OBSERVATIONS ON DISTRIBUTION OF NADH OXIDASE IN PARTICLES FROM DARK-GROWN AND LIGHT-GROWN Rhodospirillum rubrum*

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The relation between the NADH-oxidase systems of the facultative photoheterotrophe, Rhodospirillum rubrum, obtained from cells grown aerobically in the dark and grown anaerobically in the light remains to be elucidated. In a scheme proposed for light-grown cells, it has been suggested (Yamashita, et al., 1967) that cytochrome cc' and cytochrome b-563 are components. However, cytochrome cc' is absent from dark-grown cells (Taniguchi and Kamen, 1965). Moreover, kinetic analyses of oxygen uptake in whole light-grown cells reveal no correlation between the cellular oxidase and cytochrome cc' (Chance, et al., 1966).

Cultures of dark- and light-grown R. rubrum were grown and transition experiments performed as described elsewhere (Yamashita and Kamen, 1969). For sucrose gradient centrifugation, cells were collected and washed by centrifugation in the usual manner (Yamashita and Kamen, 1968) and then re-suspended in 0.01 M Tri-HCl buffer, pH 7.2, containing 1 μg per ml DNA-ase (Worthington Biochem. Corp., Type DPFF). The cells were broken in a Sorvall Ribi Cell Fractionator at 20,000 psi (15°C.).

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The lysate was centrifuged at 10,000 g for 10 min. 1 ml of the resultant supernatant was layered on a linear sucrose gradient (5-60% in 0.01 M Tris-HCl buffer, pH 7.2, 26 ml of the total volume*). The gradient tube was centrifuged in a SW 25.1 rotor at 22,500 rpm for 110 min. The sedimented materials were fractionated and estimated using methods described elsewhere (Yamashita,Kamen and Horio, 1969). NADH oxidase activity and its coupled oxidative phosphorylation were assayed as previously reported (Yamashita et al., 1967). ^{3}H-uracil, ^{14}C-phenylalanine and ^{14}C-algal protein hydrolysate were purchased from New England Nuclear Co., Boston, Mass.

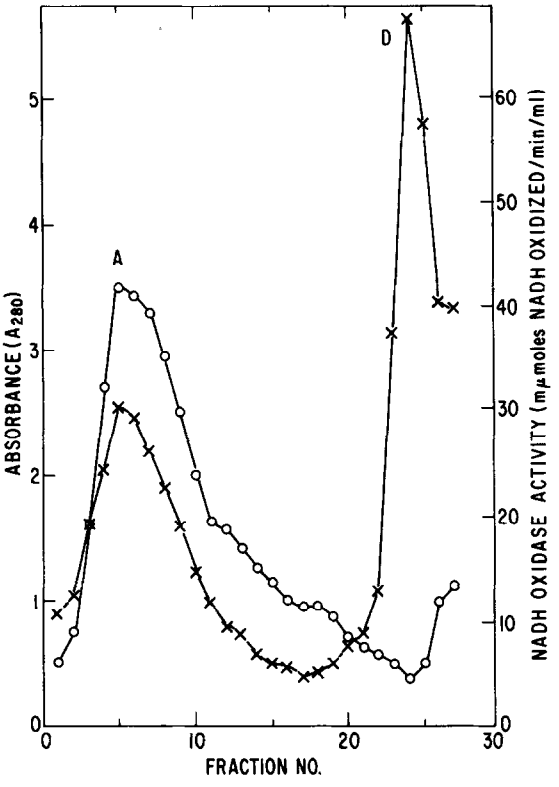

Fig. 1. Profile of sucrose gradient centrifugation of dark-grown R. rubrum. Experimental conditions, see text. Running time, 110 min. (x), absorbance at 280 mμ; (o), NADH oxidase activity.

* In some cases, 1 mM $MgCl_2$ was added but no effect of this addition on the profile of sucrose gradient centrifugation was found.

In the dark and under high oxygen tension. R. rubrum grows non-photosynthetically in a respiratory mode. A profile obtained by sucrose gradient centrifugation of a dark-grown cell extract is shown in Fig. 1. Two peaks of absorbance at 280 mμ appeared. The absorption curves of each fraction (labelled "A" and "D") showed that fraction A (hereafter called "particulate fraction") had a shoulder at 270-275 mμ but no maximum at 260 mμ, while fraction D had a distinct maximum at 260 mμ. Extracts from dark-grown cells contain NADH oxidase activity and its coupled oxidative phosphorylation activity (Geller, 1963; Taniguchi and Kamen, 1965: Yamashita et al., 1967). In our experiments, the major portion of NADH oxidase activity was associated with fracion A whereas less than 5% of the total activity appeared in the soluble fraction. Geller (1963) and Taniguchi and Kamen (1965) reported similar results on sucrose gradient centrifugation of dark-grown cells. Oxidative phosphorylation coupled with NADH oxidation was strictly associated with fraction A, and not with the soluble fraction, and fraction D consisted mainly of ribosomes.

