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Nonuniform Lateral Distribution of Transmembrane ΔpH in a Coupling Membrane as Related to Its Curvature

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Abstract

The possibility is analyzed that the pH of the water space localized inside the invagination of a membrane can differ from the pH of the external bulk buffer outside the invagination. The proton flow responsible for decreased pH values inside mitochondrial cristae and membrane invaginations of cyanobacteria has been calculated. If $\Delta\psi$ (electric potential difference) inside and outside the invaginations is the same, there may exist a lateral microheterogeneity of transmembrane ΔpH, and hence $\Delta\bar{\mu}_{H^+}$. It seems that the invagination is a kind of buffer for accumulating $\Delta\bar{\mu}_{H^+}$ in membrane systems. In eutrified waters (pH > 9) and also under the conditions of a sudden decrease or increase of light, or of a respiratory substrate of O_2, ATP synthesis should proceed in the invaginated rather than in the flat regions of a membrane.

Key Words: Cyanobacteria; mitochondrial cristae; membrane microheterogeneity; proton gradient; membrane potential.

Introduction

Owing to the cristae of mitochondria and stacks of lamellae and thylakoids of chloroplasts, coupling membranes possess a great area and a great number of enzyme complexes. This paper considers the possibility that the pH in a membrane invagination differs from that in the buffer external to the invagination.

A Closed Membrane System and the Environment

Let us consider a very simple hypothetical system consisting of proton pumps incorporated in a closed lipid membrane (Fig. 1). At a certain pH_1 value inside the vesicle, maximal ΔpH will be achieved at a minimal volume of the external medium. At a certain value for total efflux of protons and

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buffering capacity of the environment, the volume factor may prove to be of primary importance for increasing the transmembrane ΔpH.

A Model of a Membrane Invagination

Let us assume that a closed membrane system that forms an invagination (schematically shown in Fig. 2) is submerged in a buffer with an H^+ concentration C_b. Let us also assume that the proton pumps that are distributed uniformly all over the membrane extrude protons at the expense of external energy source. The external pH outside the invagination will not change appreciably, since the volume of the buffer is much greater than the internal volume of the invagination. With a sufficiently deep invagination, we have a model resembling that in Fig. 1. Inside the invagination the pH will be lower than that of the external buffer. The model shown in Fig. 1 differs from that depicted in Fig. 2 in that the latter communicates with the external buffer. If an invagination is sufficiently deep and the area of the entrance is small, the steady-state concentration of protons inside the invagination is higher than that in the external buffer. The "steady state" can be achieved if the two flows, i.e., $I_{H^+}^{out}$ (proton flow through the orifice) and $I_{H^+}^{in}$ (proton flow across the membrane through the invagination walls into the invagination), are equal. It should be noted that the time within which a "steady state" will be achieved depends on the buffering capacity of the medium surrounding the membrane. This buffering capacity, however, can be neglected because we are interested in the steady-state distribution of the concentration of protons inside an invagination, provided that the total proton flow directed inside the

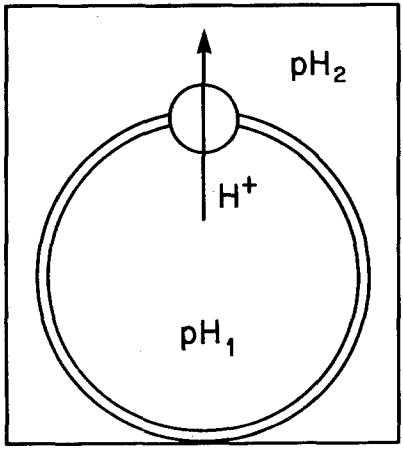

Fig. 1. A closed membrane system surrounded by a small volume of buffer. The lower the volume of the buffer, the higher is the ΔpH at the expense of the same number of extruded protons.

