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THE DEPENDENCY OF PROTON EXTRUSION IN THE LIGHT ON THE DEVELOPMENTAL STAGE OF THE PHOTOSYNTHETIC APPARATUS IN *RHODOSPIRILLUM RUBRUM*

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Summary

The rate of proton extrusion by whole cells of *Rhodospirillum rubrum* is constant on a bacteriochlorophyll basis only above cellular bacteriochlorophyll concentrations of about 10 nmol bacteriochlorophyll per mg cell protein. At specific bacteriochlorophyll cellular levels below this value, the rate of proton extrusion per bacteriochlorophyll increases. Correspondingly, membrane preparations isolated from these cells exhibit increases in the rate of proton uptake on a pigment basis. Concomitant with variations in the rates of proton extrusion by whole cells, light energy fluxes for saturating this process also vary. A fair proportionality between maximum rates of proton extrusion of whole cells and the bacteriochlorophyll cellular levels above 10 nmol per mg protein indicates that the degree of continuity of intracytoplasmic membranes and of the cytoplasmic membrane remains largely constant.

Introduction

Many members of the phototrophic bacteria contain the photosynthetic apparatus in intracytoplasmic membranes whilst the respiratory system is predominantly localized in the cytoplasmic membrane [1]. In spite of this functional difference, both types of membranes are morphogenetically related to each other in such a way that intracytoplasmic membranes, at least initially, arise by localized differentiation and invagination of the cytoplasmic membrane. Conversely, it has been suggested for *Rhodospirillum rubrum* that intracytoplasmic membranes become functionally differentiated and finally

integrated into the cytoplasmic membrane when, after transfer of phototrophically-grown cells from anaerobiosis in the light to aerobiosis in the dark (i.e., chemotrophy), the photosynthetic apparatus and its membrane structures are no longer synthesized [1].

Upon illumination, whole cells of phototrophic bacteria extrude protons along with photochemical electron transport while isolated intracytoplasmic membranes (i.e., chromatophores) take up protons [2,3]. In general, the determination of proton movements is of special importance for an understanding of light-driven electron transport and coupled phosphorylation [3—5].

In this communication it will be shown that in *R. rubrum* the rate of proton translocation is not constant on a bacteriochlorophyll basis, rather it exhibits considerable differences depending on the developmental stage of the photosynthetic apparatus. In addition, light energy fluxes required to saturate proton extrusions of whole cells also depend on the developmental stage of the photosynthetic apparatus. As experiments have been performed with whole cell suspensions, the data provide information on the degree of physical continuity of cytoplasmic and intracytoplasmic membranes.

Materials and Methods

Rhodospirillum rubrum (DSM No. 1068) was cultivated on malate medium as described before [6]. The cultures were adjusted to different bacteriochlorophyll specific contents, either by transferring chemotrophically grown cells to phototrophic conditions (i.e., anaerobiosis in the light) or, conversely, by transferring phototrophically grown cells to chemotrophic conditions (i.e., aerobiosis in the dark) [1].

For measurements of light-induced proton translocations, cells were harvested, washed twice and resuspended at a concentration of 300 μg protein per ml glass-distilled water [7]. After addition of NaCl or KCl (200 mM), the pH of the suspensions was adjusted to 5.3. The samples were constantly stirred in a water bath at 30°C and illuminated with white incandescent light. pH changes were monitored with a sensitive glass electrode coupled to a pH meter (E 300 B, Metrohm) and a recorder (RE 541, Servogor).

Rates of proton extrusion were calculated on the basis of initial pH changes taking place during the first 10 s of illumination. To calculate the buffer capacity of the suspensions, a known volume of HCl was added. No difference in buffer capacity was found during or after illumination. All data on cellular proton extrusion presented in this paper were determined as maximum rates from double-reciprocal (Lineweaver-Burk) plots of different rates of proton extrusion versus the respective energy fluxes. These plots were also used to calculate energy fluxes for half maximum saturation of cellular proton extrusion. Light energy fluxes were measured with a Kettering Radiometer (Model 68, Laboratory Data Control) at the surface of the sample cuvette. Different light energy fluxes were obtained at a fixed geometry of the set-up by placing different neutral gray filters (NG, Schott and Gen., Mainz) between the light source and the sample. For measurements of light-induced proton uptake by crude membrane preparations, membranes were isolated and incubated as described [8,9]. Light energy flux was $5 \cdot 10^2\ W \cdot m^{-2}$. Bacteriochlorophyll

was determined in methanol [10]. Protein was determined according to the method of Lowry et al. [11].

