BIOCHEMICAL JOURNAL LETTERS

The acidic environment in endocytic compartments

After coupling of ligands like low-density lipoproteins, transferrin, growth factors etc., to their corresponding receptors on the cytoplasmic membrane and the internalization via clathrin-coated pits, receptor–ligand complexes enter endocytic compartments with an acid environment. The acidification of the lumen is achieved by H^+-ion-pumping ATPases in the endosomal membrane. The endosomal acidity is important for the dissociation of (some) ligands from their receptors so that receptors can be recycled and used for further rounds of internalization. Different approaches to measure the apparent pH in endosomes indicated that the compartments are less acidic at earlier stages of endocytosis than at later ones. These differences were suggested to have a central role in the processing and sorting of internalized ligands since, *in vitro*, various receptor–ligand complexes dissociate at different pH values [1,2].

The finding that acidification during endocytosis shows stage-specific differences implies that mechanisms for its regulation have to exist. Several possibilities for this regulation were discussed, like different numbers of ATPase molecules per vesicle, modulation of their activity (for instance by covalent modifications) or regulation of the ion-permeabilities of endosomal membranes [1]. Subsequently, it was observed that in some cell strains 'early' endosomes can regulate their lumenal acidity by modulation of the membrane potential, e.g. by Na^+/K^+-ATPases [3,4] or by Cl^- channels which themselves can be modulated by second messengers [5].

Since H^+-ATPases are electrogenic, which means they pump H^+ ions without the movement of counter-ions, an interior positive membrane potential acts against a further acidification. The attempts to quantify the degree of acidity in different endocytic compartments in terms of pH values are reasonable for larger, vacuolar-type compartments. For very small compartments along the endocytic route, however, it seems to be questionable whether the classical pH-concept can be applied. Coated vesicles or carrier vesicles, for instance, with diameters of about 100 nm and a corresponding volume of 5×10^{-19} l, would contain mathematically only 0.3 free H^+ ion for maintaining a pH of 6.0 (assuming volume $= 4r^3\pi/3$, 10^{-6} mol H^+/l and 6.022×10^{23} ions/mol). That means not even one single free H^+ ion would have to be in such a vesicle to make it acidic.

In principle, the same considerations apply to small substrates that are part of the complex system of endosomes. They could be important for the entire process of endocytosis, since recent

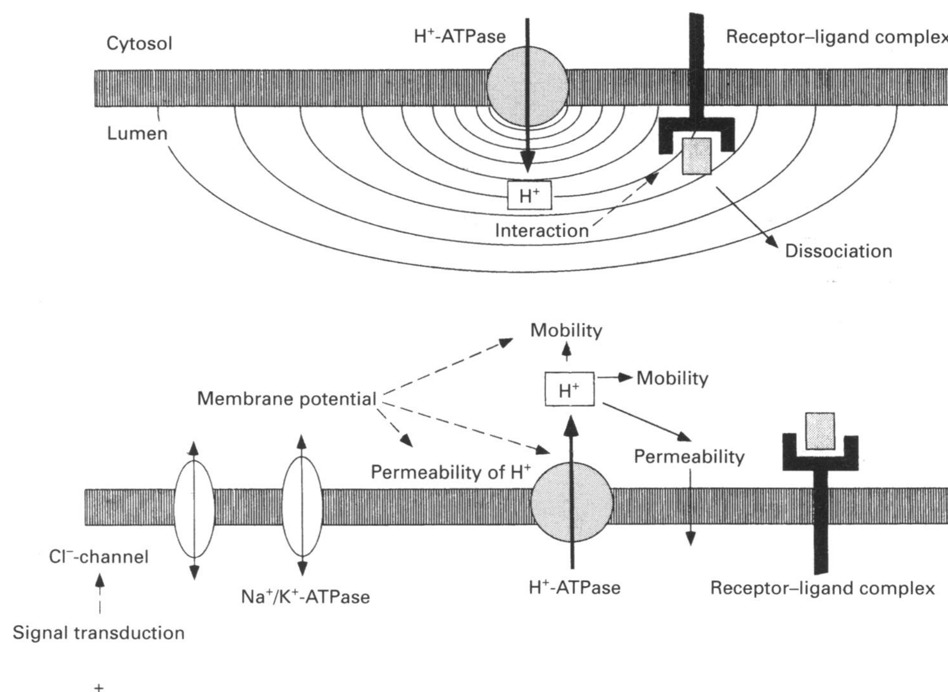

Figure 1 Model of the acidic micro-environment in endocytic compartments

The contour lines around the H^+-ATPase in the upper part of the Figure indicate the density of the H^+-orbital (the probability of occurrence of H^+ ions). The distance of a receptor–ligand complex from the H^+-ATPase and therefore the position in the H^+-orbital would be important for the probability of dissociation. The lower part shows the possible influence of the membrane potential, which is modulated by ion channels or electrogenic ion pumps, on the H^+-ATPase, the permeability of H^+ ions through the endosomal membrane and their mobility in the lumen.