Profiles obtained by sucrose gradient centrifugation of light-grown R. rubrum showed two reddish purple colored bands in the upper part of the tube. Similar observations have been reported by several authors (Newton and Newton, 1959; Cohen-Bazire and Kunisawa, 1963; Worden and Sistrom, 1964; Holt and Marr, 1965b). These have been called "light" and "heavy" particles. In addition to these colored components, we observed a very faint colored band near the bottom of the tube. It was at almost the same position as that of fraction A from dark-grown cell extracts. Thus, it appeared that the light-grown cell extracts produced mainly chromatophores. To establish the presence of the rapidly sedimenting, pale component in the light-grown cell extract, dark-grown R. rubrum was exposed to light under anaerobic conditions for 12 hours to adaptively synthesize bacteriochlorophyll. The extract of these light-adapted cells was sedimented in a sucrose gradient and the profile obtained is shown in Fig. 2. Three peaks for the absorbance at 280 mμ

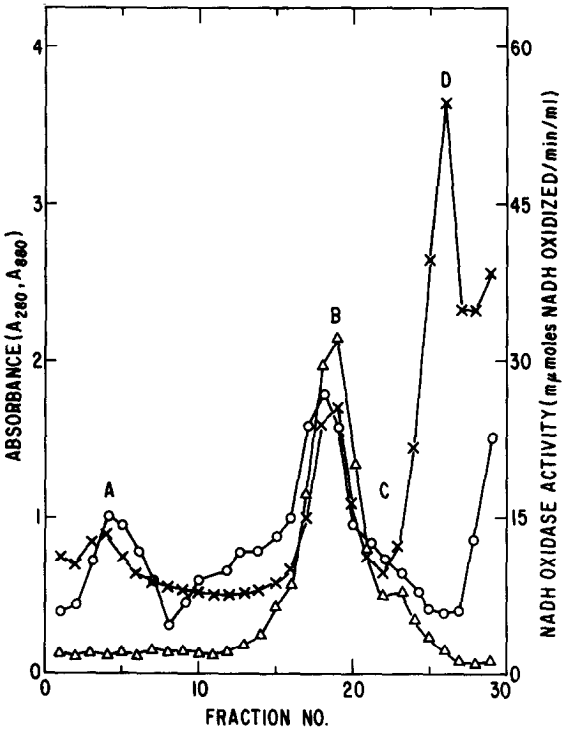

Fig. 2. Profile of sucrose gradient centrifugation of light adapted R. rubrum. Dark-grown cells were incubated for 12 hours under illumination of 70 ft-candles. Thereafter, the cell-free extract was prepared and sedimented. Other experimental conditions were the same as in Fig. 1.
(x), absorbance at 280 mμ; (Δ), absorbance at 880 mμ; (o), NADH oxidase activity.

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appeared ("A". "B", and "D") but bacteriochlorophyll ($A_{880m\mu}$) was associated with only fraction B and a shoulder (labeled "C"). Absorption curves for the fractions A and D were the same as those in dark-grown cell extracts. Fraction B had absorption maxima at 270 mμ. NADH oxidase activity appeared in A, B, the shoulder C, and in the soluble fraction, but oxidative phosphorylation activity was associated only with the particle fractions.

After transition from dark to light metabolism, it has been found that

amino acid incorporation occurs exclusively in the chromatophore fraction, that is, the newly synthesized protein is associated with the colored particles shown in Fig. 2. Also, it has been found that uracil-incorporation and bacteriochlorophyll formation increase (Yamashita and Kamen, 1968). In Fig. 3 is shown the light-stimulation of amino acid incorporation observed after transition under non-growing conditions. In general, synthesis of bacteriochlorophyll exhibits a lag time in the light, but amino acid incorporation is quickly stimulated after onset of transition. During this transition, protein estimated by Lowry's method (Lowry, 1951) does not show any increase. Gray (1967) has reported that Rhodopseudomonas spheroides preferentially incorporates amino acid into chromatophores.

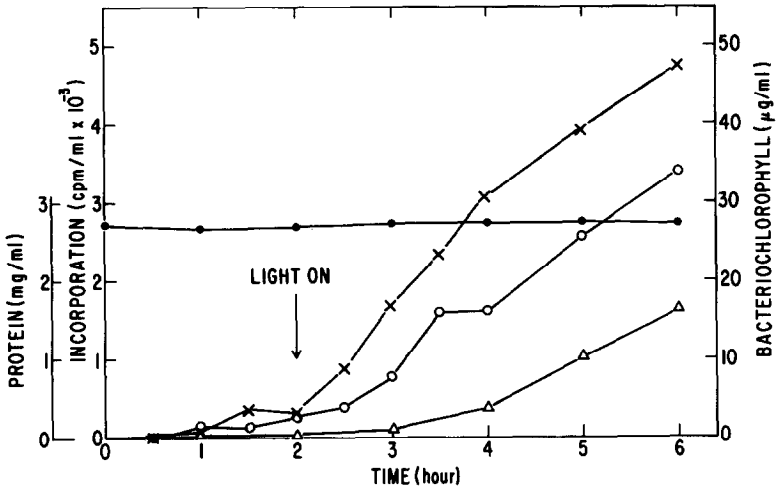

Fig. 3. Incorporation of uracil and amino acid.

