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Photophosphorylation and Oxidative Phosphorylation in Intact Cells and Chromatophores of an Aerobic Photosynthetic Bacterium, *Erythrobacter* sp. Strain OCh114

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Light-induced ATP synthesis was studied in intact cells and chromatophores of *Erythrobacter* sp. strain OCh114. ATP synthesis was measured by both the pH method and the luciferin-luciferase luminescence method. The rate of ATP synthesis was moderate (a typical value of 0.65 mol of ATP per mol of bacteriochlorophyll per min), and synthesis was inhibited by antimycin A. ATP was synthesized under illumination only under aerobic conditions and not under anaerobic conditions. This characteristic was similar to that of other light-induced energy transduction processes in this bacterial species, such as oxidation of reaction center, oxidation of cytochrome c_{551}, and translocation of H^+, which were not observed under anaerobic conditions. This phenomenon was reconciled with the fact that the *Erythrobacter* sp. could not grow anaerobically even in the light. The characteristics of oxidative phosphorylation and ATP hydrolysis were also investigated. The respiratory ratio of chromatophores was 2.3. Typical rates of oxidative phosphorylation by NADH and by succinate were 2.9 mol of ATP per mol of bacteriochlorophyll per min (P/O = 0.22) and 1.1 mol of ATP per mol of bacteriochlorophyll per min (P/O = 0.19), respectively. A typical rate of ATP hydrolysis was 0.25 mol of ATP per mol of bacteriochlorophyll per min in chromatophores. ATPase and adenylate kinase are also involved in the metabolism of adenine nucleotides in this bacterium.

All the bacterial species containing bacteriochlorophyll (Bchl) had been believed to be able to grow anaerobically under illumination by using light energy, until exceptional bacteria were isolated in 1978 (6, 15). They had Bchl *a* but could not grow photoanaerobically and have been called aerobic photosynthetic bacteria. They include *Erythrobacter* spp. (6, 18), "*Protaminobacter ruber*" (15), *Pseudomonas* sp. strain AM-1 (15), and *Pseudomonas radiora* (12) and cannot be grouped with the known species of photosynthetic bacteria.

Some reports suggest that *Erythrobacter* spp. can utilize light energy under aerobic conditions. CO_2 incorporation and ATP levels in intact cells were enhanced by light (17), and the bacterial cell yield was increased about twofold above that of a dark-grown culture when the aerobically grown culture was illuminated at a high light intensity after a preceding dark period (24 h) (K. Harashima, K. Kawazoe, and I. Yoshida, Abstr. 5th Int. Symp. Photosynthetic Prokaryotes, Grindelwald, Switzerland, 1985). We also showed the light-dependent translocation of H^+ (14). These reports suggest that in this bacterium the photochemical systems are operative. Indeed, *Erythrobacter* spp. and "*Protaminobacter ruber*" show photochemical activities (5, 13, 20), such as reversible photooxidation of reaction center Bchl and cytochromes. Why cannot this bacterium grow anaerobically under illumination? We showed that light-induced oxidation of the reaction center, oxidation of cytochrome c_{551}, and H^+ translocation stopped under anaerobic conditions (14). We also suggested that the inability of this bacterium to grow photoanaerobically was due to the reduction of the primary electron acceptor (Q_A) before illumination, which was partly due to the relatively high midpoint potential of Q_A in this bacterium (14).

In this communication we report photophosphorylation activity at a moderate level in intact cells and isolated chromatophores. Light-induced ATP formation ceased under anaerobic conditions, as did other photosynthetic reactions. ATP was also synthesized by oxidative phosphorylation when phosphorylation was coupled to the oxidation of a substrate (NADH or succinate). ATP hydrolysis and adenylate kinase activities were observed in isolated chromatophores of this bacterium.

MATERIALS AND METHODS

Culture of cells and preparation of chromatophores. *Erythrobacter* sp. strain OCh114 (ATCC 33942) was cultured at 26 to 29°C in enriched PPES-II medium (19) for 2 to 3 days in the dark with vigorous stirring. The composition of PPES-II medium was the same as described previously (14).

