Regulation of glycosylation of Lamp-1 in the bovine renal epithelial cell line NBL-1 by changes in the concentration of extracellular phosphate

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We have identified the bovine renal homologue of Lamp-1 (lysosomal-associated membrane glycoprotein 1). It has very similar physical characteristics to other Lamp-1 proteins from a wide variety of tissues and species. Partial sequence analysis has shown it to be 61 % identical with human Lamp-1 and about 50 % identical with rat and mouse Lamp-1. The extent of glycosylation of bovine Lamp-1 alters in response to changes in the concentration of extracellular phosphate. Bovine renal epithelial cells (NBL-1) grown in normal or phosphate-starved medium contain Lamp-1 of 120 kDa. However, if cells are grown in medium containing 8–10 mM phosphate, they contain Lamp-1 of only 100 kDa. The core protein and mRNA levels have been shown to remain constant under both conditions. Therefore the only conclusion is that the extent of Lamp-1 glycosylation must be changing in response to the extracellular concentration of phosphate. Unlike Carlsson and Fukuda [(1990) *J. Biol. Chem.*

265, 20488–20495], who showed that the human Lamp-1 protein contained polylactosaminoglycan residues, we have been unable to demonstrate the partial deglycosylation of bovine Lamp-1 by endo- β -galactosidase. This enzyme removes polylactosaminoglycan groups from glycoproteins, and therefore indicates that the carbohydrate structure of bovine Lamp-1 is probably different from that of other Lamp-1 proteins. At present the physiological importance of bovine renal Lamp-1 and the changes in its extent of glycosylation are unknown. In this paper we postulate that Lamp-1 may be involved in the cycling of plasma-membrane proteins to the lysosome. This is based on the finding that the only other known effect of high extracellular phosphate on NBL-1 cells is to cause a decrease in the $V_{\max}$ of plasma-membrane-associated Na^+ -dependent phosphate transport [Helps and McGivan (1991) *Eur. J. Biochem.* **200**, 797–803].

INTRODUCTION

Lysosomal associated membrane glycoproteins (Lamps) are a family of proteins which have been found in many species and cell types [1–7]. As their name suggests, they are primarily located in the lysosome of the cell, but have also been found in the plasma membrane of several cell types [3–5]. All Lamps have a very similar structure, which consists of a core protein of about 400 amino acids (40 kDa). The C-terminus of the protein is located in the cytoplasm of the cell; this region also contains a single transmembrane α -helix which anchors the Lamp in the lysosomal membrane. Most of the core protein is located in the lysosomal space and is heavily glycosylated, presumably to protect the protein from proteolytic degradation.

The glycosylation of Lamp has been widely studied [3,4,6,8,9]. There are known to be 18 N-linked glycosylation sites on the protein, some of which are modified with variable numbers of *N*-acetyl-lactosamine residues. The resulting oligosaccharides are termed polylactosaminoglycans and are found on a wide variety of proteins, including blood-group antigens and tumour-associated antigens [10]. The result of this extensive glycosylation is that the final molecular mass of Lamp is between 110 and 130 kDa. Despite this extensive knowledge of the structure of Lamps, their precise role still remains to be elucidated.

In this paper we present evidence that exposure of the bovine renal epithelial cell line NBL-1 to medium containing 0 phosphate or 10 mM phosphate for 24 h causes newly synthesized Lamp-1 to change its molecular mass from 120 kDa to 100 kDa, respectively. This change in molecular mass is caused entirely by a difference in the glycosylation state of bovine Lamp-1, since both

the mRNA levels and core protein remain unchanged during exposure to high and low phosphate concentrations. The difference in glycosylation could be due to a change in the number or length of oligosaccharides associated with Lamp-1.

MATERIALS AND METHODS

Cell culture

NBL-1 cells were obtained from Flow Laboratories (McLean, VA, U.S.A.) at passage 121 and cultured in Ham's F-12 medium supplemented with 10 % foetal-calf serum, 2 mM L-glutamine, 100 units/ml penicillin G, 0.1 mg/ml streptomycin, 0.25 $\mu\text{g}/\text{ml}$ amphotericin B and 50 $\mu\text{g}/\text{ml}$ gentamycin, at 37 °C in a humidified gas phase of CO_2/air (1:19). Cells were grown in 75 cm^2 flasks for continuous culture and in 35 mm-diameter Petri dishes for experiments. Cells were trypsin-treated once a week, seeded into Petri dishes and fed every other day until confluent (5–7 days), when they were used for experiments.