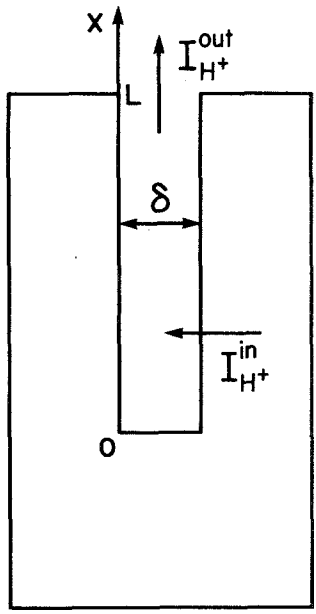

Fig. 2. A model of a membrane invagination: $I_{H^+}^{in}$ is the proton flow inside the invagination supplied by proton pumps; $I_{H^+}^{out}$ is the proton flow out of the invagination at the expense of the concentration gradient; L is the depth and δ is the width of the invagination.

invagination is constant for a sufficiently long time. In this case the proton flow from the invagination to the external buffer will depend solely on the pH inside it and on the pH of the external buffer.

Let us consider a rectangular cavity. Denoting the width of the invagination by δ, the depth by L, the total flow of protons inside the invagination per square centimeter of the membrane by $I_{H^+}{}^{in}$, the proton diffusion coefficient by D, and neglecting in the first approximation the buffering capacity of the invagination, we have the equation for the steady-state concentration $C(X)$ averaged over the width of the invagination

$$\delta D \frac{d^2C(X)}{dX^2} + 2\, I_{H^+}{}^{in} = 0 \tag{1}$$

The boundary conditions are

$$\left.\begin{aligned} C(L) &= C_b \\ \frac{dC(x)}{dx}\Big|_{x=0} &= 0 \end{aligned}\right\} \tag{2}$$

The solution is

$$C(X) = \frac{-I_{H^+}{}^{in}}{\delta D} X^2 + \frac{I_{H^+}{}^{in}}{\delta D} L^2 + C_b \tag{3}$$

Membrane Invaginations of Cyanobacteria, Higher Plants, Mitochondria, and Gram-Positive Bacteria (Mezosomes)

Lamellar, tubular, vesicular, and twisted membrane formations have been reported in some cyanobacteria (Echlin, 1964; Edwards, 1969; Whitton and Peat, 1969; Ingram and Thurston, 1970; Peshkov and Shadrina, 1977).

Analysis of electron micrographs has allowed the conclusion (Gromov and Mamkaeva, 1976) that the cytoplasmic membrane forms a continuous helical extention (Fig. 3). It may be visualized as a compactly coiled invagination. In some cases its depth L may reach 10^5 Å. Mesosomes of Gram-positive bacteria may also form helical structures.

In thylakoids of higher plant chloroplasts, the interthylakoid space lacks ATPases (Anderson, 1981), and invagination thus cannot be used for ATP synthesis. At the same time, in cyanobacteria, owing to the communication between thylakoids and the cytoplasmic membrane, the interthylakoid space communicates with the periplasm, so that the inside of a thylakoid may be viewed as an invagination. The idea of a continuous membrane in cyanobacteria was prompted by some observations in which cytoplasmic membrane

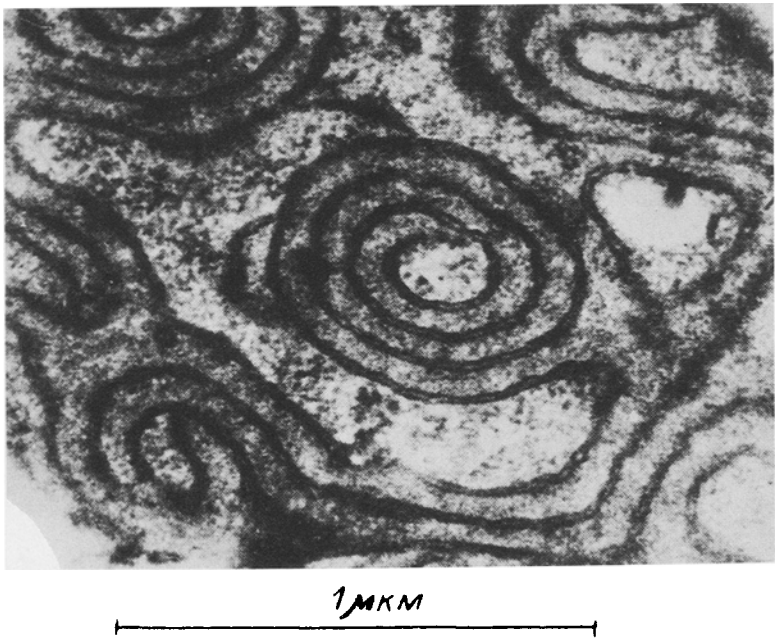

Fig. 3. A helical invagination of the cytoplasmic membrane of *Nostoc calnicola*. The depth of an incoiled invagination may reach $L \approx 10^{-3}$ cm. Photograph taken from Gromov (1978).

