A Quantitative Model of Traffic between Plasma Membrane and Secondary Lysosomes: Evaluation of Inflow, Lateral Diffusion, and Degradation

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Abstract. We present here a mathematical model that accounts for the various proportions of plasma membrane constituents occurring in the lysosomal membrane of rat fibroblasts (Draye, J.-P., J. Quintart, P. J. Courtoy, and P. Baudhuin. 1987. *Eur. J. Biochem.* 170: 395–403; Draye, J.-P., P. J. Courtoy, J. Quintart, and P. Baudhuin. 1987. *Eur. J. Biochem.* 170:405–411). It is based on contents of plasma membrane markers in purified lysosomal preparations, evaluations of their half-life in lysosomes and measurements of areas of lysosomal and plasma membranes by morphometry. In rat fibroblasts, structures labeled by a 2-h uptake of horseradish peroxidase followed by a 16-h chase (i.e., lysosomes) occupy 3% of the cellular volume and their total membrane area corresponds to 30% of the pericellular membrane area. Based on the latter values, the model predicts the rate of inflow and outflow of plasma membrane constituents into lysosomal membrane, provided their rate of degradation is known. Of the bulk of polypeptides iodinated at the cell surface, only 4% reach the lysosomes every hour, where the major part (~83%) is degraded with a half-life in lysosomes of ~0.8 h. For specific plasma membrane constituents, this model can further account for differences in the association to the lysosomal membrane by variations in the rate either of lysosomal degradation, of inflow along the pathway from the pericellular membrane to the lysosomes, or of lateral diffusion.

THE high endocytic activity of mammalian cells results in a continuous internalization, intracellular flow, and recycling of the bulk of their pericellular surface (30). The major part of internalized plasma membrane polypeptides is recycled within minutes from a prelysosomal (endosomal) compartment without significant mixing with the membrane of secondary lysosomes (6). In rat fibroblasts, some plasma membrane constituents, traced by antibodies, were shown to reach the lysosomal compartment and to be recycled back to the cell surface (27, 28). However, a recent study performed with a mouse macrophage cell line, demonstrated that the transfer of plasma membrane constituents into lysosomes was limited and selective (15). The membrane composition of purified lysosomal preparations has been analyzed and found to be clearly distinct from the bulk of plasma membrane (7, 20). Moreover, several polyclonal (26) or monoclonal antibodies (8, 20, 21) have been isolated, which produce a prominent labeling of the lysosomal membrane, with little or no labeling of the pericellular surface, except at the ruffled border of osteoclasts (2).

Since the recycling pathway of internalized plasma membrane in mouse fibroblasts may include the lysosomal compartment (33), we have quantified the pool of plasma membrane constituents in lysosomes of rat fibroblasts, analyzed the composition of the lysosomal membrane, and estimated its turnover, to determine the proportion of constituents which can escape degradation and be recycled. At equilibrium, 4% of plasma membrane polypeptides covalently labeled by ^{125}I at the cell surface, were found to be associated with the lysosomal membrane (13). In rat fibroblasts, ~50% of 5′-nucleotidase is intracellular (36), but its distribution among the different intracellular organelles remains unknown. We have found that the proportion of 5′-nucleotidase and of alkaline phosphodiesterase I in the lysosomal membrane is strikingly different (12).

To account for such differences in composition, we present a model of membrane flow between the plasma membrane and lysosomes. This model is based on morphometrical data of the endocytic compartment of rat fibroblasts and on the measured half-life of plasma membrane ^{125}I-polypeptides. It takes into account three possible parameters for individual plasma membrane constituents: the rate of inflow into the prelysosomal vesicles, the rate of exchange (i.e., lateral diffusion into the lysosomal membrane), and the rate of degradation. Part of this work has previously appeared under abstract form (11).

The plasma membrane is defined here as the pericellular membrane together with intracellular membranes whose

constituents rapidly exchange with the pericellular membrane, since these two compartments are not resolved in this work.

Materials and Methods

Cell Culture

Rat embryo fibroblasts were obtained and cultured as described by Tulkens et al. (32). Experiments were carried out with confluent cultures grown on 35- or 150-mm plastic dishes in culture medium made of DME, supplemented with 10% newborn calf serum (lot n° U802901; Gibco Laboratories, Paisley, UK).