Preparation of bovine renal brush-border membrane vesicles and their partial fractionation by lectin affinity chromatography

Bovine renal brush-border membrane vesicles were purified by the MgCl_2 precipitation method of Booth and Kenny [11]. Briefly, this involved removing the cortex from a fresh bovine kidney (wet wt. 400–500 g) and homogenizing in 300 mM sucrose/5 mM EGTA/12 mM Tris, pH 7.4, in a Waring blender at top speed. MgCl_2 (12 mM) was added to the homogenate and stirred on ice for 15 min before centrifugation at 2500 *g* for 15 min. The supernatant was collected and centrifuged at 31 000 *g* for 30 min.

Abbreviations used: Lamp-1, lysosomal-associated membrane glycoprotein 1; MEGA-10, decanoyl-*N*-methylglucamide; SSC, saline sodium citrate (0.15 M NaCl/0.015 M sodium citrate).

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The membrane pellet was resuspended in 200 ml of half-concentration homogenization buffer, and the above procedure was repeated. The final pellet of brush-border membrane vesicles was resuspended in 250 mM sucrose/10 mM Hepes, pH 7.4, centrifuged at 31 000 *g* for 30 min and resuspended in the above buffer at a concentration of 10–20 mg/ml.

The brush-border membranes were partially fractionated on a column of wheat-germ lectin by the following method. Membranes (5–10 mg/ml) were dissolved by using an equal volume of 5% MEGA-10 (decanoyl-*N*-methylglucamide) and centrifuged at 100 000 *g* for 30 min to remove insoluble material. The supernatant was applied to a 5 cm × 1 cm column of wheat-germ lectin–Sepharose, and the column was washed with 10 mM Tris/0.5% MEGA-10, pH 7.4, until the A_{280} of the eluate was zero. Bound protein was removed from the column by eluting with 100 mM *N*-acetylglucosamine/10 mM Tris/0.5% MEGA-10, pH 7.4, and the protein concentration was determined by the Bradford assay [12].

Monoclonal-antibody production

Monoclonal antibodies were produced against a variety of bovine renal brush-border membrane proteins in an attempt to produce antibodies to the Na⁺-dependent phosphate transporter. One of the monoclonal antibodies has subsequently been shown, by N-terminal amino acid sequencing, to bind to bovine renal Lamp-1.

The method employed to raise monoclonal antibodies used the hybridoma technology of Kohler and Milstein [13]. This involved injecting Balb/c mice over a 6-week period at 2-week intervals with 100 µg of the wheat-germ-lectin-purified protein fraction (shown previously to bind the Na⁺-dependent phosphate-transport protein [14]). The fraction was mixed with an equal volume of complete Freund's adjuvant for the first injection and incomplete adjuvant for the second two injections. Immune spleen cells were isolated and fused to the NSO myeloma cell line P3-NSO/1Ag 4.1 by using poly(ethylene glycol) as previously described [13]. Hybridoma tissue-culture supernatants were screened against NBL-1 cell membranes from cells incubated for 24 h in 0 and 10 mM phosphate medium by e.l.i.s.a. Antibodies showing different reactivity were screened by Western blotting and cloned by limiting dilution.

Immune ascites were induced by priming Balb/c mice intraperitoneally with 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane), leaving for 10 days and then injecting intraperitoneally 5 × 10⁶ hybridoma cells in 0.5 ml of PBS. After 10–14 days the ascitic fluid was removed and purified on a column of Protein G–Sepharose [15]. The resulting purified ascites (6 mg) were coupled to CNBr-activated Sepharose [16] and stored in PBS containing 0.01% Thimerosal to prevent bacterial growth.

Immunoaffinity purification of the 120 kDa protein recognized by the anti-Lamp-1 antibody

NBL-1 cell membranes (from one 75 cm² flask) were dissolved by using an equal volume of 5% MEGA-10/50 mM Tris, pH 7.4. The solution was centrifuged at 100 000 *g* for 30 min to remove insoluble material before being loaded on the antibody column. The solubilized membranes were recycled through the column two or three times, and it was then washed with PBS containing 0.1% MEGA-10 until the A_{280} of the eluate was zero. Protein bound to the column was eluted with 50 mM diethylamine, pH 11, containing 0.1% MEGA-10. The fractions containing

protein were pooled and adjusted to pH 7 with HCl before being concentrated to 50–100 µl (Centricon concentrator, Amicon). For N-terminal amino acid sequencing, 25 µl of this sample was run on SDS/PAGE (10% gel) and blotted to Pro-blott. The protein band was located by Coomassie Blue staining, excised and sequenced with an Applied Biosystems 477A/120A automated protein sequencer.