contacted with a double membrane of thylakoids. Ris and Singh (1961) observed these contacts in *Oscillatoria princips*. The contacts were later reported to exist in several organisms (Allen, 1968; Edwards *et al.*, 1968; Miller and Lang, 1968) and were clearly seen in *Synechococcus sp.* (Gromov and Mamkaeva, 1976). Some authors (Pankratz and Bowen, 1963; Schnepf, 1964; Jost, 1965) believe that the cytoplasmic membrane is involved in the formation of thylakoids.

It may be possible to estimate the total average proton flow that arrives in an invagination by proceeding from data on the topography of photosynthetic membranes and the efficiency of their enzymes. A thylakoid disc of spinach contains 200 electron-transfer chains occupying $S = 10^5$ Å^2 each. Let us calculate a proton flow resulting from the operation of photosynthetic redox chains, assuming that the average time of proton transfer (τ) is 20 msec [oxidation of plastoquinone by photosystem 1 is the limiting step (Junge, 1977)] and that each electron-transfer chain has two reaction centers:

$$I_{ph} = \frac{2}{\tau \cdot S} \approx 10^{13}\ \mathrm{H^+/cm^2 \cdot sec}$$

The same estimate can be made for cristae of the coupling membrane of mitochondria. It is an established fact that electron-transfer chains are uniformly distributed within the membrane. The distance between the centers of the neighboring chains is about 200 Å, so that each 40–50 thousand square angstroms of the internal membrane surface has one respiratory chain. Heart, flight muscle, and kidney contain a greater number of cristae than liver mitochondria, owing to which the inner membranes have a much larger surface and a greater number of electron-transfer chains. Mitochondria from cells with a low respiration rate, for example liver cells, have a smaller number of cristae. According to Mitchell (1966), the area of the inner membranes of rat liver mitochondria is approximately 40 m^2 per gram of mitochondrial protein. For heart mitochondria this value is 200–250 m^2, and for mitochondria from the flight muscle of shoe fly, the value is as high as 400 m^2. Let us assume that the activity of the respiratory chain (N) is approximately $2 \cdot 10^{-7}$ g-atoms of oxygen per minute per milligram of mitochondrial protein or per 0.04 m^2 of membrane surface (S) (Wikström and Krab, 1980). The proton flow, I_{ox}, at the expense of respiration will be

$$I_{ox} = 8\frac{N \cdot N_A}{S} \approx 2 \cdot 10^{13}\frac{\mathrm{H^+}}{\mathrm{sec \cdot cm^2}}$$

N_A being the Avogadro number. It is assumed that 8 protons are transferred across the membrane per atom of oxygen. It should be noted that the total proton flow into an invagination is considered due to the operation of all the $\Delta\bar{\mu}_{H^+}$ generators with no proton flow arising from the active consumers of $\Delta\bar{\mu}_{H^+}$

or from the passive flow through the membrane. The major active consumer of $\Delta\bar{\mu}_{H^+}$ is ATP synthetase. The data on submitochondrial particles allow the conclusion that the flow of protons required for ATP synthesis does not exceed 10^{13} $H^+/sec \cdot cm^2$ (Kozlov, 1981).