Reaction was started by the addition of 0.025 μc per ml of ^{14}C-algal protein hydrolysate and 0.5 μc per ml of ^{3}H-uracil. At the time indiated by an arrow, cells were submitted to illumination. Estimations of the incorporated radioactive materials, protein and bacteriochlorophyll were carried out as reported previously (Yamashita and Kamen, 1968).

(x), amino acid incorporation. (o), uracil incorporation; (Δ), bacteriochlorophyll; (•), protein.

After dark-grown cells were incubated for one hour with 20 μg per ml of ^{12}C-phenylalanine, they were illuminated and 0.1 μc per ml of ^{14}c-phenylalanine was added simultaneously. At various times, aliquots of cell suspensions were removed and disrupted in the Ribi Cell Fractionator. Each cell-free extract was subjected to sucrose gradient centrifugation. The results are shown in Fig. 4. At two hours' incubation, no chromatophore fractions were found, but a trace of radioactive phenylalanine was incorporated in the fraction at the position expected for chromatophores. After 4.5 hours, chromato-

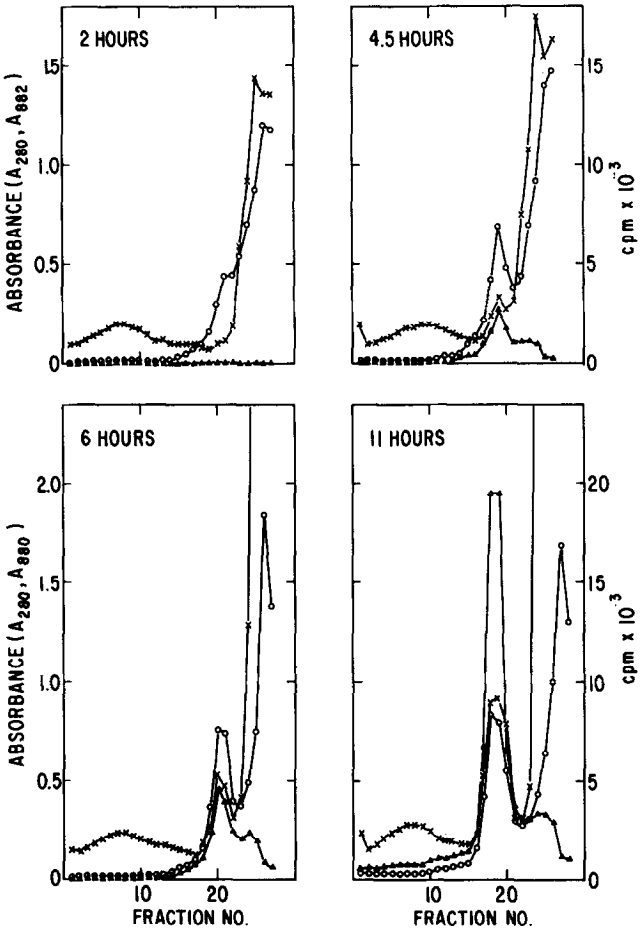

Fig. 4. Incorporation of amino acid into particle fraction during transition, measured by ^{14}C-phenylalanine incorporation.
Experimental conditions are described in text.
(x), absorbance at 280 mμ; (Δ), absorbance at 880 mμ (o), radioactivity.

phores were distinctly seen and thereafter all radioactive amino acid incorporation occurred in the chromatophore fraction. However, there was no incorporation in the heavy particle fraction (A). This preferential protein synthesis in chromatophores was also observed relative to the estimation of absorbance at 280 mμ.

Electron microscopic studies on the structure of cells of R. rubrum demonstrate that chromatophores can be formed by invagination of the cell membrane (Cohen-Bazire and Kunisawa, 1963; Boatman, 1964; Holt and Marr, 1965a). Keister et al. (1968) observed the specific synthesis of NADH oxidase protein and suggested the reorganization of cell membrane of R. rubrum. Taken with our results, it is reasonable to suggest that the protein fraction in the cell membrane is the locale for newly synthesized protein made in the light.

Localization of NADH oxidase on both the particle fraction from dark-grown R. rubrum and in chromatophore fractions from light-grown cells appears to occur and indicates that this enzyme system could be essential for electron transport in both dark- and light-grown cells.

Finally, it may be noted that the oxidase systems from both types of cells show the same dependence on substrate (NADH) and response to inhibitors, indicating they possess the same NADH dehydrogenase (Yamashita, Yoshimura and Horio, unpublished; Yamashita, Kamen and Horio, 1969).

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