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AN ALTERNATIVE HYPOTHESIS FOR THE DIRECTION OF HYDROGEN ION MOVEMENT AND ENERGY TRANSDUCTION IN H. HALOBIUM

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SUMMARY: It is generally accepted that purple membrane of H. halobium functions as a light-driven hydrogen ion pump translocating hydrogen ions from inside the cell to the external medium. However, experimental data from this laboratory together with those obtained by others have always shown an initial alkalinization of the external medium in the light. Additionally, we have found that oxygen can also induce an alkalinization of the bathing solution in the dark. These results can be readily explained if the direction of hydrogen-ion translocation is reversed, that is that both light and oxygen generate an electrochemical gradient of hydrogen ions, which is outwardly directed for ATP synthesis.

Following the initial reports on H. halobium by Oesterhelt and Stoeckenius (1), numerous studies have been published and authoritatively reviewed (2,3). In all these published papers, the purple membrane of H. halobium is accepted as functioning as a light-driven hydrogen ion pump transducing light into a gradient of hydrogen ions across the cell membrane. The electrochemical gradient of hydrogen ions is then used to synthesize ATP in accordance with the chemiosmotic hypothesis (4). Further, it is accepted that, with apparently only one exception (5), this light-driven pump (i.e., bacteriorhodopsin in the purple membrane) translocates hydrogen ions from the cytoplasmic side of the cell to the external medium. The experimental findings on which this suggestion is based rely on the following: (i) light-induced pH changes resulting in a net acidification of the medium (6-16). (ii) oxygen consumption measurements (6,17), and (iii) light-driven ATP synthesis (9,11,12,15,18-21).

Although the generally accepted interpretation of the data as given in the afore-mentioned papers is plausible and persuasive, it is by no

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means compelling. One of the most troublesome questions that has been raised is that of the direction of hydrogen ion movement (5). If it were granted that the primary function of the purple membrane is to pump hydrogen ions, it would seem to make more sense to move hydrogen ions from the bathing medium to the cell interior rather than in the opposite direction as proposed. The purpose of this paper is to discuss the results of our recent experiments on *H. halobium* in conjunction with the data obtained by others, which appear to support the alternative explanation.

MATERIALS AND METHODS: *H. halobium* was grown in a complex basal salt media using the method described by Becher and Cassim (22). Ten ml of the halophilic cells were tested for pH changes in the dark and light by a Beckman Expandomatic pH Meter. The samples were carefully controlled at 25.0^{o}C and were illuminated by fluorescent lamps. The cell suspension was unbuffered and the initial pH was set at 7.0.

RESULTS AND DISCUSSION: Fig. 1(A) shows a pH/time course of *H. halobium* cells when made anaerobic in the dark. A short burst of oxygen was seen to raise the pH of the medium. This initial alkalinization was followed by a faster acidification. After oxygen was shut off, there was no apparent change in the rate of oxygen consumption until the cells had exhausted all remaining oxygen. After that a rapid increase in hydrogen ion concentration was detected before the pH of the medium slowly reversed itself toward its initial value as can be seen in Fig. 1(A). Similar but less dramatic pH/time course curves were observed when *H. halobium* cell suspensions were illuminated A typical response curve is shown in Fig. 1(B). Here an initial transient alkalinization of the medium was recorded, which was followed by a net pH decrease as long as light was on. When the light was switched off, a more rapid change of acidity was detected before the pH slowly drifted back toward its initial value. Additionally, we have observed that both light-induced and oxygen-induced pH transients were abolished by uncouples such as 2,4 dinitrophenol and CCP. Our findings (23) described here are fully in agreement with those reported earlier (6-16).

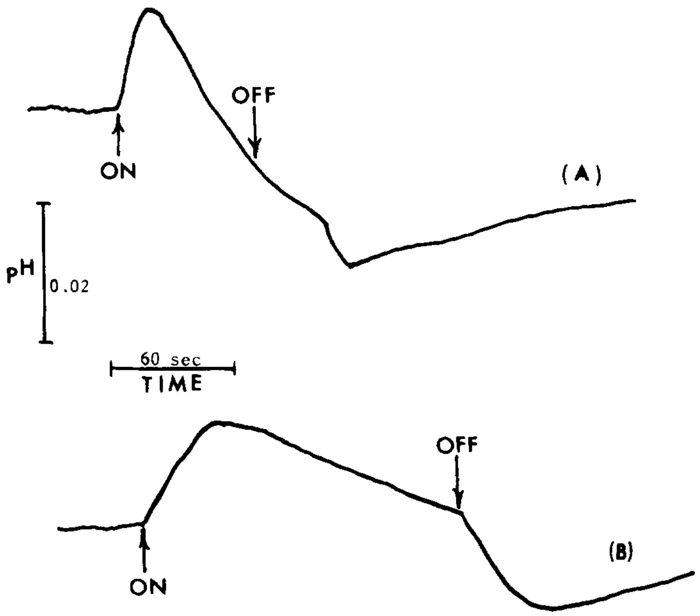

Fig. 1. Hydrogen-ion translocation. (A) Oxygen-induced pH changes when H. halobium cells in unbuffered basal salt solution were initially under anaerobic conditions then made aerobic by saturating with air (upward arrow). (B) Light-induced pH changes when H. halobium cells were illuminated with white light in unbuffered basal salt solution at pH 7.

