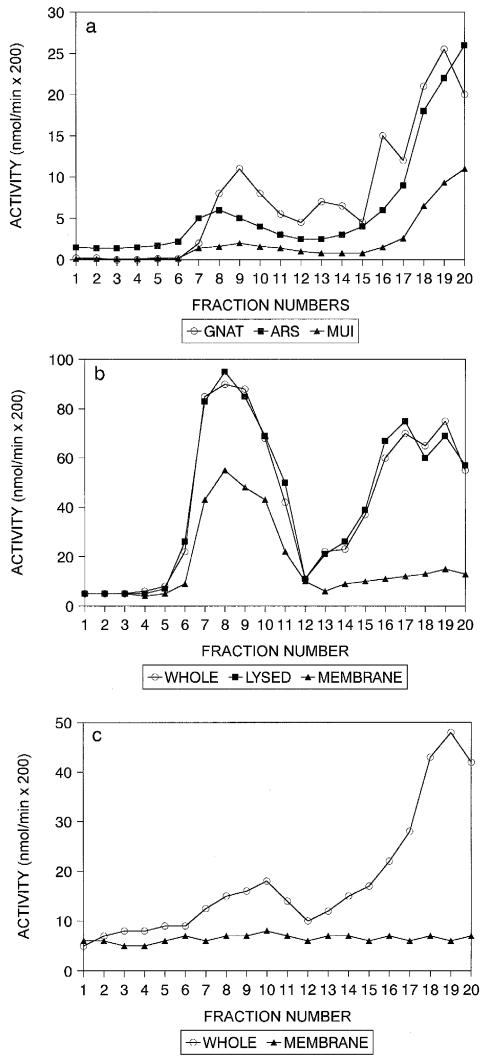


Fig. 3. Nature of enzyme released from MBR 39 bound organelles. Organelles from a fibroblast post-nuclear supernatant were bound to MBR 39 antibody, as described in Section 2. Organelles bound to MBR 39 were lysed by freeze-thawing the 96 well plate twice, and the supernatant assayed (released enzyme) for either acid phosphatase (ACP) or β-hexosaminidase (HEX) enzyme activity after centrifugation at $100\,000 \times g$, to remove any detached organelles or free membrane. The wells containing lysed organelles (MBR 39, lysed), were washed twice with 0.02 M Tris-HCl containing 0.25 M NaCl and 1% (w/v) ovalbumin before assaying either acid phosphatase or β-hexosaminidase enzyme activities. Blank, represents the substrate blank for the enzyme assayed. Enzyme activities were expressed as nmol/min and represent an average of duplicate results.

3.4. MBR 39 reacts with high density organelles characteristic of lysosomes

The subcellular distribution of MBR 39 reactive organelles was investigated using fibroblast granular fractions which had been separated on Percoll density gradients (Fig. 4). The analysis of acid phosphatase activity on fractionated organelles demonstrated two main peaks of enzyme activity, the first in low density organelles, characteristic of endosomes and the second in high density organelles, characteristic of lysosomes (Fig. 4b). Freeze-thawed organelles showed the same acid phosphatase enzyme activity as intact organelles indicating no latency in the assay of intact organelles (Fig. 4b). When the fractions from lysed organelles were centrifuged ($100\,000 \times g$) and washed in buffer A to give membrane only fractions, the acid phosphatase activity distributed almost exclusively into the endosomal peak (Fig. 4b). Thus, the soluble or lysosomal form of acid phosphatase could be released by organelle disruption, while under the same conditions the endosomal form of acid phosphatase remained associated with the membrane fraction.

The monoclonal antibody MBR 39 was used to bind organelles from the same Percoll gradient. The organelles binding to MBR 39 were characterized by the detection of acid phosphatase activity and found to be predominantly in the lysosomal peak, with only a small amount reacting with endosomal fractions (Fig. 4c). That MBR 39 bound organelles were lysosomes, was shown by the release of free acid phosphatase when the organelle fractions were freeze-thawed prior to binding with antibody (Fig. 4c). The lysosomal nature of these fractions was further substantiated by the coincident distribution of the lysosomal enzyme markers α-L-iduronidase, *N*-acetyltransferase and arylsulfatase with MBR 39 bound acid phosphatase (Fig. 4).