The internal amino acid sequence of bovine Lamp-1 was obtained by incubating affinity-purified Lamp-1 with N-glycanase (peptidyl-N-glycosidase F) in the absence of EDTA at 37 °C for 2–4 h. This not only removed the N-linked glycans, but also, due to its metalloprotease activity, cleaved Lamp-1 to give a protein fragment of 32 kDa. This protein fragment was subjected to SDS/PAGE on a 10% gel, transferred to Pro-blott and sequenced as described above.

Labelling of Lamp-1 in NBL-1 cells with [³⁵S]methionine during exposure to 0 and 10 mM phosphate

NBL-1 cells (1 × 75 cm² flask) were incubated in Ham's F-12 medium containing 0 or 10 mM phosphate and 5 µCi of [³⁵S]methionine/ml (sp. radioactivity > 1000 Ci/mmol) for 24 h. The cells were washed once with PBS and removed with a cell scraper before solubilization with MEGA-10. Immunoaffinity purification and SDS/PAGE were performed as described above. After Coomassie Blue staining, gels were soaked in Amplify (Amersham) for 20 min before drying and exposure to Hyperfilm-MP (Amersham).

SDS/PAGE and Western blotting

SDS/PAGE electrophoresis was performed by the method of Laemmli [17] and Western blotting by the method of Towbin et al. [18].

Localization of Lamp-1 in NBL-1 cells by immunofluorescence

Cells were grown to 50% confluency on glass cover-slips and washed for 3 × 5 min in PBS (cell-side up). The cells were fixed with methanol at –20 °C and incubated for 5 min at this temperature. The methanol was removed and the cells were washed in PBS before blocking in 0.2% BSA in PBS for 30 min. The cover slips were then placed cell-side down on to 70 µl of anti-Lamp-1 hybridoma tissue-culture supernatant and left for 30 min at room temperature. Cover slips were washed for 3 × 5 min in PBS, cell-side up. The second antibody (goat anti-mouse, conjugated with tetramethylrhodamine), diluted 1:500 in 0.2% BSA in PBS, was added in exactly the same way as the first antibody, and the mixture was incubated for 30 min in the dark. The cover slips were washed for 3 × 5 min in PBS before being rinsed in water, drained and mounted on glass slides by using 20–30 µl of Moviolt solution.

PCR, DNA sequencing and Northern-blot analysis

PCRs were carried out by standard protocols. The sequence of the PCR primers used is shown below. DNA sequencing was performed with a Sequenase V 2.0 kit from U.S. Biochemical Corp.

Primer 1

5' AAC/TGGG/AACCTGCCTGCTGGCC 3'

Primer 2

5' GAGGACGATGAGGACCAGCCC 3'

Northern-blot analysis of NBL-1 RNA was carried out by standard protocols. A 20 μ g portion of RNA isolated from NBL-1 cells by guanidine thiocyanate denaturation and extraction with phenol/chloroform/3-methylbutan-1-ol was denatured and run on a 1%-agarose/formaldehyde gel. The RNA was transferred to Hybond-N (Amersham), which was dried at 80 °C and pre-hybridized in 5 $\times$ SSC (SSC = 0.15 M NaCl/0.015 M sodium citrate)/5 $\times$ Denhardt's containing 0.5% SDS and 0.1 mg/ml salmon sperm DNA for 2 h at 65 °C. The DNA probe was labelled with [³²P]dCTP by using a random-primed labelling kit from U.S. Biochemical Corp. and added to the prehybridization mixture. Hybridization was allowed to continue overnight at 65 °C before washing the blot twice in 2 $\times$ SSC/0.1% SDS at room temperature for 20 min, once in 1 $\times$ SSC/0.5% SDS at 65 °C for 20 min and once in 0.1 $\times$ SSC/0.5% SDS at 65 °C for 20 min. The blot was exposed to Hyperfilm-MP (Amersham) for 24 h before being developed.