To prepare chromatophores, cells were harvested and suspended in a mixture of 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) pH 8.0, 100 mM KCl, 5 mM $MgCl_2$, and a small amount of DNase. The suspended cells were broken in a French pressure cell at 130 MPa, and chromatophores were isolated by the method of Doi et al. (3). The precipitated chromatophores were resuspended in the medium described above (without DNase) and used for experiments.

Measurement of pH changes of the suspension of chromatophores and intact cells. Light-induced pH changes of the suspension of chromatophores and intact cells were measured with a pH electrode (Horiba no. 6028) with a Hitachi-Horiba pH meter (model F-7_{ss}). Continuous light was provided from a halogen lamp (650 W) through a red cutoff filter (Toshiba VR-69; light intensity, 400 W/m^2). The

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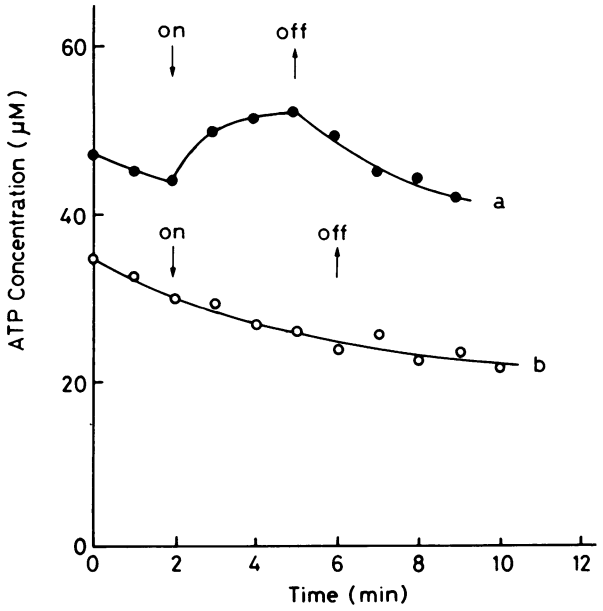

FIG. 1. Time course of ATP synthesis by chromatophores under illumination in aerobic and anerobic conditions. The reaction mixture contained 5 mM HEPES buffer, pH 8.0, 100 mM KCl, 5 mM $MgCl_2$, 1 mM KH_2PO_4, 0.17 mM ADP, 12 mM sodium succinate, 0.12 mM PMS, and chromatophores. The Bchl concentration was 19 μM. The arrows show the times that light was turned on and off. ATP concentrations in chromatophore suspensions were determined by the luciferin-luciferase method. See text for details. Trace a, Aerobic conditions. The suspension was stirred in an open vessel. The O_2 concentration was ca. 33 to 46% saturation. Trace b, Anaerobic conditions. Glucose oxidase (500 μg/ml), D-glucose (2.5 mM)and catalase (50 μg/ml) were added to the suspension, which was covered by liquid paraffin and stirred continuously. The O_2 concentration was below 2.0% saturation.

initial pH was adjusted to 6.4. The temperature was maintained at 25°C.

ATP extraction and determination. In membrane suspensions, the ATP concentration was determined directly by the luciferin-luciferase assay in ATPase-inhibiting arsenate buffer (100 mM, pH 7.75) containing 40 mM $MgSO_4$. A typical assay mixture contained 0.1 ml of chromatophore suspension and 0.5 ml of firefly extract (2.0 mg/ml) (Sigma Chemical Co.) in arsenate buffer. The ATP concentration was calibrated by using a known amount of ATP. Chemiluminescence of the luciferase reaction was detected with an Aminco Chemo-Glow photometer (American Instrument Co.). In intact cells ATP was released by extraction with 90% (vol/vol) dimethyl sulfoxide (DMSO) by the method of Leps and Ensign (7). One volume of the suspension was rapidly mixed with 9 volumes of DMSO and incubated at 25°C for approximately 2 min. One volume of the DMSO extract was then diluted with 5 volumes of 10 mM morpholinopropanesulfonate buffer, pH 7.4. The concentration of ATP in the diluted extract was determined by the luciferase reaction as for membrane preparations.

The glass electrode method was also used to determine photophosphorylation activity by measuring the alkalinization of the reaction mixture due to ATP formation from ADP and P_i (11). The initial pH was adjusted to 7.4 and the n value ($\Delta H^+/\Delta P_i$) of 0.891 was used for determination of the amount of ATP formed.