Iodination of Cell Surface Polypeptides

Cell surface polypeptides were iodinated at 4°C by the lactoperoxidase procedure, as described by Hubbard and Cohn (16). Cells were rinsed 3 times with PBS, and incubated for 15 min in PBS containing glucose (5 mM), lactoperoxidase (5 mU/ml), glucose oxidase (0.5–1.0 mU/ml), and Na[^{125}I] (5–100 μCi/ml). Cells were then washed at 4°C, twice for 10 min with culture medium supplemented with 10% of newborn calf serum, and finally four times with PBS. The cell-associated TCA-precipitable ^{125}I is hereafter defined as ^{125}I-label.

Cell Fractionation

Fibroblasts were homogenized in 0.25 M sucrose containing 1 mM EDTA and 3 mM imidazole, pH 7, as described in reference 13. Postnuclear particles were layered either on 32 ml linear sucrose (1.1–1.3 g/ml in density) or preformed linear Percoll gradients (15–65% vol/vol), and centrifuged in a rotor (model VTi50; Beckman Instruments, Inc., Palo Alto, CA) at 17 × 10^6 *g* min for sucrose gradients, and at 1.8 × 10^6 *g* min for Percoll gradients. 14 fractions were collected, weighed, and analyzed for density, protein, radioactivity, and enzyme activities.

Accumulation of HRP into Lysosomes and DAB Shift Procedure

Cells were incubated for 2 h in a medium containing 200 μg/ml ^{3}H-HRP, extensively washed, and chased 16 h in HRP-free medium. Postnuclear particles were first equilibrated on preformed linear Percoll gradients (15–65% vol/vol) and preparations enriched in *N*-acetyl-β-glucosaminidase were isolated between 1.10 and 1.13 g/ml in density. A density shift of HRP-containing structures was then induced by incubating those preparations in 2.8 mM 3,3′-diaminobenzidine tetrahydrochloride (DAB)[1] with 6 mM H_2O_2, as previously described (9), followed again by equilibration in a linear sucrose gradient (1.15–1.30 g/ml in density).

Morphology

For electron microscopy, fibroblasts were fixed by 2% glutaraldehyde in 0.1 M Na cacodylate buffer, pH 7.4, for 60 min at room temperature. Peroxidase cytochemistry was performed by the procedure of Graham and Karnovsky (14). Cells were washed twice for 10 min in 0.05 M Tris-HCl, pH 6.0, preincubated for 15 min in the same buffer containing 2 mg/ml DAB. Reaction was started by the addition of H_2O_2 (0.02%). Cells were extensively rinsed 60 min later in Tris-HCl buffer and postfixed for 1 h at 4°C in a 1% osmium tetroxide and 2% potassium ferrocyanide solution. A multipurpose test grid similar to that described by Weibel et al. (34) was used for the determination of volume fraction or membrane area. Stereological analysis was performed as described previously (25).

Protein Determination and Enzymatic Assays

Protein was measured according to Lowry et al. (22) using BSA as standard. In some experiments, cells were cultured for 24 h with 0.05–0.10 μCi/ml [^{14}C]leucine, and chased for 18 h. Those conditions led to the incorporation of 1–2 nCi/mg cell protein. Activities of the following marker enzymes were determined using established procedures: 5′-nucleotidase (1), alkaline phosphodiesterase I, galactosyltransferase (4), cathepsin B (3), and *N*-acetyl-β-glucosaminidase (19).

Determination of Radioactivity

Incorporated ^{125}I was determinated after the "dish-batch" method described by Hubbard and Cohn (16). Radioactivity was measured in a liquid scintillation counter (LS 7500 DPM; Beckman Instruments, Inc.) after dissolution of the sample in Aqualuma cocktail (Lumac Systems, Basel, Switzerland).

Reagents

Horseradish peroxidase (type II), glucose oxidase, and DAB were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium boro[^{3}H]hydride, [^{14}C]formaldehyde, and sodium [^{125}I] were purchased from the Radiochemical Centre (Amersham, UK). Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Lactoperoxidase was from Calbiochem-Behring Corp. (San Diego, CA). Other chemicals were purchased from E. Merck (Darmstadt, FRG) or Koch-Light (Colnbrook, UK).

