Reconstitution of Purple Membrane Vesicles Catalyzing Light-driven Proton Uptake and Adenosine Triphosphate Formation

(Received for publication, August 20, 1973)

Efraim Racker and Walther Stoeckenius

From the Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14850, and Cardiovascular Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

SUMMARY

The purple membrane from Halobacterium halobium was incorporated into phospholipid vesicles. On illumination (2 × 10⁴ ergs per cm² per s) the reconstituted vesicles took up protons (50 to 200 ng of ions H⁺ per mg of purple protein) which were released in the dark. Addition of valinomycin accelerated both the rate of uptake in the light and the release of protons in the dark. Uncouplers of oxidative phosphorylation abolished the uptake of protons. Inclusion of the mitochondrial oligomycin-sensitive ATPase during reconstitution yielded vesicles which catalyzed light-dependent phosphorylation. These reconstituted vesicles represent a simple model system for a biological proton pump capable of generating ATP from ADP and P_i.

The purple membrane forms part of the surface membrane of Halobacterium halobium cells (1). It contains only one protein, bacteriorhodopsin, which resembles the visual pigment of animals (2). It was recently reported that bacteriorhodopsin responds to a light flash with a transient shift of its absorption peak from 560 nm to 415 nm and a release and uptake of protons (3). In starved bacteria which are rich in purple membrane illumination results in H⁺ excretion, inhibition of respiration (3), and increases in intracellular ATP (4). It was proposed that a vectorial orientation of bacteriorhodopsin in the cell membrane generates a chemiosmotic gradient upon illumination (3). In this communication we describe phospholipid vesicles which are formed in the presence of the purple membrane and which transpose protons from outside to inside in the direction opposite to that observed in intact bacteria. Inclusion of the oligomycin-sensitive ATPase of bovine heart mitochondria during reconstitution with the purple membrane yields vesicles which catalyze light-dependent ATP formation from ADP and P_i.

MATERIALS AND METHODS

Growth of H. halobium and isolation of the purple membrane were performed as described previously (1, 5). The isolated membranes were stored in basal salts (growth medium without nutrients). Crude soybean phospholipids after extraction with acetone (6) or purified phosphatidylethanolamine and phosphatidylcholine (7) were used. Cardiolipin was purchased from General Biochemicals, Chagrin Falls, Ohio. The hydrophobic protein fraction (25 to 50 P) from bovine heart mitochondria and coupling factors were prepared as described previously (6).

Measurements of pH were performed with a glass electrode (A. H. Thomas 4855-L15) attached to a Corning pH meter model 12, with expanded scale connected to a Bristol 1-millivolt potentiometric recorder. The glass electrode was immersed in 1 ml of 150 mm KCl to which the reconstituted vesicles were added in a conical glass cell containing a small magnetic bar. A water jacket circulating water at 25° surrounded the glass cell. The mixture was stirred magnetically and was illuminated with a Kodak projecto, model 550, equipped with a 500-watt quartz halogen lamp. The light was passed through a OG-5 Schott filter. Calibration with standard HCl pulses was performed at the end of each experiment.

ATP formation was measured as described previously (8) in the presence of sufficient excess of hexokinase to suppress the P_i-ATP exchange catalyzed by the hydrophobic protein fraction. Protein was determined according to Lowry et al. (9). Variations in light intensity were measured with a Yellow Springs Instrument Co. model 95 radiometer.

RESULTS AND DISCUSSION

As shown in Fig. 1 illumination of phospholipid vesicles reconstituted with the purple membrane gave rise to an increase in external pH, indicating light-driven proton uptake by the vesicles. When the light was turned off the pH returned to the original level. On addition of valinomycin, in the presence of K⁺, both the rate of H⁺ uptake in the light and release in the dark were accelerated. The total extent of H⁺ uptake was sometimes increased by valinomycin, and sometimes diminished, depending on the composition of the vesicles. Uncouplers of oxidative phosphorylation, like 1799 (bis-(hexafluoroacetonyl)-acetone), eliminated the light-induced H⁺ uptake, although relatively high concentrations were needed. On the other hand, very low concentrations of nigericin (1 µg per ml) completely inhibited H⁺ uptake. These observations strongly support the suggestion that proton movement takes place across the membrane.