Materials

Foetal-calf serum and antibiotic/antimycotic (10000 units/ml penicillin G, 10 mg/ml streptomycin and 25 μ g/ml amphotericin B), Ham's F-12 medium (normal and phosphate-free), RPMI 1640 medium and gentamycin (50 mg/ml) were bought from Gibco (Paisley, Scotland, U.K.). [³⁵S]Methionine (sp. radioactivity > 1000 Ci/mmol) was purchased from Amersham. All other chemicals were of analytical grade.

MEGA-10 was synthesized by the method of Hildreth [19].

RESULTS

Figure 1 shows a silver-stained SDS/PAGE gel of the NBL-1 cell protein fraction which binds to the antibody column. We have shown by N-terminal protein-sequence analysis that the protein bound to this column is bovine Lamp-1. The gel clearly shows that bovine renal Lamp-1 has a molecular mass of 120 kDa, which is very similar to that described for other Lamp-1 proteins from different species and tissue [1–7]. The gel also shows the effect of N-glycanase on the molecular mass of Lamp-1. In the presence of this deglycosylating enzyme (specific for N-glycans), the molecular mass of Lamp-1 is decreased from 120 kDa to 38 kDa. This indicates that the core protein contains about 350 amino acids and that all the glycosylation is present as N-linked glycans. All of these characteristics are consistent with those of other Lamp-1 proteins, indicating that the bovine renal Lamp-1 described here is part of the Lamp-1 family of proteins.

To identify firmly that the protein being purified by the immunoaffinity column was actually Lamp-1, the N-terminal and some internal amino acid sequence were obtained. The alignment of this sequence to human Lamp-1 (its closest match) is shown in Figure 2: there is significant similarity between human and bovine Lamp-1 in both the N-terminal and internal sequences, although the proteins are from different tissues and species. Bovine Lamp-1 shows slightly less similarity to rat and mouse Lamp-1, and even less to chick Lamp-1.

A Western blot of NBL-1 cell membranes isolated from cells exposed for 24 h to various concentrations of phosphate is shown in Figure 3. The blot shows that the amount of Lamp-1 is decreased by incubation of cells for 24 h in high-phosphate medium, and also that there is an increase in the amount of a 100 kDa protein under the same conditions. This is supported by the data shown in Figure 4.

Figure 4 is a densitometric scan of the Western blot shown in Figure 3. It shows that there is 3-times as much of the 120 kDa

Figure 1 Silver-stained SDS/PAGE gel of bovine renal Lamp-1 before and after treatment with peptidyl-N-glycosidase F

Bovine renal Lamp-1 was isolated by immunoaffinity chromatography from bovine renal brush-border membrane vesicles. Portions (2, 4 and 6 μ g) were loaded on a 10% gel, subjected to SDS/PAGE and then silver-stained. Equal quantities of protein were also subjected to glycosidase-F treatment for 2–4 h at 37 °C before loading on the same gel. Tracks: (a) 2 μ g of bovine Lamp-1; (b) 4 μ g of Lamp-1; (c) 6 μ g of Lamp-1; (d) 2 μ g of deglycosylated Lamp-1; (e) 4 μ g of deglycosylated Lamp-1; (f) 6 μ g of deglycosylated Lamp-1.

Figure 2 Sequence similarity between NBL-1 Lamp-1 N-terminus and internal amino acid sequence and human Lamp-1 protein sequence

The N-terminal and some internal amino acid sequence was determined from immunoaffinity-purified bovine renal Lamp-1 by running the protein on SDS/PAGE (10% gel) and blotting on to a Pro-blott membrane. After staining with Coomassie Blue, Lamp-1 was excised and its amino acid sequence determined by using an Applied Biosystems automated protein sequencer. The protein sequence was aligned with that of human Lamp-1 by using protein alignment programs from Daresbury, U.K.

Figure 3 Western blot of NBL-1 cell membranes from cells incubated in 0–10 mM phosphate for 24 h and probed with the anti-Lamp-1 antibody

NBL-1 cells were incubated for 24 h in medium containing 0, 3, 7 or 10 mM phosphate. Cells were harvested and dissolved in MEGA-10 before 20 μ g of each preparation was run on SDS/PAGE (10% gel). The gel was transferred to nitrocellulose and probed with the anti-Lamp-1 antibody. Tracks: (a) cells grown in 0 mM phosphate medium; (b) cells grown in 3 mM phosphate medium; (c) cells grown in 7 mM phosphate medium; (d) cells grown in 10 mM phosphate medium.