Mathematical Model

On the basis of the scheme depicted in Fig 1, the following equations can be derived. The mass of a given constituent at the lysosomal membrane is the result of three factors. (*a*) The inflow can be represented as

$$k_a Q_p, \tag{1}$$

where k_a is the fraction of plasma membrane transferred to the lysosomal membrane per unit time (h^{-1}), and Q_p is the mass of the constituent associated with the plasma membrane. (*b*) The outflow, resulting from recycling to the plasma membrane and which is assumed to be equal to the inflow, equals

$$-k_a S_p Q_l / S_l, \tag{2}$$

where S_p and S_l are the surface area of the plasma and the lysosomal membrane respectively (cm^2), and Q_l is the mass of the constituent associated with the lysosomal membrane. (*c*) The loss, resulting from degradation in the lysosomes, equals

$$-k_d Q_l, \tag{3}$$

where k_d is the first order degradation constant of the constituent in the lysosomes (h^{-1}).

Assuming that degradation is limited to lysosomes, k_d of any membrane constituent is equal to k'_d, the apparent degradation constant measured in whole cells, multiplied by $[1 + Q_p/Q_l]$.

Combining equations 1, 2, and 3, the rate of change per unit time of the mass of a given constituent associated with the lysosomal membrane is

$$dQ_l/dt = k_a Q_p - [S_p/S_l]k_a Q_l - k_d Q_l. \tag{4}$$

Defining Q_{tot} as the total mass of the constituent associated with the cells at a given time and Q_0 as the initial value of Q_{tot}, and assuming first order degradation, we have

$$Q_{tot} = Q_0 e^{-k'_d t}. \tag{5}$$

Combining with equation 4 yields

$$dQ_l/dt = [k_a - k'_d]\, Q_0 e^{-k'_d t} - [1 + \gamma]k_a Q_l, \tag{6}$$

where γ stands for S_p/S_l, the ratio between the area of plasma and lysosomal membranes.

Upon integration of equation 6, one obtains

$$Q_l = Q_0 \frac{[k_a - k'_d]}{[1 + \gamma]k_a - k'_d}\left\{e^{-k'_d t} - e^{-[1+\gamma]k_a t}\right\}. \tag{7}$$

Replacing Q_0 by its value in equation 5, the ratio (R) of the amount of the constituent associated with lysosomes divided by the amount associated with cells is

$$R = Q_l/Q_{tot} = \frac{[k_a - k'_d]}{[1 + \gamma]k_a - k'_d}\left\{1 - e^{-[(1+\gamma)k_a - k'_d]t}\right\}. \tag{8}$$

The ratio R can be measured, and its value at equilibrium, R_{eq}, can easily be computed from equation 8. It is equal to

$$R_{eq} = [1 - k'_d/k_a]/[1 + \gamma - k'_d/k_a]. \tag{9}$$

1. *Abbreviation used in this paper*: DAB, 3,3′-diaminobenzidine tetrahydrochloride.

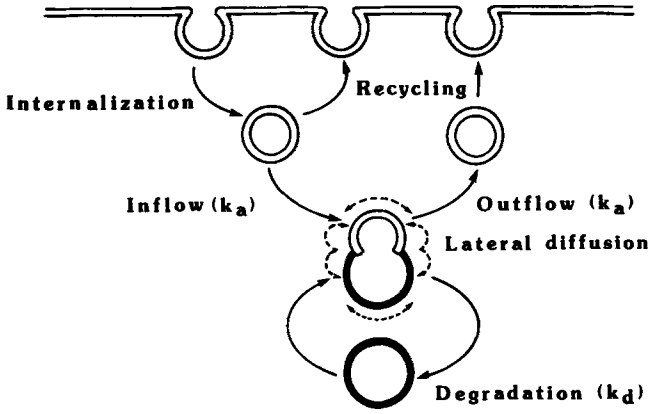

Figure 1. Model of exchange and degradation of plasma membrane constituents during endocytosis. Plasma membrane (defined as the pericellular membrane together with intracellular membranes whose constituents rapidly exchange with the pericellular membrane) is represented as open bilayer; lysosomal membrane is shown as filled bilayer. Cell surface constituents are internalized into vesicles, which either recycle back to the cell surface or fuse with the lysosomes. In this case, exchange of constituents between plasma and lysosomal membranes occurs by lateral diffusion (*broken lines*). The rate of inflow of plasma membrane into lysosomes (k_a) is expressed as a fractional area of plasma membrane per unit time (h^{-1}); k_d is the degradation rate constant of a constituent in the lysosomes (h^{-1}). De novo synthesis of membrane constituents is not taken into account in this representation.

