The Effect of Transfer from Low to High Light Intensity on Electron Transport in \textit{Rhodospirillum rubrum} Membranes

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Abstract. The effects of transfer from low to high light intensity on membrane bound electron transport reactions of \textit{Rhodospirillum rubrum} were investigated. The experiments were performed with cultures which did not form bacteriochlorophyll (Bchl) for about two cell mass doublings during the initial phase of adaptation to high light intensity. Lack of Bchl synthesis causes a decrease of Bchl contents of cells and membranes. Also, the cellular amounts of photosynthetically active intracytoplasmic membranes decrease.

In crude membrane fractions containing both cytoplasmic and intracytoplasmic membranes the initial activities of NADH oxidizing reactions increase only slightly (about 1.2 times) per protein, but the initial activities of succinate oxidizing reactions decrease (multiplied by a factor of 0.7). On a Bchl basis activities of NADH oxidizing reactions increase 3.4 times while activities of succinate dependent reactions increase 1.9 times. With isolated intracytoplasmic membranes activities of NADH as well as succinate dependent reactions increase to a comparable extent on a Bchl basis (about 1.8 times) and stay nearly constant on a protein basis. Cytochrome c oxidase responds like succinate dependent reactions. The data indicate that in cells growing under the conditions applied NADH oxidizing electron transport systems are incorporated into both, cytoplasmic and intracytoplasmic membranes, while incorporation of succinate oxidizing systems is confined to intracytoplasmic membranes only.

Activities of photophosphorylation and succinate dependent NAD\(^+\) reduction in the light increase per Bchl about 1.8 times. On a Bchl basis increases of the fast light induced \textquotedblleft on\textquotedblright\ reactions at 422 nm and of \textit{b}-type cytochrome levels become three times greater then increases of cytochrome \textit{c}\(_2\) reactions and levels. These results infer that although electron transport reactions of intracytoplasmic membranes change correlated to each other, Bchl, cytochrome \textit{c}\(_2\) and \textit{b}-type cytochromes cellular levels are independent of each other. Furthermore, the data indicate that cytochrome \textit{c}\(_2\) rather than \textit{b}-type cytochrome is involved with steps rate limiting for photophosphorylation.

Key words: \textit{Rhodospirillum rubrum} — Membrane bound functions — Bchl-levels — NADH and succinate dependent electron transport systems — Photophosphorylation — Light induced cytochrome reactions — Cytochrome \textit{c}\(_2\) — \textit{b}-type cytochrome.

Like other members of the Rhodospirillaceae cells of \textit{Rhodospirillum rubrum} form photosynthetically active intracytoplasmic membranes under anaerobic light conditions (or low aeration) (Oelze and Drews, 1972). Accordingly bacteriochlorophyll (Bchl) is one of the most prominent constituents of intracytoplasmic membranes. In addition to the photosynthetic apparatus intracytoplasmic membranes exhibit low but significant respiratory activities. Yet, within the cell most of the respiratory system is located in the cytoplasmic membrane (Throm et al., 1970).

The cellular amount of intracytoplasmic membranes is not constant, it varies dependent on the light intensity applied for phototrophic cultivation. Electronmicroscopic investigations revealed that relative to high light intensities low light intensities cause an increase of the cellular contents of intracytoplasmic membranes and vice versa (Cohen-Bazire and Kunisawa, 1963; Holt and Marr, 1965). Correspondingly,
the cellular Bchl contents vary also. Such variations influence the proportions of intracytoplasmic to cytoplasmic membranes as well as the proportions of the respective functions.

In agreement with this we demonstrated before that transfer of low light grown cells of \textit{R. rubrum} to high light intensity results in an increase of respiratory activities relative to photosynthetic activities (Irschik and Oelze, 1973). Moreover, it was shown that the Bchl contents decreased not only of whole cells but also of intracytoplasmic membranes. This again was accompanied by changes in the functional patterns of intracytoplasmic membranes.

For the present communication we extended our previous investigations to obtain a more detailed picture concerning the effects of high light intensity on various activities of the photosynthetic apparatus and the respiratory electron transport system.

\textbf{MATERIALS AND METHODS}

\textit{Rhodospirillium rubrum}, strain FR1 (Lehrstuhl für Mikrobiologie, Freiburg), was cultivated under high light intensity (4 x 10^5 erg/cm^2 s) as described before (Irschik and Oelze, 1973). Bacteria were harvested by centrifugation. For measurements of light dependent reactions and respiratory activities cells were resuspended in 0.1 M glycyl-glycine buffer (pH 7.6). Cells used for quantitative determination of cytochromes were resuspended in distilled water.

For membrane isolation cells were homogenized by two passages through a French-pressure cell at 16000 lb/inch^2 and 4°C. Homogenates were separated from whole cells and large debris by centrifugation at 15900 x g for 20 min. After this supernatant were centrifuged at 229400 x g for 60 min. The resulting sediment was used either as crude membrane fraction or subjected to Ficoll gradient centrifugation for isolation of purified intracytoplasmic membranes (Oelze et al., 1969).

All of the enzyme activities assayed spectrophotometrically. NADH oxidase was measured at 340 nm by the oxidation of 0.15 mM NADH (Throm et al., 1970). NADH dehydrogenase (EC 1.6.99.3) and succinate:cytochrome c reductase (EC 1.3.99.1) activities were measured by assay of DCIP reduction at 578 nm.

Activities of the respiratory chain were measured on the basis of the extinction coefficient of 20.6 mM^{-1} cm^{-1} at 578 nm. NADH:cytochrome c reductase (EC 1.6.99.3) and succinate:cytochrome c reductase (EC 1.3.99.1) were assayed in 0.05 M phosphate buffer (pH 7.6) supplemented with 2 mM sodium azide, 0.05 mM horse heart cytochrome c, and either 0.15 mM NADH or 6 mM sodium succinate. Cytochrome c reduction was measured with an Eppendorf spectrophotometer (filter Hg 546 nm). Cytochrome c oxidase (EC 1.9.3.1) was determined at 550 nm (Wharton and Trz goloff, 1967). Horse heart ferrocytochrome c was obtained by reduction with ascorbate. Oxidized and excess ascorbate were removed by chromatography on Sephadex G-25. The reaction mixture contained 0.05 M phosphate buffer (pH 7.6) and 0.05 mM ferrocytochrome c. Activities were calculated by using the extinction coefficients of 21 mM^{-1} cm^{-1} (filter Hg 546 nm) and 19 mM^{-1} cm^{-1} at 550 nm.

\textbf{RESULTS}

Cells of \textit{Rhodospirillium rubrum} grown anaerobically under low light intensity were used to inoculate fresh culture medium at a low optical density. These cultures were transferred to high light intensity and cultivated at 30°C. As shown before Bchl synthesis is inhibited under these conditions for a while although cells continue to grow (Irschik and Oelze, 1973; Table 1). Consequently the specific Bchl contents of whole cells, of the crude membrane fraction, and even of intracytoplasmic membranes decrease (Table 1). As the total Bchl contents of the culture remain constant the decrease of the specific Bchl contents of cells as well as of membranes must be the results of additional protein formation. These proteins might be correlated to membrane bound physiological activities other than photosynthesis (Irschik and Oelze, 1973).

Activities of the respiratory chain were measured with crude membrane and purified intracytoplasmic membrane fractions.