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# BACTERIORHODOPSIN: PHOTOSIGNAL TRANSDUCTION AND PHOTOENERGY TRANSDUCTION IN DIFFERENT BIOLOGICAL SYSTEMS

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Living organisms use light as a source of energy and as a source of information. They have developed highly specialized photoenergy and photosignal transducing devices which serve these functions. Membranes are essential parts of both photosignal and photoenergy transducing systems.

In photoenergy transduction a substantial part of the absorbed energy is conserved for times very long compared to the lifetime of excited states and converted finally to chemical free energy of ATP and other forms in which it can be stored for further use by the organism. In photosignal transduction light typically triggers an event which dissipates much more energy than is absorbed in the form of light. The additional energy had been stored previously by the organism through some energy transducing systems.

## LIGHT ENERGY TRANSDUCTION

We have recently described a new light energy transducing system in halobacteria (1, 2, 3; see also 4). It is located in the cell membrane and converts light energy absorbed by a rhodopsin-like protein, bacteriorhodopsin, into a chemiosmotic gradient. The bacteriorhodopsin is the only protein required; it functions as a light-driven proton pump. The pump is apparently electrogenic, and the total energy in the gradient consists of a potential and an osmotic gradient. The cell can use the gradient to synthesize ATP and probably also to drive other energy-requiring processes such as active transport and possibly locomotion directly from the gradient. Cells respiring in the dark also produce a chemiosmotic gradient which is indistinguishable from the light-produced gradient, and energy-requiring systems of the cell appear to operate from the gradient regardless of whether the primary source of energy is substrate oxidation or light. Modifications of the cell's metabolism may, of course, be required to adapt it to the different conditions

 

of photosynthesis and respiration. This implies a photosensing function in the cell.

Comparing bacteriorhodopsin-mediated to chlorophyll-mediated photosynthesis, one is struck by the simplicity of the bacteriorhodopsin system, which appears to require only the insertion of one new protein into the cell membrane, when the cell switches from substrate oxidation to photosynthesis. By contrast chlorophyll-mediated photosynthesis requires special organelles, which contain an electron-transport chain different from the respiratory chain. Also, antenna and accessory pigments are used to absorb light energy in larger quantity and over a wider spectral range, and this energy is funneled into the specialized reaction centers where the first energy conversion occurs, resulting in an oxidized chlorophyll and a reduced acceptor. This is true even for the simplest known chlorophyll-mediated systems, such as that of the nonsulfur purple bacteria.

Perhaps the most interesting and also the most obscure step in the energy conversion is the transition from the excited state of the reaction-center chlorophyll to the first observable chemical reaction, the transfer of an electron. Here the typically very short-lived photointermediate is linked to the relatively long-lived chemical intermediate. The energy captured by accessory pigments and antenna chlorophyll is transferred to the reaction-center chlorophyll, which has a lower-energy excited state, as indicated by its longer wavelength absorption band. It acts as an efficient trap preventing the back reaction. In bacteriorhodopsin the corresponding step is presumably the $bk_{610} \rightarrow bL_{550}$ transition (4). $bK_{610}$ is formed even at liquid nitrogen temperature and in less than 10 nsec (presumably in a few picoseconds if the analogy to prelumirhodopsin is correct). $bL_{550}$ appears with a half time of 2 $\mu$sec at room temperature.

Interestingly enough the result of the folllowing steps in the reaction cycle appears to be the same in both cases – a translocation of protons across the membrane barrier and the generation of a chemiosmotic gradient – at least if we follow Mitchell's theory of photophosphorylation for chromatophores and chloroplasts. In bacteriorhodopsin no other reasonable mechanism for energy conversion has so far been proposed. The conversion to a chemiosmotic gradient constitutes another significant step on the way to energy storage with longer time constants. The gradient may be used directly for energy-requiring processes such as active transport and possibly locomotion, or it may be converted to a storage form for energy with a still longer lifetime, ATP.