The lysosomal enzyme activities were also shown to be contained within the organelles bound by MBR 39 by the binding experiments with unfractionated organelle preparations (Fig. 2). The small amount of MBR 39 bound acid phosphatase activity, detected in low density endosomal fractions (Fig. 4c), appeared to be soluble acid phosphatase that was released upon organelle disruption. This may indicate either a small proportion of the MBR 39 antigen is localized in endosomes, or that there were some lysosomes co-fractionating with endosomes in the low density region of the gradient. The latter was supported by the observation that acid phosphatase reactivity did not bind to MBR 39 from this region of the gradient for lysed organelles (i.e., representing soluble lysosomal acid phosphatase, not the membrane bound endosomal form; Fig. 4c).

3.5. MBR 39 reacts with the cytosolic side of the lysosome

Without sequence information it is difficult to definitively identify the MBR 39 bound protein as an integral membrane protein. We have however attempted to determine whether the epitope detected by MBR 39 is on the cytosolic or the lumenal side of the

Fig. 4. MBR 39 primarily detects high density organelles characteristic of lysosomes. To establish the lysosomal reactivity of MBR 39, fibroblast granular fractions were subfractionated on Percoll density gradients and the purified organelles analysed for MBR 39 interaction and for the co-distribution of lysosomal markers. The lysosomal enzymes *N*-acetyltransferase (GNAT), arylsulfatase (ARS) and α-L-iduronidase (MUI) localized mainly in high density organelles (fractions 16–20, Fig. 4a). The organelles reacting with MBR 39(detected by acid phosphatase (ACP) activity), were also mainly in fractions 16–20 (Fig. 4c) and therefore coincident with high density lysosomes. The soluble mature form of acid phosphatase (lysosomal) was distinguishable from the membrane bound (endosomal) form of acid phosphatase (Fig. 4b). MBR 39 did not bind acid phosphatase from preparations of organelles which had been lysed and only reacted with organelles containing the soluble mature form of acid phosphatase (Fig. 4c). Whole, represents intact organelles; lysed, represents freeze-thawed organelles; membrane, represents lysed organelles where membrane fractions were separated from the soluble organelle contents. Figs. 4a and 4b, represents the direct assay of enzyme activities on organelle fractions, while Fig. 4c represents interaction with the monoclonal antibody MBR 39. All enzyme activities were expressed in nmol/min$\times$200.

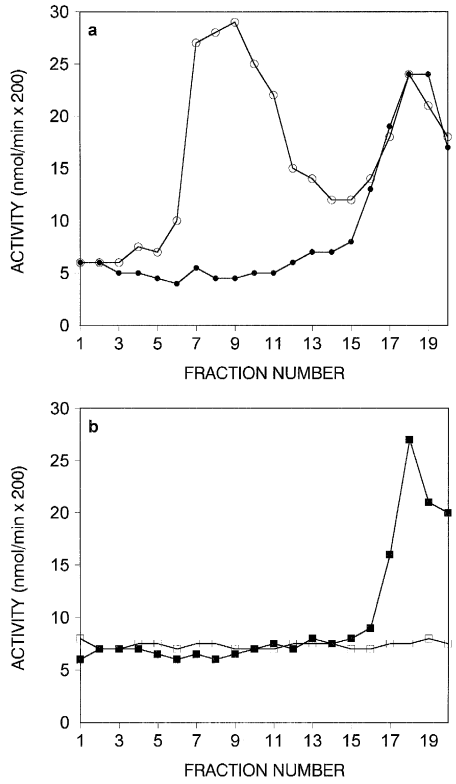


Fig. 5. MBR 39 reacts with the cytosolic side of the lysosome. Fibroblast granular fractions were subfractionated on a Percoll density gradient and the organelle fractions either assayed for acid phosphatase activity (○: Fig. 5a), and β-hexosaminidase activity (●: Fig. 5a) or reacted in an organelle binding assay with either anti-hLAMP-1 (□: Fig. 5b) or MBR 39 (■: Fig. 5b) monoclonal antibodies. Acid phosphatase activity localized into both a high density peak and a low density peak, while β-hexosaminidase localized selectively in the high density organelles characteristic of lysosomes. Antibody to hLAMP-1 failed to bind organelles containing acid phosphatase. In contrast MBR 39 bound high density organelles as measured by their acid phosphatase content. The latter organelles were coincident with those organelles containing β-hexosaminidase enzyme activity.