NMR measurement. ^{31}P nuclear magnetic resonance (NMR) measurements were performed as described previously (8) with a JEOL GX-400 spectrophotometer at 161.8 MHz. Spectra were obtained by accumulation of 2,500 transients with a 45° pulse at intervals of 0.22 s. The resonances of adenine nucleotides and P_i were assigned by comparison of chemical shift values with those of authentic samples. Chemical shift values were obtained from external methylenediphosphonic acid solution. Illumination of the samples was provided by a 1-kW xenon lamp. The light was passed through a Wratten 88A filter and introduced into the sample by an optical fiber and a quartz light guide as described previously (9). Oxygen was provided during the measurements by bubbling O_2 at the bottom of the sample tube (2.0 ml/min). All measurements were performed at 25°C.

Other procedures. The oxygen concentration of the suspension was measured with an oxygen electrode (Rank Brothers). Bchl concentration was determined in an acetone-methanol extract by the method of Clayton (2).

RESULTS

H^+ translocation and ATP formation in chromatophores and intact cells. We had already observed light-dependent

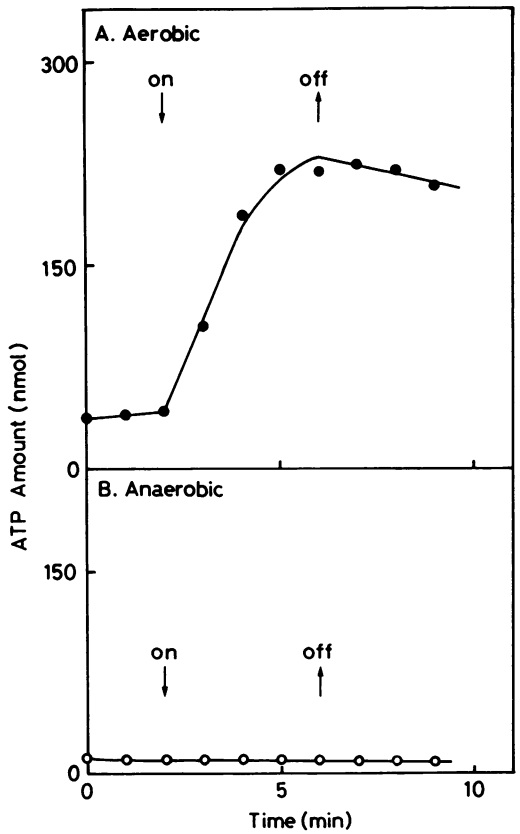

FIG. 2. Time course of ATP synthesis of intact cells under illumination in aerobic and anaerobic conditions. The reaction mixture contained 5 mM HEPES buffer, pH 8.0, 100 mM KCl, 10 mM $MgCl_2$, and intact cells. The Bchl concentration was 29 μM. The arrows show the times that light was turned on and off. The amount of ATP in intact cells in the reaction mixture (3.0 ml) was determined in the DMSO extract by the luciferin-luciferase method. (A) Aerobic conditions. The suspension was vigorously stirred in an open vessel for 5 min prior to measurement and during measurement. (B) Anaerobic conditions. The suspension was covered with liquid paraffin.