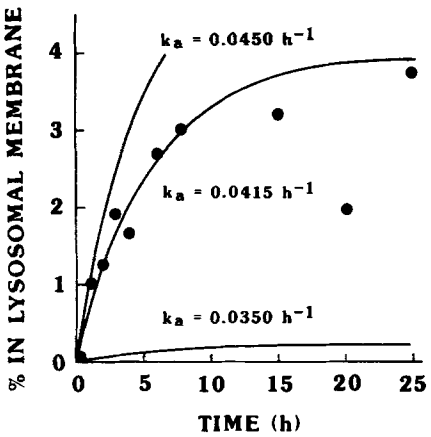

Figure 2. Intracellular processing of ^{125}I-label. Cells were iodinated, washed, and incubated at 37°C for the indicated times before homogenization. Postnuclear particle fractions were prepared and layered on top of preformed Percoll gradients (15–65% vol/vol). The minor component of the ^{125}I-label that became increasingly associated with the lysosomal *N*-acetyl-β-glucosaminidase distribution was estimated from the fractions equilibrating between 1.10 and 1.13 g/ml. These fractions correspond to the lysosomal preparations characterized in references 12 and 13. The ^{125}I content was corrected for contamination by the pericellular membrane by substracting the amount present in the fractions isolated immediately after cell labeling (0.65% of the label in the homogenate). The ratio between the ^{125}I and *N*-acetyl-β-glucosaminidase contents in the lysosomal preparations, both expressed as percent of the homogenate, was taken as a measure of the amount of plasma membrane constituents associated with lysosomes. Curves obtained from equation 8 are fitted with the experimental results, using the measured apparent degradation rate k'_d = 0.0346 h^{-1}, the rounded measured ratio of the plasma membrane to the lysosomal membrane ($\gamma = 4$), and three different values for k_a, the rate of inflow.

R_{eq} is of course limited to positive values and hence $k'_d/k_a < 1$, since γ is always >0.

This derivation assumes that fusion betwen incoming endocytic vesicles and lysosomes is followed by complete equilibration of membrane constituents by lateral diffusion, which may not be necessarily the case. If the rate of exchange is reduced (i.e., if lateral diffusion is limiting), the surface area of lysosomes will behave as if it was smaller than its actual value. We will thus consider that γ is reflecting the effective, rather than the physical, area available for exchange of constituents by lateral diffusion.

For endogenous constituents (5′-nucleotidase and alkaline phosphodiesterase I), we will restrict ourselves to values at equilibrium, and predict their kinetics of redistribution into the lysosomes, if they had been labeled at the cell surface by an as yet unavailable selective, nonperturbing, covalent procedure. In this case however, the model does not take into account newly synthesised constituents introduced either into plasma or lysosomal membranes.

Results

Fate of ^{125}I Polypeptides upon Incubation at 37°C

Cell surface polypeptides of rat fibroblasts were first labeled at 4°C with ^{125}I by the lactoperoxidase procedure and incubated at 37°C. When cells were homogenized at various intervals after labeling and postnuclear particles were equilibrated in linear Percoll gradients, most of the ^{125}I-label remained closely associated with 5′-nucleotidase and alkaline phosphodiesterase I (13). A minor component of the ^{125}I-label became increasingly associated with the lysosomal enzyme *N*-acetyl-β-glucosaminidase. The ^{125}I-label redistributed slowly to the lysosomes and approached equilibrium at 4–6 h. Results are summarized in Fig. 2. As can be seen, at most 4% of this label could be attributed to the lysosomal compartment.

Association of Plasma Membrane Markers with Lysosomes

To study the association with the lysosomal membrane of 5′-nucleotidase and alkaline phosphodiesterase I, two classical plasma membrane markers, postnuclear particles were first equilibrated in linear preformed Percoll gradients. Preparations enriched 28-fold in *N*-acetyl-β-glucosaminidase, and containing more than 50% of the total cell activity of *N*-acetyl-β-glucosaminidase, were obtained at densities between 1.10 and 1.13 g/ml. Some of these preparations were further incubated in 2.8 mM DAB, with or without H_2O_2, and equilibrated again in a linear sucrose gradient. The shifted material was enriched 33-fold in *N*-acetyl-β-glucosaminidase (35–40% of the cell activity). Comparing all lysosomal preparations (Fig. 3), a linear correlation appears between the amount of *N*-acetyl-β-glucosaminidase and that of either 5′-nucleotidase or alkaline phosphodiesterase I. It should be noted that the values before and after the DAB-induced density shift are not appreciably different, indicating that the cytochemical reaction caused no significant enzyme inactivation. This analysis indicates that ~1.5% of 5′-nucleotidase, but as much as 16% of alkaline phosphodiesterase I of the homogenate are associated with the lysosomes. The major part (65–75%) of the plasma membrane markers present in the lysosomal preparations isolated after the linear Percoll