lysosomal membrane. Organelles from a fibroblast granular fraction separated on Percoll gradients, were incubated with either MBR 39 or a polyclonal anti-hLAMP-1 antibody. Fractions containing high density organelles with soluble acid phosphatase activity were bound to MBR 39 but did not interact with hLAMP-1 antibodies (Fig. 5). As the hLAMP-1 antibody has been shown to react with the lumenal side of the lysosome, it is suggested that the MBR 39 epitope is on the opposite or cytosolic side of the lysosome. The failure of hLAMP-1 to bind high density lysosomal organelles, which have been shown to contain soluble acid phosphatase, indicates that the organelles from this region of the Percoll gradient are intact and in the correct orientation (rather than disrupted and reformed in the reverse orientation, which would expose the hLAMP-1 reactive domain that has been shown to be on the lumenal side of the lysosome).

3.6. Immunochemical characterization of the polypeptide detected by MBR 39

Salt washed placental lysosomal membrane fractions [20], were solubilized in reducing sample buffer (containing 1% w/v SDS), electrophoresed and Western blotted using MBR 39 monoclonal antibody (Fig. 6). Two polypeptide species of molecular mass 63 kDa (major polypeptide) and 73 kDa (minor polypeptide) were identified by MBR 39 reactivity. The polypeptides identified showed only very weak cross reactivity with an IgM monoclonal antibody

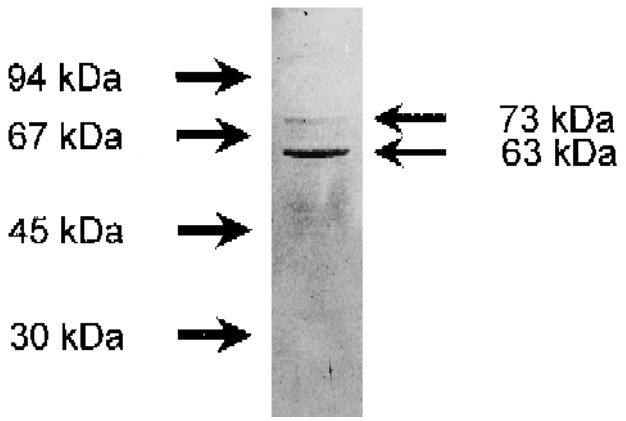


Fig. 6. Immunochemical characterization of the MBR 39 reactive protein. Purified placental lysosomal membranes (prepared as previously described in Brooks et al., 1993 [20]) were washed in 1 M KCI and 0.25 M NaCl (in 0.02 M Tris buffer (pH 7.0)) and immunoblotted using MBR 39 monoclonal antibody and a peroxidase labelled sheep anti-mouse Ig antibody. The molecular mass markers were as indicated on the left of the figure and are in kilodaltons (kDa). The results indicate that the MBR 39 reactive protein is membrane associated and is detected as two polypeptides of molecular mass, 63 kDa (major) and 73 kDa (minor). In a negative control immunoblot, another monoclonal antibody of the same IgM subclass (which reacts with another lysosomal membrane protein) had only very weak cross reactivity with these and other polypeptides (data not shown).

(same subclass as MBR 39) which reacted with another lysosomal membrane protein (not shown). As the MBR 39 reactive protein was not extracted from lysosomal membrane fractions by either 1 M KC1 or 0.25 M NaCl washes, but was solubilized in 1% SDS, this protein appears to be either tightly associated with the lysosomal membrane or possibly an integral membrane protein.