These experiments were performed in the presence of 150 mm KCl at pH 5.45 with no pH adjustment or addition of buffer. The low buffering capacity of the system resulted in high sensitivity. At more alkaline pH, the buffering capacity increased and the pH changes became correspondingly smaller. However, the absolute values of H⁺ uptake showed little variation between pH 5.2 and 7.4. Optimal responses were obtained at high light intensities (2 × 6 ergs per cm² per s). No differences were noted when air was replaced by argon.

These experiments can be explained as follows. In the bacterial membrane the bacteriorhodopsin is asymmetrically oriented and in the light causes protons to move from the inside to the outside (3). In the reconstituted vesicles the orientation of the purple protein in the membrane is preferentially the opposite, giving rise to proton movements from the outside to the inside. A similar preferential orientation in one direction was previously observed in experiments on the reconstitution of cytochrome oxidase vesicles (10). The final pH level reached in the light...
Fig. 1 (left). Seven micromoles of crude soybean phospholipids (6) were suspended in 0.2 ml of 150 mM KCl containing 2% sodium cholate and sonicated to clarity as described previously (7). After addition of 0.1 ml of a suspension of purple membrane (4.2 mg of protein per ml of basal salt) the mixture was again sonicated for 10 s and dialyzed for 18 hours against 100 volumes of 150 mM KCl with one change of fluid after about 15 hours. Proton movements were measured in a final volume of 1 ml as described under "Materials and Methods" with 20 μl of the reconstituted vesicles (containing 28 μg of protein). Sequence of additives represents a steady state between the light-driven proton movement and a passive back diffusion. Uncouplers of oxidative phosphorylation, which increase the permeability for protons, increase the back diffusion rate and thus reduce the response to a low level.

Instead of the crude mixture of soybean phospholipids (Fig. 1), purified phosphatidylethanolamine and phosphatidylcholine can be used in the reconstitution. Vesicles thus formed catalyzed H+ uptake proportional to the amount of vesicles used in the assay (Fig. 2). No H+ uptake was detectable when the purple membrane was assayed without added phospholipids.

When the hydrophobic protein fraction from bovine heart mitochondria, which contains the oligomycin-sensitive ATPase system (6), was incorporated together with the purple membrane, vesicles were obtained which catalyzed light-dependent ATP formation, provided coupling factors were added prior to assay. It can be seen from Table I that there was no ATP formation in the absence of light or in the presence of bis-(hexafluoroacetonyl)acetone. Marked inhibition of ATP formation was observed in the presence of an energy transfer inhibitor or in the absence of coupling factors. There was no significant ATP formation in the absence of the hydrophobic protein fraction (not included in this set of experiments). In these experiments crude soybean phospholipids were used for reconstitution. When pure phosphatidylcholine and phosphatidylethanolamine were substituted, as described in the legend to Table I, except that 0.003 ml of hydrophobic protein (25 μg per ml) was also added during reconstitution. To 20 μl of the dialyzed vesicles, 40 μg of F1 and 49 μg of oligomycin sensitivity-conferring protein were added. After 15 min at 25°, 1.0 ml of an assay solution containing 50 units of yeast hexokinase, 2 μM ATP, 32 μM glucose, 5 mM Tris sulfate, 0.3 mM ATP, and 1 mg of bovine serum albumin in 0.1 M sucrose at pH 7.4, were added, and the mixture was placed in a water bath at 30° and illuminated as described in the legend to Fig. 1. After 20 min the reaction was stopped with 0.1 ml of 50% trichloroacetic acid and analyzed for radioactive glucose-6-P (8). The abbreviation used is: 1799, bis-(hexafluoroacetonyl)acetone.

### Table I

<table>
<thead>
<tr>
<th>Additions</th>
<th>Glucose-6-P</th>
<th>Glucose-6-P/μg of bacteriorhodopsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>15.5</td>
<td>594</td>
</tr>
<tr>
<td>Illuminated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minus coupling factors</td>
<td>2.9</td>
<td>110</td>
</tr>
<tr>
<td>Plus 1799 (10−4 M)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plus rutamycin (4 μg)</td>
<td>4.0</td>
<td>170</td>
</tr>
<tr>
<td>(Dark)</td>
<td>0.0</td>
<td>23</td>
</tr>
<tr>
<td>Minus purple membrane</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

REFERENCES

Reconstitution of Purple Membrane Vesicles Catalyzing Light-driven Proton Uptake and Adenosine Triphosphate Formation
Efraim Racker and Walther Stoeckenius


Access the most updated version of this article at http://www.jbc.org/content/249/2/662

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/2/662.full.html#ref-list-1