Figure 4 Densitometric scan of the Western blot shown in Figure 3

The Western blot shown in Figure 3 was scanned with a Chromoscan 3 (Joyce-Loebl) scanning densitometer set on the reflectance setting. Tracks (a)–(d) are respectively scans of the lane containing NBL-1 cell membranes from cells incubated in 0 mM phosphate, 3 mM phosphate, 7 mM phosphate or 10 mM phosphate.

protein in cells incubated in zero-phosphate medium than there is in cells incubated in 10 mM phosphate medium. Hence, much less of the 120 kDa form of Lamp-1 is present when cells are exposed to excess phosphate. This is firstly because under these conditions no new 120 kDa protein is being synthesized (see below), and secondly the turnover rate of the protein may be increased. Conversely, the amount of the 100 kDa protein is much greater in cells incubated under conditions of excess phosphate. The increase in the amount of the 100 kDa protein does not account totally for the decrease in the 120 kDa protein seen under these conditions. This may be partly explained by an increase in the turnover rate of the 120 kDa protein. Also, it is possible that the antigenic epitope may be partially masked on the 100 kDa protein under conditions of SDS/PAGE and Western blotting by the change in glycosylation state.

To determine whether the 100 kDa protein was the result of protein synthesis *de novo*, or whether it was a breakdown product of the 120 kDa protein, it was decided to label metabolically Lamp-1 with [35 S]methionine under conditions of phosphate starvation and excess. An autoradiograph of the protein fraction which was purified on the anti-Lamp-1 antibody column and run on SDS/PAGE is shown in Figure 5. It is clear that cells incubated under conditions of phosphate starvation produce only the 120 kDa form of Lamp-1, whereas those exposed to 10 mM phosphate produce only the 100 kDa form of Lamp-1. When both forms are deglycosylated, they yield the same core protein of 38 kDa, indicating no change in protein structure. The only plausible conclusion from this result is that the extent of Lamp-1 glycosylation is decreased when cells are exposed to excess concentrations of phosphate.

In an attempt to produce a cDNA probe against bovine Lamp-1, a 520 bp PCR product was synthesized from NBL-1 cDNA by using PCR primers designed against similar regions of rat, mouse and human Lamp-1 cDNA. The cDNA probe was

Figure 5 Autoradiograph of NBL-1 cell membrane proteins purified by immunoaffinity chromatography from cells incubated in 0 and 10 mM phosphate medium in the presence of [35 S]methionine

NBL-1 cells were incubated in medium containing 0 and 10 mM phosphate in the presence of [35 S]methionine for 24 h. The cells were then harvested and dissolved in MEGA-10 before immunoaffinity purification using the anti-Lamp-1 antibody column. Tracks (a) and (b) contain labelled proteins bound to the antibody column from cell membranes isolated from cells grown in 0 mM phosphate and in 10 mM phosphate respectively. Tracks (c) and (d) contain the same protein fraction as tracks (a) and (b) respectively, except that it has been treated with peptidyl-N-glycosidase F.

used to screen a Northern blot of RNA isolated from NBL-1 cells incubated in 0 and 10 mM phosphate for 24 h. The results

Figure 6 Localization of bovine Lamp-1 in NBL-1 cells by using the anti-Lamp-1 antibody and immunofluorescence

NBL-1 cells were grown to 50% confluency on glass cover-slips and fixed with methanol. Cells were stained with the anti-Lamp-1 antibody and a second anti-mouse rhodamine-labelled antibody. Staining was detected by fluorescence of the rhodamine-labelled antibody.

(not shown) clearly showed that there was no difference in the levels of mRNA encoding bovine Lamp-1 between cells incubated in high and low phosphate. This result supports the finding that only the extent of Lamp-1 glycosylation is altered in response to excess phosphate and that the mRNA and core-protein levels remain the same under these conditions.

The PCR product encoding the C-terminal half of the protein was sequenced, and hence yielded nearly half of the bovine Lamp-1 cDNA sequence. From the data it is clear that bovine renal Lamp-1 most resembles human Lamp-1 from chronic myelogenous leukaemia cells (61% identity). There is about 50% identity with Lamp-1 from both mouse and rat (called Igpl20) tissue, whereas the identity with chick brain Lamp-1 (called LEP100) is only 47%.