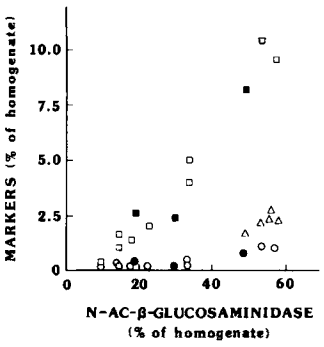

Figure 3. Relation between plasma membrane markers and lysosomes. In the purified preparations, the amounts of 5′-nucleotidase and alkaline phosphodiesterase I were plotted as a function of *N*-acetyl-β-glucosaminidase content. All values are expressed as percent of the homogenate. Values obtained before and after the DAB-induced density shift are identified by open and filled symbols, respectively. The amount of ^{125}I incorporated at cell surface by the lactoperoxidase procedure and found at equilibrium in the lysosomal compartment at steady-state (>6 h of cell incubation at 37°C after labeling), is also indicated for comparison. ■ and □, alkaline phosphodiesterase I; ● and ○, 5′-nucleotidase; △, ^{125}I.

gradients could be sedimented at 3×10^6 *g* min after five freezing-thawing cycles in hypotonic medium, indicating their association with the lysosomal membrane (12).

Morphometry of Endocytosis

Two experimental protocols (16 h continuous uptake, or 2 h pulse followed by 16 h chase) were designed to label either endocytic structures together with lysosomes, or lysosomes only. In both experiments, the area of the pericellular membrane was also estimated (Table I). When cells were incubated 16 h without chase, lysosomes and endosomes occupied together 4.3% of the cellular volume and were limited by a membrane whose area corresponded to 44% of the pericellular membrane area. Lysosomes accounted for 3.3% of the cellular volume, and their membrane area corresponded to 30% of the pericellular membrane.

Application of the Model to Iodinated Cell Surface Polypeptides

As previously reported (13), when cell surface polypeptides of rat fibroblasts were first labeled at 4°C with ^{125}I by the lactoperoxidase procedure and incubated at 37°C, cell-associated radioactivity disappeared with diphasic kinetics. About 35% of the label was lost with a half-life of <1 h and the remaining 65% with a half-life of 20 h. Diphasic kinetics were also reported for the polypeptides iodinated at the surface of L cells (17), and at the lysosomal membrane of mouse peritoneal macrophages exposed to latex-lactoperoxidase (24). The rapidly released component corresponds mostly to TCA-precipitable material, and may include constituents of dead cells as well as extrinsic membrane proteins shed from the pericellular surface. The slow component was used for the determination of the rate of degradation of plasma membrane polypeptides. For the average plasma membrane constituents, the apparent rate of degradation (k'_d; i.e., by reference to the total cell pool) was rather slow. The measured half-life of cell surface labeled ^{125}I polypeptides in fibroblasts being close to 20 h, k'_d is thus 0.0346 h^{-1}.

For the evaluation of the ratio between the amount of markers in lysosomes with respect to the whole cell content (equation 8), we need estimates of 2 other parameters γ and k_a. The γ value, i.e., the surface ratio of the plasma membrane (cell surface + endosomal surface) to the lysosomal membrane, was determined from morphometrical data (see Table I) as [100 + 14]/30 = 3.8, hereafter rounded to 4. The last parameter, k_a, was thus evaluated by computer fitting. As was shown in Fig. 2, a good fit is obtained at k_a equal to 0.0415 h^{-1}. This implies that 4.15% of the plasma membrane iodinated polypeptides reached the lysosomal compartment per hour. The fit is sensitive to small changes in the value of k_a and constraints are rather stringent, indicating that reasonable confidence can be attributed to the estimate.

With this value, the model would further predict that, once in lysosomes, the ^{125}I polypeptides were degraded with a half-life of ~0.8 h (k_d). As can be expected, the actual rate of degradation in the lysosomes is thus considerably higher than the apparent rate of degradation at the level of the cell. Dividing k'_d by k_a, one may calculate that ~83% of the ^{125}I polypeptides reaching the lysosomal compartment are degraded. We conclude that the remainder is recycled. Of course, we cannot exclude that a part of the ^{125}I-label is associated to nonlysosomal structures or lysosomal-like structures equilibrating between 1.10 and 1.13 g/ml in Percoll gradient, and that the low recycling may partially originate from them. In the model, despite the high degree of lysosomal degradation of iodinated plasma membrane polypeptides, we have also assumed that the outflow equals the inflow.