4. Discussion

A monoclonal antibody, MBR 39 was generated against purified preparations of placental lysosomal membrane and has been shown to react with a membrane associated protein on the cytosolic side of the lysosome. The MBR 39 reactive protein was not readily extractable by high ionic strength solubility but was detergent extractable indicating that it was either tightly associated with the lysosomal membrane or an integral lysosomal membrane protein [20]. Western blot analysis under reducing conditions demonstrated reactivity with two polypeptide species of molecular mass, 63 kDa (major) and 73 kDa (minor). Organelle binding studies indicated that the MBR 39 protein was located in lysosomes that contained the soluble lysosomal enzymes β-hexosaminidase, α-L-iduronidase, arylsulfatase and acid phosphatase. However, MBR 39 did not appear to react with either organelles containing Golgi markers or with the cell surface. Subcellular fractionation indicated that MBR 39 protein was distributed primarily in high density organelles which co-locate with soluble lysosomal enzyme markers, as well as an integral lysosomal membrane protein *N*-acetyltransferase. MBR 39 reacted with a subset of low density organelles characteristic of endosomes, but these organelles were shown to contain soluble acid phosphatase, suggesting they were lower density lysosomes. Thus, MBR 39 appears to present as a specific marker for lysosomal organelles.

The intracellular traffic of membrane associated molecules is crucial in the understanding of the structural and functional basis of endosomal and lysosomal heterogeneity and thus, the biogenesis of the vacuolar compartment. Endosome-associated antigens have been characterized [4,18,34–36], giving rise to considerable information on the individual compartments involved in the transfer of internalized molecules to lysosomes and the membrane composition and thus, structure/function of these specialized organelles. The endosomal antigens reported thus far, appear to be distinct from the protein detected by MBR 39, both in molecular size [18,36] and in their intracellular distribution. However, while MBR 39 showed predominantly lysosomal reactivity, it is likely that the molecule traffics through the endosomal compartment during its normal processing, as is the case for other lysosomal proteins and lysosomal membrane proteins.

The characterization of membrane proteins of the lysosome has also revealed several proteins which show predominantly lysosomal intracellular distribution [9,10,14,37]. However, the antigens identified to date do not appear to exhibit the same biochemical properties as the protein detected by MBR 39. The localization of the MBR 39 protein to the cytosolic side of the lysosome potentially provides a unique marker for lysosomal biogenesis studies. Moreover, the MBR 39 protein may have a key functional role in the lysosome due to its observed specific localization in lysosomal organelles.

The biogenesis of the lysosomal enzyme acid phosphatase (tartrate inhibitable form) has been characterized [38–41]. Lysosomal acid phosphatase is initially passaged from the endoplasmic reticulum to the trans-Golgi and to endosome vesicles (in $\sim$30 min) and at this stage has a short 19-amino-acid cytoplasmic tail, a membrane spanning domain and a highly glycosylated ectoplasmic domain [41]. This precursor form of the enzyme has been proposed to rapidly recycle between the endosome pool and the cell surface (5–6 h retention) before processing, via proteolytic clipping of the cytoplasmic tail and ectoplasmic domain, results in a soluble mature form of lysosomal acid phosphatase which is delivered to the lumen of the lysosome [41]. Percoll fractionation studies reported in this paper support the proposed processing differences between endosomally located membrane bound form and lysosomally located soluble form of acid phosphatase.

Peters et al. [41] concluded that endosome cell surface recycling may be an obligatory passage for the processing/maturation of many lysosomal enzymes. In our membrane binding studies and by immunofluorescence analysis we could demonstrate

little or no cell surface reactivity with MBR 39. Moreover, we concluded that at most, only minimal amounts of the MBR 39 protein resides in the endosome pool and that it is mainly localized in lysosomal organelles.

Acknowledgements

The authors gratefully acknowledge Christine Boulter for expert technical assistance, Kathy Nelson for maintenance of fibroblast cell cultures. This work was supported by a program grant from the National Health and Medical Research Council of Australia.

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