Figure 6 shows a photograph of one NBL-1 cell which has been methanol-fixed and probed with the anti-Lamp-1 antibody and a rhodamine-labelled second antibody. The picture clearly shows that bovine Lamp-1 is found mainly in lysosomes or other intracellular vesicles such as endosomes. Very little, if any, Lamp-1 can be seen associated with the plasma membrane of the cell. This finding is again in agreement with that for Lamp-1 from other tissues and species, since in these cells Lamp-1 is located primarily in the lysosomes.

DISCUSSION

In this paper we have shown that Lamp-1 isolated from bovine renal epithelial cells has similarity to human, rat, mouse and chick Lamp-1 proteins. It shares their high degree of N-glycosylation, which can be altered by exposing the cells to 10 mM phosphate for 24 h. Bovine Lamp-1 has a molecular mass of 120 kDa in both normal and phosphate-starved NBL-1 cells, but in cells exposed to excess phosphate this value is decreased to 100 kDa. This change in the molecular mass of Lamp-1 is due solely to a decrease in the extent of glycosylation of the protein, since the core protein remains the same size (38 kDa) under both conditions. The mechanism for the decrease in the molecular mass of Lamp-1 under conditions of excess phosphate have not

been studied; however, it can be concluded that either the number of glycosylation sites or their length must be decreased to account for the observation.

Partial sequence analysis of the PCR product of bovine renal Lamp-1 show that it contains four cysteine residues which align with the conserved cysteine residues found in other Lamp-1 proteins. These residues have been proposed to be essential in the folding of Lamp-1 by the formation of disulphide bridges [20]. Hence, it can be concluded that bovine Lamp-1 probably has a very similar structure to Lamp-1 from other species.

The change in the molecular mass of bovine Lamp-1 is very similar to that shown by Carlsson and Fukuda [8] for Lamp-1 isolated from chronic myelogenous leukaemia and HL-60 cells. These authors showed that Lamp-1 could be decreased in molecular mass from 120 kDa to about 100 kDa by treatment with endo- β -galactosidase. This treatment removes polylactosaminoglycans from Lamp-1, hence indicating that about 20 kDa of human Lamp-1 is composed of polylactosaminoglycans. These carbohydrate groups are also found in many other cell types [21,22]. This finding is very similar to that observed in the present study of bovine Lamp-1 treated with excess phosphate. However, in the present study we have not been able to show, by treatment with endo- β -galactosidase (results not shown), that the change in molecular mass of bovine Lamp-1 is due to polylactosaminoglycans.

It should be noted that the change in the glycosylation state of Lamp-1 under conditions of phosphate excess is not the only change which takes place in NBL-1 cells. We have previously reported the effect of phosphate starvation on Na⁺-dependent transport of phosphate into NBL-1 cells [14]. Under conditions of phosphate starvation, the V_{max} is doubled and the K_m halved compared with cells incubated in medium containing 10 mM phosphate. This effect is dependent on protein synthesis, and hence is probably due to an increase in the number of transporters in the plasma membrane of the cell. We have no evidence at the present time that the change in glycosylation of Lamp-1 and the decrease in Na⁺-dependent phosphate transport, which both occur during exposure to excess phosphate, are linked. However, since no precise role has yet been defined for Lamp-1, it is possible that Lamp-1 may be involved in returning the transporter from the plasma membrane to the lysosome under conditions of excess phosphate. It has been shown for certain Lamp-1 proteins that between 7 and 10% are located in the plasma membrane of the cell [23]. It has also been shown for Lamp-1 from chick (called LEP100) that it cycles between lysosomes, endosomes and the plasma membrane [6,24]. Hence it would be possible for Lamp-1 to associate with the phosphate transporter and maybe cause its internalization and targeting to the lysosome.

The fact that no plasma-membrane staining could be observed with immunofluorescence studies of bovine Lamp-1 does not preclude its possible role in the regulation of phosphate transport. The phosphate transporter comprises a very small percentage of the plasma-membrane protein, and hence would only require a very small number of associated Lamp-1 molecules to cause its recycling. This small number of Lamp-1 molecules would probably not be visible above the background staining produced by immunofluorescence.

To date, the exact role of Lamp-1 in cellular function has still to be elucidated. The present study using bovine renal epithelial cells has shown that under conditions of phosphate excess the glycosylation state of Lamp-1 is altered. This is caused not by the partial deglycosylation of native Lamp-1, but by the synthesis of new Lamp-1 containing less oligosaccharide units. We have shown that this is not due to a change in the content of polylactosaminoglycans, as is the case for other Lamp-1 mole-

cules, and hence must be due to a change in the number or type of other carbohydrate moieties.

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