Discussion

Recycling via Lysosomal Membrane

The estimated value for k_a (~4% of the plasma membrane

Table I. Morphometry of Endocytosis

	Fractional volume (percent of cellular volume)	Membrane area per unit volume
		μm^{-1}
Experiment I		
Structures labeled after 16 h uptake	4.26 ± 0.01	0.53 ± 0.05
Pericellular membrane	–	1.20 ± 0.17
Experiment II		
Lysosomes: (2 h uptake, 16-h chase)	3.30 ± 0.06	0.47 ± 0.11
Pericellular membrane	–	1.57 ± 0.27

Rat fibroblasts were incubated in the presence of 1 mg/ml horseradish peroxidase at 37°C using two distinct protocols. Firstly, cells were continuously exposed to horseradish peroxidase for 16 h, washed, and fixed. Horseradish peroxidase-containing structures were demonstrated by cytochemistry. Secondly, cells were exposed to horseradish peroxidase for 2 h and chased for 16 h, before fixation and cytochemistry. This second protocol labels bona fide lysosomes (12). The total section areas analyzed were 251 μm^2 or 435 μm^2, in experiments I and II, respectively. Values are given ± SEM.

being transferred to the lysosomes per hour) is much smaller than the rate of internalization of the pericellular membrane. Stereological studies from the literature indicate that fibroblast-derived cells, such as L cells, internalize their cell surface roughly every 2 h (29). This comparison strongly suggests that the major recycling route (>90%) of the pericellular membrane does not involve a true lysosomal stopover (see also references 5 and 6). The model presented here leads us to the conclusion that once plasma membrane constituents have been transferred to the lysosomal membrane or to the membrane of endocytic structures equilibrating at the same density as lysosomes, ~83% are degraded, the rest being recycled back to the cell surface either from lysosomes or from endocytic structures having identical equilibration densities in Percoll gradients.

Contribution of the Bulk of Plasma Membrane Constituents to the Lysosomal Membrane

During incubation at 37°C, the ^{125}I-label is progressively delivered to the lysosomal compartment. After 25 h of incubation, i.e., at steady state, only ~4% of cell-associated ^{125}I-label is recovered in lysosomes (R_{eq}; see also reference 13). This low fraction of the bulk of plasma membrane constituents is comparable with the low value (<5%) obtained in rat fibroblasts using antiplasma membrane antibodies (28). It is also very similar to the small size (2.5%) of the slowly exchangeable pool of internalized membrane glycoconjugates (6) and of the fraction recovered in the lysosomal compartment of p338D$_1$ macrophages (15). These values are in contrast with the ratio of the lysosomal to the plasma membrane surfaces (our measured ratio is 0.3 in cultured rat fibroblasts).

Application to Classical Plasma Membrane Markers

We have shown that ~1.5% of 5′-nucleotidase and ~16% of alkaline phosphodiesterase I are associated with purified lysosomes. The occurrence of enzyme activities considered as markers for other organelles in our highly purified lysosomal preparations can be interpreted either as contamination by these organelles, or as constituents truly associated with lysosomes. Evidences for true association have been discussed in detail elsewhere (12) and it is reasonable to assume that these membrane constituents indicate continuity or exchange within a physiological route. For these two membrane proteins, we have thus the ratio at equilibrium between the lysosomal membrane and the plasma membrane (R_{eq}).

Although the model was initially derived to follow the fate of iodinated cell surface proteins, whose kinetics of exchange with the lysosomal membrane could be followed, it can also be applied to endogenous plasma membrane markers, whose R_{eq} can be measured. For the inflow of membrane (k_a), it appears reasonable to assume the same rate as that deduced for iodinated proteins. It was further assumed that the lateral diffusion was also nonlimiting for the markers used, hence γ was taken as equal to 4. In a first hypothesis, differences in 5′-nucleotidase and alkaline phosphodiesterase I content were thus accounted for solely by adjusting the degradation constant k_d. This is shown in Fig. 4 *A* and is referred to in Table II as the differential degradation hypothesis.