The efficiency of energy conversion in chlorophyll-mediated bacterial photosynthesis has been estimated to be ~15% (5), depending on the system investigated and the storage step considered – ATP synthesis or proton translocation. Earlier steps would necessarily have higher efficiencies. In H. halobium only proton translocation or, to be exact, acidification of the medium is easily accessible experimentally and has been sufficiently studied to obtain an estimate. The measurements are complicated by the fact that under most conditions a brief alkalinization precedes the acidification (Fig. 1). However, the recorded change does essentially consist of two partially superimposed exponential processes (Fig. 2), a transient inflow of protons and a sustained ejection of protons from the cell. Alternatively, conditions have been found where the second process only is observed. Measuring the initial rate of proton ejection and the bacteriorhodopsin absorption of the same cell suspension we calculate an average quantum yield of 1.6 photons per proton translocated. This is certainly an overestimate because limitations in the instrumentation will lead to an underestimate of the initial rate and an overestimate of the bacteriorhodopsin absorption. Furthermore, some of the cells certainly have an impaired permeability

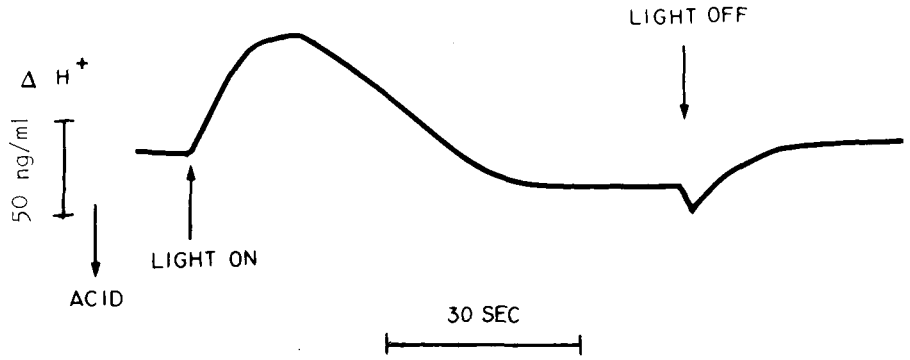


Fig. 1. Typical light-induced pH change in a suspension of Halobacterium halobium. Cell concentration $5 \times 10^9$ cells/ml. $O.D._{570} = 0.15$ (1 cm). Light intensity is $1 \times 10^6$ ergs/cm$^2$ · sec (530–650 nm).