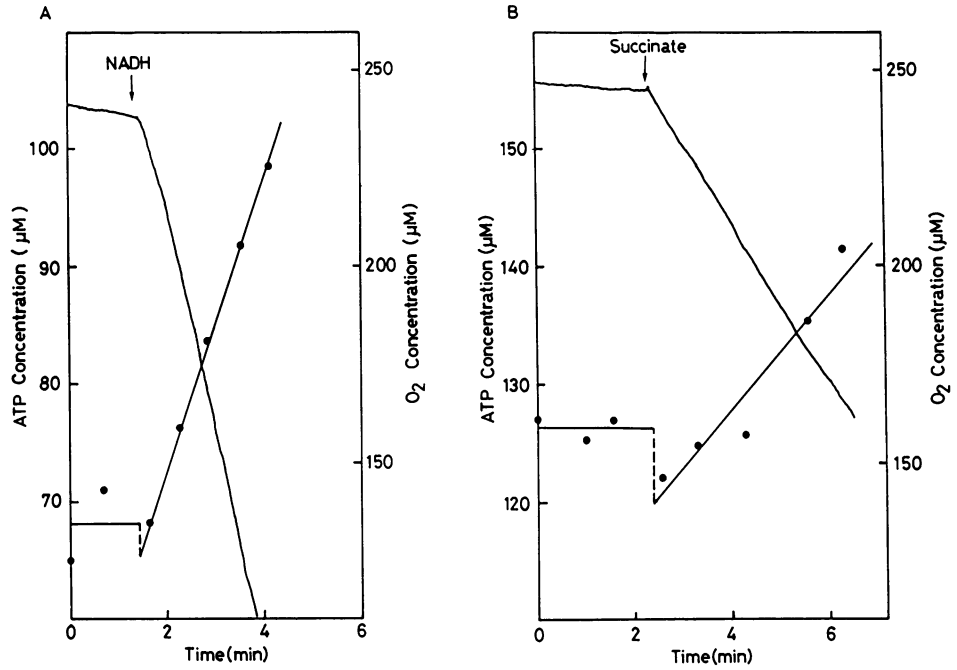

FIG. 3. Time course of ATP synthesis coupled to NADH and succinate oxidation in chromatophores in the dark. Symbols: ●, ATP concentration; ——, O_2 concentration. (A) Time course of ATP synthesis and O_2 uptake induced by 3 mM NADH. The reaction mixture contained 5 mM HEPES buffer, pH 8.0, 100 mM KCl, 10 mM $MgCl_2$, 1 mM KH_2PO_4, 0.33 mM ADP, and chromatophores. The Bchl concentration was 4.2 μM. (B) Time course of ATP synthesis and O_2 uptake induced by 7.5 mM sodium succinate. Experimental conditions were the same as in panel A.

H^+ efflux from intact cells (14). In chromatophores, H^+ ions were reversibly taken up after continuous illumination without addition of reagents, although the magnitude of the H^+ uptake was small. Addition of 1 mM phenazine methosulfate (PMS) greatly increased H^+ uptake. The low uptake in the absence of PMS may be due to the depletion of electron transfer components, for example, soluble cytochrome c_{551}, during the preparation of chromatophores. Valinomycin (2 μM) stimulated H^+ translocation about 50%, and 10 μM carbonylcyanide *m*-chlorophenylhydrazone inhibited translocation. Enhancement of H^+ translocation by valinomycin suggested that a membrane potential was formed by illumination in the *Erythrobacter* sp. chromatophores as in other photosynthetic bacteria (4, 21). KCN (10 mM) did not affect the light-induced proton uptake. These data indicated that an electrochemical potential difference of H^+ was formed by illumination in an aerobic photosynthetic *Erythrobacter* sp., as reported for another Bchl-containing aerobic bacterium, "*Protaminobacter ruber*" (20).

In the presence of ADP, P_i, and $MgCl_2$, ATP was formed under continuous illumination of the chromatophore suspension, as shown by steady alkalinization of the reaction mixture. The rate of ATP formation with PMS was 0.65 mol of ATP per mol of Bchl per min. ATP formation was inhibited by the addition of 10 μM antimycin A.

ATP formation was also confirmed by the luciferin-luciferase assay (Fig. 1). The typical rate of photophosphorylation in chromatophores under aerobic conditions was in the range of 0.25 to 0.5 mol of ATP per mol of Bchl per min.

Dependence of photophosphorylation on the presence of oxygen. In intact cells, photooxidation of cytochromes and reaction center Bchl and H^+ translocation by light have been observed. We reported previously that these light-induced processes ceased under anaerobic conditions and became observable again when the suspension was re-aerated. This type of experiment was also carried out for photophosphorylation. Figure 2 shows photophosphorylation in intact cells under aerobic and anaerobic conditions. We could detect ATP formation by light under aerobic conditions (0.55 mol of ATP per mol of Bchl per min) (Fig. 2A) but not under anaerobic conditions (Fig. 2B). This result further confirmed that under anaerobic conditions photosynthetic energy transduction could not take place in this *Erythrobacter* sp. With chromatophores we obtained results similar to those shown in Fig. 2 under aerobic (Fig.1, trace a) and anaerobic (Fig. 1, trace b) conditions. The latter conditions were obtained by adding glucose oxidase. D-glucose, and catalase to the reaction mixture and by covering the reaction vessel with liquid paraffin. Under aerobic conditions (O_2 concentration, ca. 40% saturation), ATP formation by light was observed, but we could not detect photophosphorylation under anaerobic conditions (O_2 concentration, below 2.0% saturation).