investigations showed that the shape of early endocytic compartments (at least of some cell types) is much more tubulo-reticular than previously assumed, with tubules of just 30–50 nm in diameter [6,7]. The observations of the endocytic traffic in living Hep-2 cells by video-image microscopy that were carried out by Hopkins et al. [6] indicated that the endosomal system of these cells consists of a network of interconnected tubules through which boluses move that carry receptor–ligand complexes. The boluses could be identical to multivesicular bodies or carrier vesicles that are observed in electron microscopy, since the connecting tubules that do not appear in electron-microscopical pictures seem to be sensitive to the chemical fixatives usually used for the preparation for microscopy.

These findings led to a new conception of endosomes as interconnected networks where boluses move along tubules trawling for receptors [8]; an idea that is somehow contradictory to the classical models of endocytosis with separate endosomes. If we want to apply the classical pH-concept to such tubular endosomes of 30 nm diameter, we come to the conclusion that a pH of 6.0 would mean only one free H^+ ion in a section of more than 2 μm. Therefore it seems obvious that we have to think in terms of probabilities of occurrence of H^+ ions rather than concentrations for the submicroscopical environment of these compartments. According to the pH-theory there could be an OH^- ion in the tubule as well, but the probability for it would be much lower than for an H^+ ion. Since the molecular basis for the pH-dependent dissociation of receptor–ligand complexes is not clear, we cannot conclude that certain conditions that are necessary *in vitro* are equally important for the dissociation within the endosomal system *in vivo*. It is imaginable that a certain frequency of collisions between H^+ ions and receptors is necessary for a conformational change of the receptor and the dissociation of the ligand. On the other hand, a certain probability of occurrence of H^+ ions relative to OH^- ions could influence the probability of the open state of the receptor and the dissociation of the ligand would just occur if the receptor remained in its open state over a given period of time. We could think of the probability distribution of H^+ ions as an H^+-ion orbital. The density of the orbital at a defined site would be proportional to the activity of H^+ ions at this position. Thus the distance between H^+-ATPases and receptors would be important for the interaction of H^+ ions with the receptor–ligand complex and its dissociation.

Besides the lack of exact knowledge about the interaction of receptors with H^+-ions *in vivo*, we have no detailed understanding about the behaviour of H^+ ions themselves in the endosomal micro-environment. The pH value describes the activity of H^+ ions only for ideal aqueous solutions with sufficient precision. Within endosomes, however, we have high protein concentrations and a large number of ionic charges, and we do not know the activity coefficient of H^+ ions in these solutions. It is unlikely that the classical rules of free diffusion apply to the case of a heterogeneous phospholipid-bilayer-enclosed environment like the endosomal lumen. It is possible that the activity coefficient or the probability of occurrence of H^+ ions is higher in the proximity of the membrane compared with more distal regions, due to the inside positive membrane potential. The radial mobility of H^+ ions in the tubule could be reduced by the membrane potential, whereas the overall mobility could be influenced by the charged phospholipid groups. On the other hand, ion gradients along the membrane tubule or axial potential differences could regulate the axial mobility of H^+ ions as well. A belt of Na^+/K^+-ATPases, for instance, could generate a positive zone which acts as a barrier for the movement of H^+ ions or in other terms lowers the density of their probability distribution.

Since the endosomal membrane has a high permeability for H^+ ions as long as counter-ions are available [9], H^+ ions pumped into the endosomal lumen by H^+-ATPases can get out again with a certain probability, leading to a lateral decrease of the orbital density depending on the distance from the H^+-ATPase.

These characteristics as well as the possibility of barrier zones for H^+-movement would mean that different regions of complex endocytic compartments or tubulo-reticular endosomes can be differently acidic, even if they are linked. If this possibility proves to be true, it would defeat one of the main criticisms of the concept of an endosomal reticulum, namely the argument that it cannot explain the occurrence of differently acidic endosomal compartments and the functional role of pH differences for endocytic traffic.

Regardless of which model of endocytosis is closer to reality, we should be careful not simply to transfer idealized concepts which were developed for the inorganic chemistry of aqueous solutions, like the pH-concept, to the submicroscopical level of biological systems if we want to consider the complexity of these systems.

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