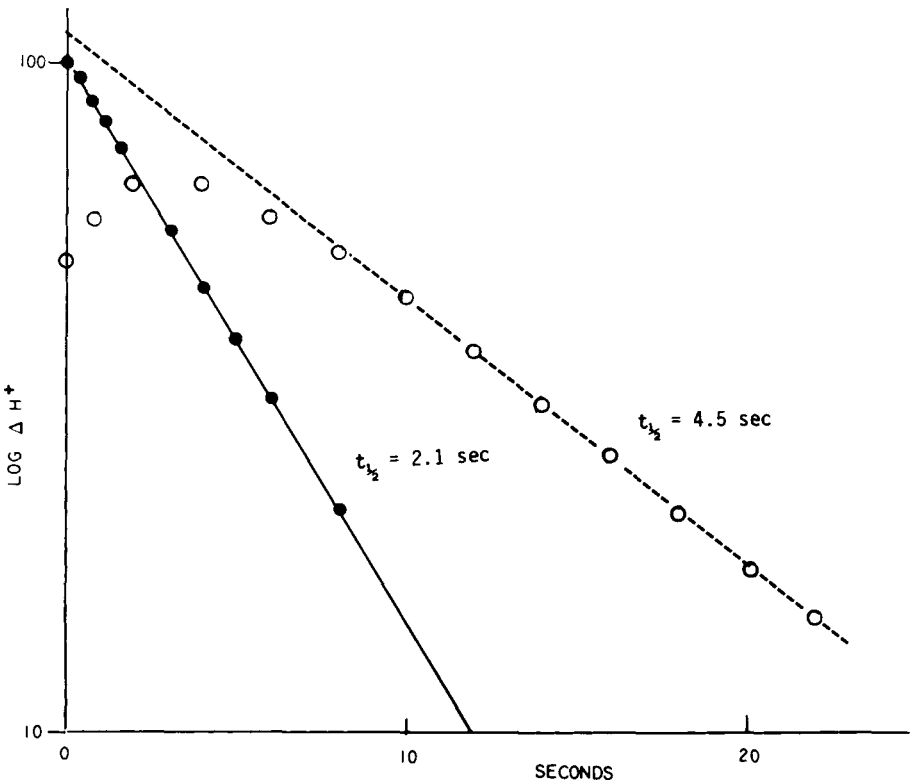


Fig. 2. Kinetic analysis of the light-on portion of the response in Fig. 1:
o o o o o: $\log \Delta H^+_{total}$ (experimental)
– – – – – – –: $\log \Delta H^+_{acid}$ extrapolated exponential component (slow acidification)
●–●–●–●: $\log \Delta H^+_{alk}$ (fast alkalinization) calculated from
$\log \Delta H^+_{alk} = \log \Delta H^+_{total} - \log \Delta H^+_{acid}$

barrier and are unable to sustain a gradient even though their bacteriorhodopsin is still functioning. This leads us to the conclusion that the actual quantum yield is probably 1.0. Assuming the stoichiometry $2H^+$/ATP, according to Mitchell, and an average energy content of 45 Kcal/einstein for the photon absorbed by bacteriorhodopsin this would yield an energy conversion efficiency of 10%. Estimating the pH gradient established across the cell membrane in the light gives a similar value. This, too, must be an underestimate in spite of some uncertainty about the buffering capacity of the cell interior, because no dissipating processes – neither the ATP synthesis nor other processes driven by the chemiosmotic gradient directly – have been taken into account. However, these rough estimates should suffice to show that light energy transduction in the bacteriorhodopsin-mediated system is comparable in efficiency to that in "conventional" photosynthesis and can constitute a sufficient energy source for H. halobium. This conclusion is also supported by the observation that anaerobic cells in the light reach as high or higher intracellular ATP concentrations than aerated cells in the dark.

## LIGHT SIGNAL TRANSDUCTION

The characteristic feature of a sensory mechanism is not the storage of energy over relatively long periods of time, but rather amplification of a very low energy conversion event. The best understood light signal transducing system is the eye. It is presently thought that absorption of light by the membrane-bound visual pigment causes a conformational change in the molecule, which then changes the permeability of the membrane, releasing an ion gradient and dissipating a comparatively large amount of energy. A second similar amplification step may occur before the amplified signal is transmitted to the next cell.

The most striking similarity between bacteriorhodopsin and the visual pigment is, of course, in their molecular structure. Both contain retinal as the chromophore, and in both it is bound as a Schiff base to a lysine residue of the protein. They show very similar light reactions, and in both cases the pigment is firmly bound to a membrane (1, 4). The significant difference is that bacteriorhodopsin undergoes a cyclic photoreaction which requires the absorption of one photon as the only energy input, whereas the visual pigments require additional energy to complete the cycle and regain the initial state.

In view of the similarities it is interesting to find that bacteriorhodopsin may also function as a signal transducer. Halobacteria are flagellated motile organisms, which swim usually in a straight line. Illuminated cells reverse the direction of swimming when the light intensity in the wavelength region absorbed by bacteriorhodopsin is suddenly decreased. An increase in the intensity of blue light has the same effect but appears to be linked to a different sensor (W. Stoeckenius, unpublished). This phototactic response has been recently investigated in more detail (K. Foster and H. C. Berg, unpublished).

The characterization of a sensory mechanism given above is complicated by the smallness of the bacteria. One photon absorbed by bacteriorhodopsin has more than enough energy to reverse the direction of swimming of a single cell (6). An amplification step therefore may not be necessary, and the distinction between an energy transducing and a signal transducing mechanism becomes difficult to make because a conversion of one form of energy into another also occurs in signal transducers. The definition of a