Results

Fig. 1 shows the dependency of the maximum rates of proton extrusion in the light on the specific bacteriochlorophyll contents of whole cells of *R. rubrum*. It becomes evident that the rates are constant on a bacteriochlorophyll basis only above cellular bacteriochlorophyll levels of about 10 nmol per mg protein. But, decreasing cellular bacteriochlorophyll concentrations below this value results in increasing rates of protons extrusion. It is noteworthy that it does not matter if low specific contents in bacteriochlorophyll were achieved either by adapting chemotrophic cells to phototrophic conditions or by the reversal of this process.

On a cell-protein basis, the rates of proton extrusion increase drastically at rather low cellular pigment concentrations and, after reaching a peak level, they decrease slightly. But as cells reach bacteriochlorophyll concentrations (about 10 nmol per mg protein) from which, thereafter, constant amounts of protons are extruded on a pigment basis per min, the rates of cellular proton translocation increase largely in proportion to the specific bacteriochlorophyll

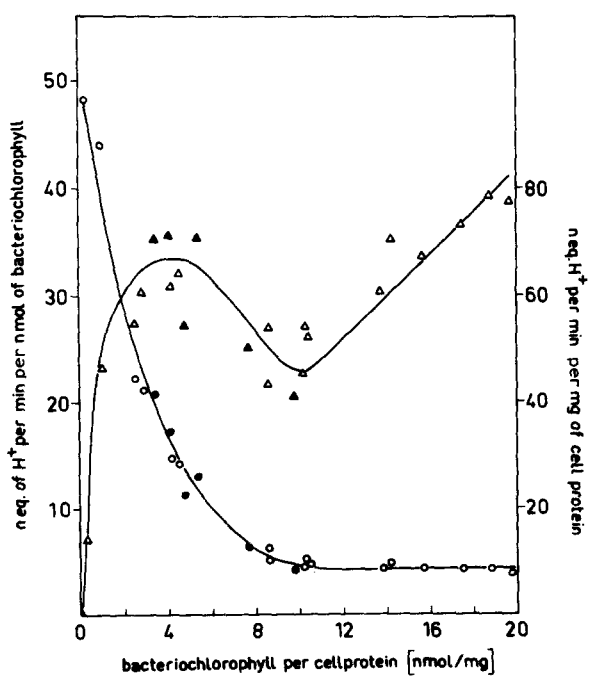

Fig. 1. Maximum rates of proton extrusion by whole cells of *Rhodospirillum rubrum* as determined with a glass electrode. (●———●, ○———○) nequiv. H^+ extruded/nmol BChl per min; (▲———▲, △———△) nequiv. H^+ extruded/mg cell protein per min. The open symbols represent values obtained with cells at different stages of adaptation from chemotrophic to phototrophic conditions, closed symbols represent values obtained with cells at different stages of adaptation from phototrophic to chemotrophic conditions. BChl, bacteriochlorophyll.

TABLE I

PROTON UPTAKE BY MEMBRANE PREPARATIONS ISOLATED FROM CELLS OF *RHODOSPIRILLUM RUBRUM* OF DIFFERENT BACTERIOCHLOROPHYLL (BChl) CONCENTRATIONS

BChl per cell (nmol/mg protein)	Proton uptake (nequiv. H^+/nmol BChl per min)
11	0.44
5.6	0.9
2.9	1.3
0.9	6.0

concentration. Differences in proton translocation as determined with whole cells might be due to differences in the intracellular milieu of solutes. To test this possibility, membrane preparations were isolated from cells of different bacteriochlorophyll contents and subjected to measurements of light-induced proton uptake. The data (Table I) show that with isolated membranes the observed relationship also exists between the rates of proton uptake and the respective cellular bacteriochlorophyll concentrations.

Concomitant with increasing rates of proton extrusion by whole cells, the requirements in light energy fluxes of this process also increase. Fig. 2 depicts the dependence on the cellular bacteriochlorophyll concentration of half-maximum light energy fluxes for proton extrusions. By comparing Figs. 1 and 2, it becomes evident that below specific pigment concentrations of about 10 nmol per mg cell protein, increasing the rates of proton extrusion also means increasing the energy requirements of this light-dependent process.

Finally, in the context of energetic considerations, it is noteworthy that variations in the extents of proton extrusions obviously do not occur at the expense of the membrane potential and vice versa. This was shown by addition of valinomycin (4 μM) in the presence of KCl (200 mM) [12]. Cells of both 5.4 and 13 nmol bacteriochlorophyll per mg cell protein exhibit maximally about 15% stimulation by valinomycin of the rates of proton translocation.