Let us estimate the passive H^+ flow across the coupling membrane. Assuming a membrane potential of $\Delta\psi \approx 0.3$ V, and assuming the specific resistance (R) of a coupling membrane to be approximately equal to $2.5 \cdot 10^6$ $\Omega \cdot cm^2$ (Mitchell, 1981) we have

$$I_{pass} = \frac{\Delta\psi}{R \cdot e} \approx 0.012 \cdot 10^{13}\ \mu^+/cm^2 \cdot sec$$

where e is the electron charge. We can see that the leakage, i.e., the passive flow, does not exceed 8% of I_{ox}. One should also bear in mind that, at a certain membrane potential value, there appears an extra flow of protons into an invagination due to the ATP hydrolysis. Assuming that each ATPase occupies $S \approx 10^4$ Å^2 and that the rate of the ATP hydrolysis (v) is approximately $4 \cdot 10^4$ turnovers per minute, we have

$$I_{ATPase} = \frac{2v}{S} \approx 1.3 \cdot 10^{15}\ \mu^+/cm^2 \cdot sec$$

We assume thereby that two H^+ are transferred across the membrane for every molecule of ATP hydrolyzed. It goes without saying that the buffering capacity of the membrane to other ions will also affect the "steady state" inside an invagination. These factors will be dealt with in the next paper. Having taken into consideration all the above factors, we estimate the total flow of protons inside the invagination ($I_{H^+}^{in}$) to be 10^{13} $H^+/cm^2 \cdot sec$.

Substituting these values into Eq. (3), we have the H^+ concentration vs. the distance from the orifice (Fig. 4) for cyanobacteria (curve I, pH of the external medium 6.5; curve II, pH 8.5) and for cristae of mitochondria (curve III, pH_b of external buffer 6.5). In all the above cases it is assumed that $D \approx 10^{-4}$ $cm^2 \cdot sec^{-1}$.

This estimate should be interpreted to mean that, over a larger part of a membrane invagination, ΔpH_{inv} exceeds $\Delta pH_{non\text{-}inv}$ (i.e., ΔpH on the membrane outside the invagination) by at least $\Delta pH = 1$, which corresponds to a membrane potential of approximately 50 mV.

The electric potential difference, $\Delta\psi$, on a continuous coupling membrane within one cell can be considered constant in the first approximation. This means that the parameter $\tau \approx RC$, which characterizes the time of redistribution of $\Delta\psi$ over the membrane, does not exceed several hundredths of a second (Kara-Ivanov, 1982) (C is the capacity of the membrane, and R is the resistance of the premembrane lateral layers of liquid). A membrane from this point of view is nothing but an electric cable (Skulachev, 1980).

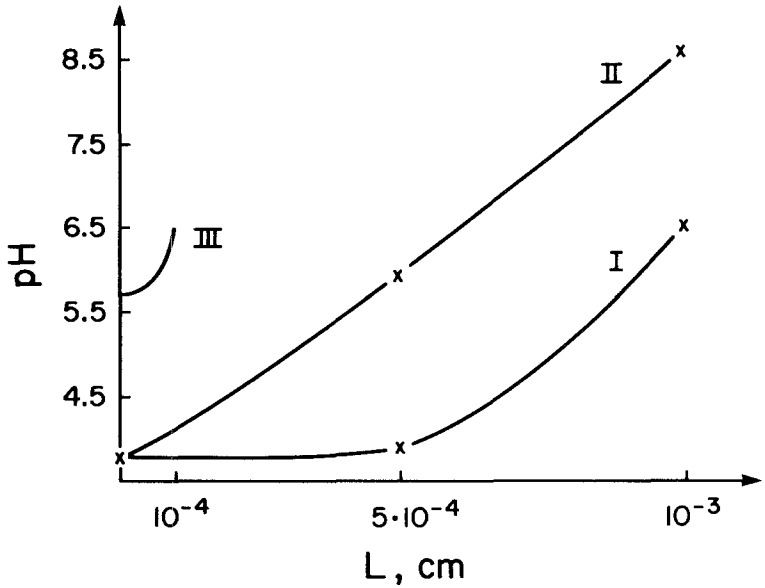

Fig. 4. The pH inside an invagination vs. the distance from the orifice. For cyanobacterium, the depth of invagination $L = 10^{-3}$ cm: (I) pH of the external solution 6.5; (II) pH of the external solution 8.5. For mitochondria $L = 10^{-4}$ cm: (III) pH of the external buffer 6.5.