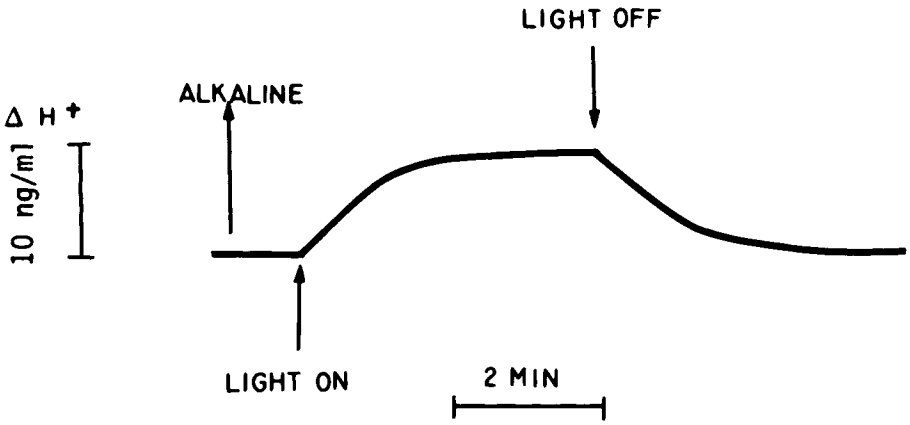


Fig. 3. Light-induced pH change. Light intensity = $5 \times 10^4$ ergs/cm$^2$ · sec. Same conditions as in Fig. 1.

sensory mechanism contains the notion that it is especially sensitive to changes in signal energy and that it transmits these as signals to other systems of the organism, modulating its behavior or metabolism. However, the same may be true for typical energy transducers and their products in an organism as small as the prokaryotic cell. Chlorophyll-containing bacteria are phototactic, and the action spectrum for phototaxis is the same as that for photosynthesis. The effect is thought to be mediated through changes in the level of ATP (7) or the "high energy intermediate" (8). Bacteriorhodopsin may similarly effect changes in other systems of the cell through changes in the chemiosmotic gradient. For instance, another sensory function for bacteriorhodopsin may be inferred from the observation that respiration is inhibited by light (1, 9). This light effect has the same action spectrum as the pH response; it is abolished by uncouplers, and the pH gradient should suffice as the link between respiration and photosynthesis.

A third sensory function of bacteriorhodopsin may, however, actually incorporate an amplification step. Light is the trigger for the initial inflow of protons (see page 778 and Fig. 3). The action spectrum for this light response is identical to the action spectrum of the proton pump and corresponds to the absorption spectrum of bacteriorhodopsin. It requires, however, much lower light intensity for its manifestation. All observations are so far consistent with the assumption that it results from a permeability change of the cell membrane for protons or another ion species, the transport of which is coupled to the proton flow. The cells apparently maintain a minimal electrochemical gradient across the cell membrane even when they are deprived of energy sources by being kept under anaerobic conditions in the dark. Evidence for this is the observation that very low light intensities or concentrations of uncouplers such as CCCP or FCCP which are too low to abolish the pumping phenomenon always cause an alkalinization of the medium in these cells. Subsequent illumination with higher light intensities then causes only an acidification of the medium without any further initial alkalinization. The postulated light-induced permeability change may not be located in the bacteriorhodopsin-containing part, the purple membrane, but in the rest of the surface membrane, because model systems consisting of phospholipid vesicles with incorporated purple membrane have never shown this phenomenon, but exhibit only the proton pumping function (3; San-Bao Hwang and

W. Stoeckenius, unpublished). Preliminary measurements indicate that the quantum yield of the alkalinization effect could be larger than 1, consistent with the assumption that it constitutes a typical sensing process.

We see that the difference between an energy transducing and a sensing mechanism is indistinct in prokaryotic cells, whereas it is quite obvious in higher organisms. We may assume that during evolution both developed from the same basic mechanisms, which, through membrane-bound pigments, converted light into other forms of energy. In the retinal-based system, the main development apparently was toward the sensory mechanism, through the addition of amplification steps, whereas in chlorophyll-based systems it was toward more efficient energy transduction through addition of a membrane-bound electron transport chain. One could envisage a more primitive system where only chlorophyll is membrane bound and the electron acceptors are both free in solution on opposite sides of the membrane, similar to the model systems developed recently (10). The high resistance barrier of the membrane appears to be essential both for energy conversion and for storage, as well as for the amplification function.

## ACKNOWLEDGMENT

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