Characteristics of oxidative phosphorylation of chromatophores. Intact cells of *Erythrobacter* spp. consume oxygen by using endogenous substrates (5). Chromatophores isolated from this *Erythrobacter* sp. also consumed oxygen in the presence of an appropriate substrate, including NADH and succinate. The O_2 uptake induced by NADH was accelerated when ADP was added (not shown here), and the respiratory ratio was 2.3.

Figure 3A shows the NADH-dependent O_2 uptake and ATP formation coupled to O_2 uptake. The rate of ATP formation was 2.9 mol of ATP per mol of Bchl per min, and the P/O ratio was 0.22. Figure 3B shows the O_2 uptake induced by succinate and ATP formation coupled to the O_2

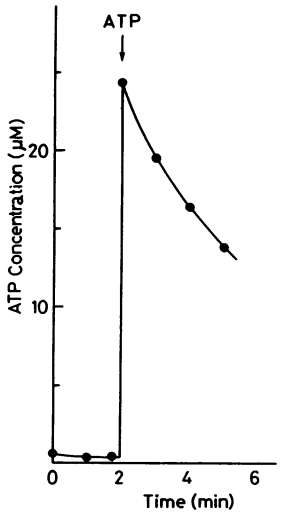

FIG. 4. Time course of ATP hydrolysis of the suspension of chromatophores in the dark. The reaction mixture contained 5 mM HEPES buffer, pH 8.0, 100 mM KCl, 5 mM $MgCl_2$, and chromatophores. The arrow shows the time of addition of 25 μM ATP. The Bchl concentration was 20 μM.

uptake. The rate of ATP formation was 1.1 mol of ATP per mol of Bchl per min, and the P/O ratio was 0.19. The P/O ratios were a little lower than those in facultative photosynthetic bacteria (1, 16).

ATP hydrolysis in the dark. As already shown in Fig. 1 and 2, the level of ATP synthesized during illumination decreased during the dark period at a rather high rate, both in the suspension of chromatophores and in intact cells of *Erythrobacter* sp. strain OCh114. Figure 4 shows the hydrolysis of added ATP in the dark in isolated chromatophores measured by the luciferin-luciferase assay. The initial rate of hydrolysis was 0.25 mol of ATP per mol of Bchl per min at an ATP concentration of 25 μM.

When a dense suspension of *Erythrobacter* sp. chromatophores (Bchl concentration, 170 μM) was incubated with ATP, the appearance of AMP, as well as of ADP and P_i, was detected by ^{31}P-NMR. Figure 5 shows the time courses of the disappearance of ATP and appearance of ADP, AMP, and P_i. ADP reached a maximum and then gradually decreased. This figure indicates the presence of adenylate kinase activity in addition to ATPase activity. Illumination of the chromatophores in the NMR sample tube had no significant effect on the level of adenylates, probably due to the low redox potential and insufficient light penetration, both due to the density of the suspension.

DISCUSSION

In this communication we reported the characteristics of energy metabolism (ATP synthesis and ATP hydrolysis) in *Erythrobacter* sp. strain OCh114. We clearly showed the existence of photophosphorylation and oxidative phosphorylation. In this *Erythrobacter* sp. no clear evidence of photophosphorylation was present, although the ATP level in intact cells increased after prolonged illumination (17). The difficulty was in the unexpected dependence of phosphorylation on the presence of O_2 in the reaction mixture, which easily became anaerobic by respiration. In this experiment we could detect photophosphorylation by two different methods. This suggests that the pigment and photochemical systems in this organism are functional and can synthesize ATP under illumination, although the activity was not as high as in other photosynthetic bacteria (11).