Alternatively a differential diffusion hypothesis could be proposed, if our results can be explained by adjusting the value of γ, the area available for exchange during membrane continuity between endocytic vesicles and lysosomes, and assuming for the marker enzymes the same degradation constant as determined for iodinated polypeptides. In this second hypothesis, despite rapid transfer of soluble content, the membrane proteins of the donor and the recipient organelles would not readily mix by lateral diffusion due to a limiting fusion-fission interval. The effect of this factor is presented in Fig. 4 *B*. Since the ratio between the area of the plasma membrane and that of lysosomes is 4, γ set at this value implies that lateral diffusion is not limiting. Fig. 4 *B* also shows that significant variations of γ have only a moderate effect on the predicted redistribution of ^{125}I polypeptides. Hence, our mathematical model offers only rough estimates of the effect of differences in the diffusion of individual membrane constituents. For alkaline phosphodiesterase I, the value of γ = 1.0, predicted for a k'_d/k_a ratio equal to that of ^{125}I polypeptides, can hardly be reconciled with the value deduced from our morphometrical results.

Finally, selective inclusion or exclusion at any of the vesicular intermediate between the plasma membrane and lysosomes, i.e., differential inflow, can also be accounted for in our model, by adjusting the value of k_a for the plateau level

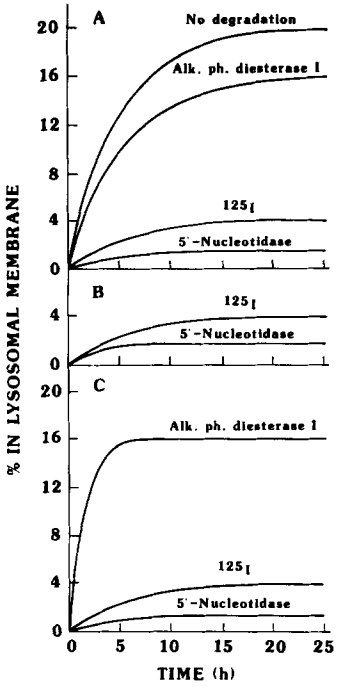

Figure 4. Effect of degradation, lateral diffusion, and inflow on the predicted redistribution of plasma membrane markers into lysosomes. This figure compares the predicted redistribution of ^{125}I-labeled plasma membrane polypeptides or that of selected plasma membrane markers if they had been labeled at the cell surface. *A* shows the effect of the degradation rate (k'_d). Curves are obtained from equation 8 using k_a = 0.0415 h^{-1} and γ = 4, as in Fig. 2. The value of k'_d was changed for each constituent, to fit the measured values of R_{eq}. The curve corresponding to k'_d = 0 h^{-1} (no degradation) is also given for comparison. *B* shows the effect of the available surface of exchange (γ). Curves are obtained from equation 8, set at k_a = 0.0415 h^{-1} and k'_d = 0.0346 h^{-1}, γ being the adjusted parameter. An increase of γ from four to ten decreases the predicted contribution of plasma membrane constituents close to that calculated for 5′-nucleotidase. *C* shows the effect of selective inclusion or exclusion along the endocytic pathway on the redistribution of plasma membrane markers into the lysosomes (k_a). Curves are obtained from equation 8, set at k'_d = 0.0346 h^{-1} and γ = 4, k_a obtained for ^{125}I (0.0415 h^{-1}) being now readjusted to fit the results obtained for the other plasma membrane markers. Increase of k_a by a factor of 3.53 brings the predicted contribution of the plasma membrane constituent to lysosomes close to that calculated for alkaline phosphodiesterase I. A minor reduction of k_a (×0.89) decreases the predicted contribution close to that calculated for 5′-nucleotidase.

Table II. Estimation of Distribution Parameters Obtained for Endogenous Marker Enzymes

Markers	Hypotheses		
	Differential degradation k'_d	Differential diffusion γ	Differential inflow k_a
	h^{-1}		h^{-1}
^{125}I Polypeptides (R_{eq} = 0.039)	0.0346	4.0	0.0415
5′-Nucleotidase (R_{eq} = 0.015)	0.0389	10.6	0.0369
Alkaline phosphodiesterase I (R_{eq} = 0.160)	0.0098	1.0	0.1465

For plasma membrane markers, the three parameters in equation 8 were considered. In each hypothesis, two parameters were set to values obtained with ^{125}I polypeptides, and the third one was estimated.

measured for the two marker enzymes, and keeping the other parameters constant (Fig. 4 *C*). The selectivity of the internalization route towards lysosomes can be characterized by the ratio of the k_a value for each of the 2 markers with respect to that of ^{125}I polypeptides. In this third hypothesis, variations of the inflow rate are inversely proportional to those proposed for the degradation constant. At plateau, these 2 parameters are indeed found in equation 9 as the ratio k'_d/k_a. However, k_a and k'_d have distinct effects on the interval required to achieve equilibrium (see also Table II).