However, the initial alkalinization has been explained in terms of the hydrogen ion gradients and membrane potentials that exist in both light anaerobic and dark cells. It is not clear why the initial alkalinization can not be caused by light-driven pump moving hydrogen ions from the external medium to the interior of the cell, as can be seen clearly in Fig. 1(B). Experiments with purple membrane-containing liposomes (24) also showed that in the light a measurable uptake of hydrogen ions resulted. This has been explained by suggesting that the "pumps" were incorporated inside out thereby the direction of hydrogen ion pumping was the opposite of that observed in intact cells. However, it is not clear why the purple membrane was incorporated preferentically the manner supposed.

Our observations on H. halobium are fully consistent with the findings of the others (2,3) in that both respiring cells in the dark, and anaerobic

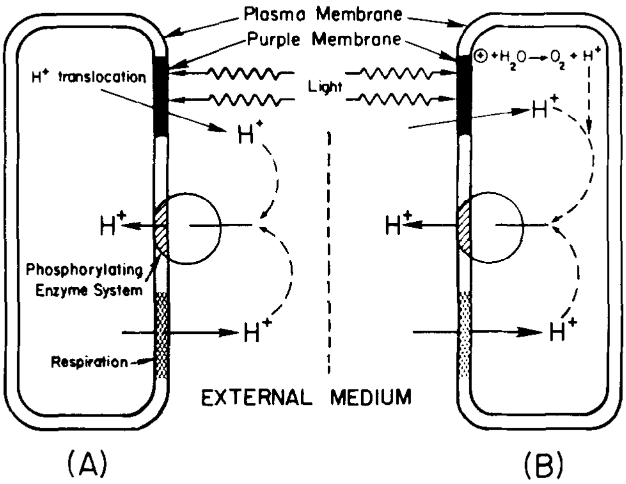

Fig. 2. Schemes for energy transduction in *H. halobium*. (A) The old model in which the direction of hydrogen ion translocation is from the inside of the cell to the external medium, (B) The new model in which either light or substrate oxidation produces an outwardly directed electrochemical hydrogen-ion gradient for ATP synthesis. This model also suggests light-initiated oxidation of water as in chloroplast thylakoids. ⊕ denotes positive holes generated by light.

cells exposed to light, acidify the medium. The generally accepted scheme is shown in a highly simplified diagram in Fig. 2A. The initial alkalinization hitheto has not been attributed to the bacteriorhodopsin pump (5). Those papers which do address this initial alkalinization question attribute its driving force to an electrochemical gradient (interior negative) which is established in the dark (11,12). Illumination is thought to trigger an unexplained, rapid influx of protons. Upon dissipation of the initial gradient, the bacteriorhodopsin light-driven hydrogen ion pump then acidifies the media. The additional burst of acidity after turning off the light (Fig. 1B) is attributed to ATP hydrolysis. The similar curve measured upon addition of oxygen to an anaerobic cell suspension (Fig. 1A) has received a similar explanation (see Fig. 2A).

In view of our data and after a closer scrutiny of the data obtained by others, an alternative scheme is proposed which is shown in Fig. 2B.

In essence, this new scheme suggests that, if the primary function of the purple membrane is to pump hydrogen ions, it pumps them from the external medium to the interior of the cell.

The initial brief alkalinization of the medium followed by acidification observed in *H. halobium* is reminiscent of the observation of Jagendorf and Hind with chloroplast thylakoids (25). In the case of thylakoids, light generates a proton gradient by translocating H^+ ions through a phosphorylating enzyme to form ATP as envisioned by Mitchell (4).

In the case of the purple membrane, as with the thylakoid membrane, the pigment embedded in an ultrathin lipid bilayer is the key element for energy transduction, which appears to use a similar mechanism (26), that is light absorbed by the pigment causing charge separation and leading eventually to a proton gradient across the membrane with the inside of the cell (thylakoid) containing more protons. In the thylakoids it is generally assumed that water oxidation results in the release of four protons per 0_2 evolved also taking place at the interior side of the membrane. Is there any evidence for oxygen evolution in *H. halobium*? As already mentioned, exposing the cells to light greatly reduced respiration as monitored by oxygen concentration (6,17). This, however, may be interpreted to mean oxygen production as a result of water photolysis by a mechanism similar to that occurring in the chloroplast thylakoids. The H^+ ions, generated by pumping and/or by water oxidation, flow outwardly through the phosphorylating enzyme in accordance with the chemiosmatic hypothesis to acidify the medium, thereby giving rise to the observed pH change and ATP synthesis (12).

The alternative explanation applied equally well to *H. halobium* in the dark (Fig. 2B). The cells synthesize ATP in the absence of light when oxygen is available. If the same phosphorylating enzyme is used, as there is no reason to doubt otherwise, the oxygen must be used for substrate oxidation to pump protons *inwardly* and thus generate the necessary electrochemical potential gradient. Essentially, it seem highly probably that a halobacterium in the dark behaves similarly to a mitochondion and like a thylakoid in the light. It should be pointed out that this alternative scheme as depicted in Fig. 2B in any way either negates the various proposed mechanisms for the hydrogen-ion pump (27-29), nor diminishes the importance of the photochemistry of H. halobium published recently (30-32). Insofar as can be acertained, the data may be equally applicable. During the photoreaction cycle a hydrogen ion is released from the cytoplasmic surface of the membrane and one taken up at the external surface.

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