We previously showed that the photosynthetic electron transfer system was inoperative in anaerobic cells of this *Erythrobacter* sp. (14). This phenomenon is reconciled with the physiological fact that this bacterium does not grow photoanerobically. Photophosphorylation also ceased under anaerobic conditions, as shown in both intact cells and chromatophores (Fig. 1 and 2). This can be considered one of the reasons why this bacterium cannot grow photoanaerobically. We previously discussed this phenomenon from the viewpoint of the lowering of the oxidation-reduction potential by anaerobiosis. No charge separation can occur in the photochemical reaction center if the primary electron acceptor Q_A is almost reduced before illumination. This explanation is probable because the midpoint potential of Q_A of this bacterium was higher than in other photosynthetic bacteria (14). Further detailed studies must be done about aerobic-anaerobic transition to solve the problem.

In oxidative phosphorylation, the P/O ratios were somewhat lower than those in other facultative photosynthetic bacteria (1, 16).

In chromatophores the oxidative phosphorylation activity was higher than that of photophosphorylation. The typical values were 4.5-fold (NADH) and 1.7-fold (succinate) that of photophosphorylation on the basis of Bchl concentration. The fluxes of ATP synthesis and the interaction of photo-

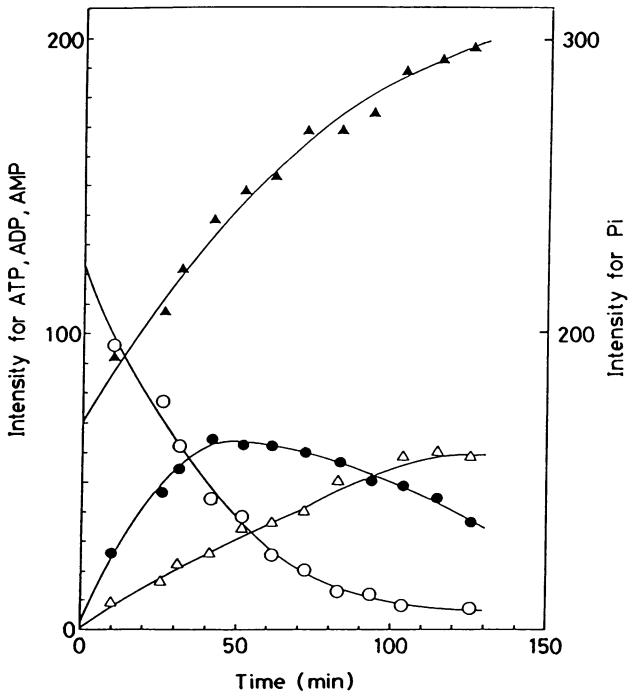

FIG. 5. Time course of adenine nucleotides (ATP, ADP, and AMP) and P_i concentrations in the suspension of chromatophores measured by ^{31}P-NMR. Intensity of adenine nucleotides was determined by using the peak height of ATP-β, ADP-β, and AMP resonances. The reaction mixture contained 50 mM KCl, 5 mM $MgCl_2$, 0.2 mM PMS, 0.1 mM valinomycin, and chromatophores. The Bchl concentration was 170 μM. ATP (1.97 mM) was added to the suspension at time zero. The initial pH of the suspension was 7.3. Symbols: ○, ATP concentration; ●, ADP concentration; △, AMP concentration; ▲, P_i concentration. Spectra were accumulated 2,500 times with a recycling time of 0.22 s.

and oxidative phosphorylation under various conditions such as light-aerobic, dark-aerobic, and light-semianaerobic conditions have not been fully analyzed as for other photosynthetic bacteria. In this *Erythrobacter* sp., O_2 uptake was reported to be largely reduced by illumination (5), as observed in other facultative photosynthetic bacteria. Under these conditions it is likely that oxidative phosphorylation is suppressed and photophosphorylation becomes the main pathway of ATP synthesis in this organism.

Many photosynthetic bacteria have adenylate kinase and ATPase activities. These enzyme systems are probably involved in the regulation of adenylate pools in intact cells (10). Activities of ATPase and adenylate kinase, determined enzymatically and by ^{31}P-NMR, were rather high in this organism (Fig. 4 and 5). Their roles in energy and adenylate metabolism are yet to be elucidated.

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