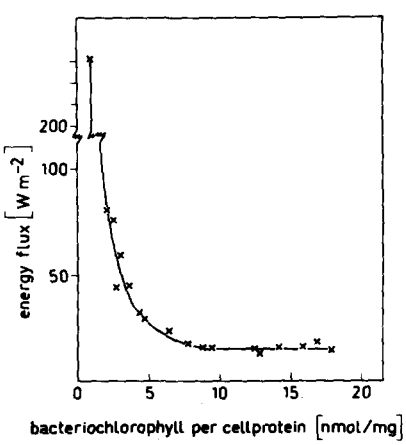

Fig. 2. The dependency on the specific cellular bacteriochlorophyll concentration of light energy fluxes required for half-maximum saturation of proton extrusion by whole cells of *Rhodospirillum rubrum*.

Discussion

Nishimura [13] localized the rate-limiting step in the photochemical electron transport system of *R. rubrum* between cytochromes *b* and *c*. In support of this, Prince and Dutton [14] suggested a hypothetical constituent 'Z' between the two cytochromes to regulate electron flow and concomitant proton translocation in *Rhodopseudomonas sphaeroides*. The variable rates of proton extrusion as well as the variable energy requirements of this process indicate that, in *R. rubrum*, the activity of the rate-limiting step is variable in particular at low cellular levels of bacteriochlorophyll which are proportional to the cellular levels of both reaction center and light-harvesting bacteriochlorophyll [15]. Low bacteriochlorophyll cellular levels can be obtained with facultative phototrophic bacteria during early stages of development of the photosynthetic apparatus, or after transfer of phototrophic cells from low to either high light intensity or to chemotrophic conditions [1,10]. Under all of these conditions, increased rates on a bacteriochlorophyll basis in photophosphorylation have been observed [16,17]. Michels and Konings [18,19] reported that although cytoplasmic membrane vesicles exhibited more than 20 times less bacteriochlorophyll than fully-developed chromatophores of *R. sphaeroides*, upon illumination, both types of membranes generated electrochemical proton gradients of nearly equal magnitude. This and the data reported herein suggest electrochemical proton gradients to be also increased at saturating light energy fluxes on a bacteriochlorophyll basis in cells of low pigment contents.

It was proposed before that the photosynthetic apparatus is formed immediately after transfer from chemotrophic to phototrophic conditions by incorporation of the necessary bacteriochlorophyll complexes (i.e., reaction center and light-harvesting moieties) into the cytoplasmic membranes [1], taking advantage of the existing constituents of the respiratory system in the early formation of the photochemical electron transport system. On the other hand, the existing intracytoplasmic membrane system and, accordingly, the pattern and distribution of the photosynthetic apparatus are differentiated under conditions of limited bacteriochlorophyll synthesis by the incorporation of constituents of the respiratory system [1,16].

The high rates of proton extrusion at low cellular pigments contents are in good agreement with the concept that constituents which are already present under chemotrophic conditions, or which are formed preferentially under conditions of limited bacteriochlorophyll synthesis (e.g., constituents of the section between cytochromes *b* and *c* of the respiratory chain [20,21]), participate in the rate-limiting step of photochemical electron transport. Provided light energy fluxes are saturating, this leads to increased rates of electron flow. But, the rates decrease as the ratio of bacteriochlorophyll per respiratory chain increases. At the final stage of development, the photosynthetic apparatus is formed from its various constituents in constant proportion. This again agrees with the constant rate of proton extrusion on a bacteriochlorophyll basis above cellular pigment concentrations of 10 nmol per mg protein. The data, furthermore, have some bearing on the physical state of intracytoplasmic membranes within the cell. Electron micrographs of thin sections of several members of the phototrophic bacteria revealed intracyto-

plasmic membranes predominantly as closed vesicles [1,10,22]. This led to the postulation that intracytoplasmic membranes, at least partially, exist in vivo as closed vesicular entities [22]. It is known from published data that the specific bacteriochlorophyll contents of intracytoplasmic membranes of *R. rubrum* stay constant above cellular bacteriochlorophyll concentrations of 10 nmol per mg protein [1]. In addition, the data of this report show that the rates of cellular proton extrusion and cellular bacteriochlorophyll concentrations above 10 nmol per mg protein are largely proportional. This implies that the proportion of intracytoplasmic membranes which are continuous with the cytoplasmic membrane stays largely constant.

Acknowledgements

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