It is not possible to propose a rational choice in favor of one of the three possibilities offered, and simultaneous variations of all parameters can of course not be excluded. For 5′-nucleotidase, available evidences suggest that the enzyme is largely excluded from coated pits on some cell types (23). Furthermore, recent data with a selective perturbant strongly suggest that in rat fibroblasts, 5′-nucleotidase is internalized by an alternative noncoated pathway, with much slower recycling kinetics (18). Since ligands internalized through coated or noncoated cell surface are subsequently mixed in a common intracellular organelle and similarly directed towards lysosomes (31), this would imply that the rate of internalization of 5′-nucleotidase is significantly decreased. However, doubling the value of γ or increasing k'_d by ~10% also yields a good fit with the experimental data. For alkaline phosphodiesterase I, the value of γ = 1.0, predicted for k'_d/k_a ratio equal to that of ^{125}I polypeptides, being too low to be reconciled with the value deduced from morphometrical results, a difference of k'_d/k_a remains as the only possibility offered by the model. Compared to ^{125}I polypeptides, the observed proportion of alkaline phosphodiesterase I in lysosomes can thus be explained either by a 3.5-fold increase of its half-life (70 h), or a preferential inflow of the same extent.

In the degradation hypothesis (Table III), the apparent half-lives of plasma membrane proteins range between 17.8 and 70.2 h, which correspond to actual half-lifes in lysosomes between 0.27 and 11.2 h. Fig. 4 *A* shows the prediction of R_{eq} obtained at various k'_d values, using the k_a and γ values established for ^{125}I polypeptides. The behavior of alkaline phosphodiesterase I is close to that of the theoretical limit represented by a nondegradable plasma membrane constituent (T/2 → ∞), while 5′-nucleotidase is close to the other extreme. In preliminary experiments, we have exposed mouse peritoneal macrophages to latex particles to accelerate transfer of plasma membrane into lysosomes (35), and observed that the decay of alkaline phosphodiesterase I was two- to threefold slower than for 5′-nucleotidase (10). Provided that representative samples of the pericellular membrane are dragged into lysosomes by their adherence to latex beads, this observation favors the hypothesis that alkaline phosphodiesterase I activity is much more resistant to proteolysis than 5′-nucleotidase. For major plasma membrane polypeptides labeled with ^{125}I, however, we found that the degradation was similar for all iodinated species (13).

The model suggests further experiments, to assess the effect of controlled alterations on the various parameters. For k_d, we will follow the effects of leupeptin, an inhibitor of lysosomal degradation. For k_a, we plan to take the advantage of the selective redistribution of epidermal growth factor

Table III. Degradation of Plasma Membrane Constituents (Differential Degradation Hypothesis)

Markers	Q_p/Q_t (Steady-state)	k'_d	T/2	k_d	T/2
		h^{-1}	h	h^{-1}	h
^{125}I Polypeptides	24.64	0.0346	20.00	0.889	0.78
5′-Nucleotidase (R_{eq} = 0.015)	65.67	0.0389	17.79	2.598	0.27
Alkaline phosphodiesterase I (R_{eq} = 0.160)	5.25	0.0098	70.16	0.062	11.22

The apparent half-life of plasma membrane constituents was determined from equation 8, with k_a set at 0.0415 h^{-1}. The actual half-life in lysosomes was calculated from k_d which is equal to k'_d, the apparent degradation constant measured on whole cells, multiplied by [1 + Q_p/Q_t]. The Q_p/Q_t ratio was calculated from the amount associated at steady-state with the plasma membrane (Q_p) and the lysosomal membrane (Q_t). Values for 5′-nucleotidase have also been calculated under the same assumptions, although there is experimental evidence for a lower k_a value.

receptors into endosomes and lysosomes by phorbol esters and epidermal growth factor, respectively, and to quantify the immunoprecipitable receptor in the homogenate and purified lysosomal preparations. Differences that could not be accounted for by the two first parameters will tentatively be attributed to variations of γ.

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