Thus, since $\Delta\psi$ is constant and ΔpH may vary considerably along the membrane, we conclude that $\Delta\bar{\mu}_{H+} = \Delta\psi + \Delta pH$ is macroheterogeneous due to the invaginations.

Functions of Investigations

Let us visualize a de-energized membrane in which generators and consumers of $\Delta\bar{\mu}_{H^+}$ start to operate. It is evident that $\Delta\bar{\mu}_{H^+}$ will accumulate faster inside an invagination than outside it, since with the same number of protons carried across the membrane ΔpH will be higher; this means that the consumption of $\Delta\mu_{H+}$, for example for ATP synthesis, will start earlier. It should not be overlooked that in reality the external energy sources that create $\Delta\bar{\mu}_{H^+}$ operate in an oscillating regime, for example, oscillation of light and dark for cyanobacteria and delivery of oxidative phosphorylation substrate in mitochondria. Oscillating light or substrate will entail a change in $\Delta\bar{\mu}_{H^+}$ outside an invagination, and in this case an invagination may serve as a kind of buffering system which retains for a longer time the electrochemical potential on the invagination membrane above the threshold value for ATP synthesis

even at subthreshold values on the membrane beyond the invagination. In reality, ATP synthesis inside the invagination will start earlier and last longer than outside it. The regulation of the cell from inside by altering the permeability of the membrane with subsequent switchover of $\delta\bar{\mu}_{H^+}$ from the $\Delta\psi$ to the ΔpH component will induce the same effect as the external oscillation of substrates—ATP synthesis will take place inside rather than outside the invaginations. It cannot be ruled out that there exists a certain "taxis" of enzyme systems—they move to the sites where they can operate at maximum efficiency. What is the mechanism of this taxis? Skulachev (1980) offered the following hypothesis. The binding of a certain membrane protein to a substrate alters the conformation of the protein in such a way that its mobility in the membrane decreases. Even if the diffusion coefficient decreases slightly, within a sufficiently long time the proteins in the membrane will be distributed in such a way that they will accumulate in the site where the concentration of the substrate is higher. In this case ATP synthetases will accumulate in invaginated regions of the membrane.

Let us consider an extreme case. Let us take a cyanobacterium ($pH_{in} = 7.5$) in a eutrified pond with pH ≥ 9 (eutrified water may be alkaline because of the consumption of CO_2). Under such conditions proton pumps ejecting H^+ outside will be switched off in the non-invaginated regions since $\Delta pH_{sm} = -1.5$ (i.e., pH on the smooth region of the membrane) and alkalinization of the internal space is inadmissible; hence ATP will not be synthesized at $\Delta\psi = 180$ mV since $\Delta\bar{\mu}_{H^+} = 180 - 90 = 90$ mV is below the threshold for ATP synthesis. To maintain a constant cytoplasmic pH, protons should be driven inside the cell against the pH gradient. This can be achieved at the expense of the membrane potential by the $Na^+/n \cdot H^+$ antiport, if $n > 1$.

The situation is different in membrane invaginations in which the acidity is higher than in the external buffer and ATP synthesis can take place at the same value of $\Delta\psi$ since $\Delta\bar{\mu}_{H^+} > 180$ mV (i.e., the $\Delta\bar{\mu}_{H^+}$ is above the threshold for ATP synthesis). The resulting conclusion is that ΔpH along the membrane is macroheterogeneous—it is higher at the site of potential generation, that is, inside the invagination, and lower outside, where potential is consumed to maintain a constant pH inside the whole system. In this case invagination reminds one of an electric power station from which $\Delta\bar{\mu}_{H^+}$ is transported to the place where it is consumed. The mechanism of this transport has been discussed previously (Kara-Ivanov, 1982; Skulachev, 1980). The above conclusion provides an explanation for the observation of Gromov mentioned above (Gromov and Mamkaeva, 1976) that under moderate illumination or in an intensely illuminated and very dense culture, membrane invagination and thylakoid stacks form helices all over the cell.

It should be noted that every system that has an invagination constantly loses energy because, due to concentration gradients, protons are extruded

from the investigation to the surrounding buffer. How does one stop this leakage? The problem can be solved by narrowing or closing the invagination "door," forming a system of thylakoid type.

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