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RELATIONSHIP BETWEEN PHOTOSYNTHETIC AND OXIDATIVE PHOSPHORYLATIONS IN CHROMATOPHORES FROM LIGHT-GROWN CELLS OF *RHODOSPIRILLUM RUBRUM*

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SUMMARY

1. Oxidation of NADH by molecular O_2, phosphorylation coupled with the oxidation, and photophosphorylations induced by ascorbate, phenazine methosulfate and NADH, have been studied with chromatophores from light-grown cells of *Rhodospirillum rubrum*.

2. Chromatophores catalyze the oxidation of NADH aerobically in the dark. NADH oxidation is markedly stimulated by the addition of an energy-trapping system (ADP *plus* P_i, or ATP–hexokinase–glucose *plus* P_i) over the whole range of pH; the rate is maximal at pH 7.4–7.8 in the absence of the energy-trapping system, and at pH 6.9 in its presence.

3. Coupled to NADH oxidation, ATP (or glucose 6-phosphate) is synthesized from ADP and P_i. Mg^{2+}, NADH, P_i and ADP are each required to almost the same extent for both oxidative phosphorylation and photosynthetic phosphorylation with NADH; the rate is maximal in the presence of 5.0 mM Mg^{2+}, and the values of K_m are 33 μM for NADH, 1.3 mM for P_i and 13 μM for ADP. The rate of oxidative phosphorylation with NADH is maximal at pH 6.8, while the rate of photosynthetic phosphorylation is maximal at pH 7.5 when induced by NADH, and at pH 8.0 when induced by ascorbate and by phenazine methosulfate.

4. *o*-Phenanthroline (0.1 mM) inhibits ascorbate-induced photophosphorylation by 80% and NADH-induced photophosphorylation by 50%, but does not significantly affect NADH oxidation and its coupled phosphorylation. These three reactions with NADH are completely inhibited in the presence of 0.01 M *o*-phenanthroline. Changes in the pH of the reaction mixture, or energy-trapping system, significantly influence the *o*-phenanthroline inhibition of photophosphorylation. Ascorbate-induced photophosphorylation, when assayed in the presence of hexokinase and glucose, is not appreciably inhibited by 0.1 mM.

5. When chromatophores are heated at appropriate temperatures, NADH oxidation and coupled phosphorylation are inactivated nearly in parallel with NADH-

Abbreviations: NOQNO, 2-*n*-nonyl-4-hydroxyquinoline-*N*-oxide; HOQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide; RHP, a variant cytochrome from *R. rubrum*; PMS, phenazine methosulfate; GTA buffer, equimolar mixture of 3,3-dimethylglutaric acid, Tris, and 2-amino-2-methyl-1,3-propanediol.

induced photophosphorylation, while ascorbate-induced and phenazine methosulfate-induced photophosphorylations are significantly more stable to heat. Preillumination with ultraviolet radiation inhibits NADH oxidative phosphorylation.

6. 0.01 M cyanide inhibits by 90% both NADH oxidation and its coupled phosphorylation; the K_m value for cyanide is approx. 0.7 mM.

7. Under anaerobic conditions, 1 μg/ml of antimycin A inhibits ascorbate-induced photophosphorylation completely and NADH-induced photophosphorylation up to 85%. The activity of NADH-induced photophosphorylation still remaining in the presence of antimycin A is further inhibited by the addition of ascorbate; the K_m value for ascorbate is approx. 0.2 mM, roughly equivalent to an E_{mid} of +0.2 V.

8. Based on these findings, relationships between NADH oxidation, its coupled phosphorylation, and photophosphorylations are discussed.

INTRODUCTION

Cells of *Rhodospirillum rubrum* grow either anaerobically in the light (light-grown cells) or aerobically in the dark (dark-grown cells). Under anaerobic conditions in the light, cells utilize light energy for growth; ATP is synthesized photochemically from ADP and P_i in the chromatophores obtained from light-grown cells[1-8]; ATP synthesis is coupled to an electron-transport system in a cyclic fashion[6-8]. Light-grown cells, however, as well as dark-grown ones, can oxidize substrates with molecular O_2. Earlier, NAKAMURA[9] discovered that the O_2 uptake of light-grown cells of *Rhodopseudomonas palustris* was inhibited under illumination. HORIO AND TAYLOR[10,11] have demonstrated, using light-grown cells of *R. rubrum*, that the light inhibition of the O_2 uptake is correlated with the function of the photochemical apparatus, namely through participation of bacteriochlorophyll and carotenoids, and, using dark-grown cells, that no *a*-type cytochrome is functional in the O_2 uptake. It appears therefore that an intimate relationship exists between the respiratory and photosynthetic electron-transport systems[11].

SMITH AND BALTSCHEFFSKY[12], and GELLER[13,14], found that extracts from either light-grown or dark-grown cells of *R. rubrum* catalyzed the phosphorylation coupled to the oxidation of various substrates, and that the oxidative and photosynthetic phosphorylations were notably different in their responses to antimycin A, 2-*n*-nonyl-4-hydroxyquinoline-*N*-oxide (NOQNO), 2-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) and compound SN5949, suggesting that the two kinds of phosphorylations might be coupled to different electron-transport systems.

Using chromatophores from light-grown cells of *R. rubrum* that can catalyze ATP synthesis coupled with oxidation of NADH by molecular O_2 in the dark, we have studied the relationships between the oxidative and photosynthetic phosphorylations.

MATERIALS AND METHODS

Cells of *R. rubrum* were grown anaerobically under illumination, and chromatophores were prepared therefrom by sonication, as described previously[15].

The amounts of $^{32}P_i$ incorporated into organic substances were determined by

the method of NIELSEN AND LEHNINGER[16] as modified by AVRON[17]; after extraction of $^{32}P_i$ with the mixture of organic solvents, the aqueous aliquots (organic fraction) were analyzed for radioactivity.

Activities of photophosphorylation were assayed by the method previously described[15], except that 67 mM ascorbate or 0.5 mM NADH was used to stimulate photophosphorylation[6]. These are called "ascorbate-induced" or "NADH-induced" photophosphorylation.

Anaerobic reactions for the assay of photophosphorylation were carried out in Thunberg tubes. Standard components of the reaction mixture were as follows: in the main chamber, 1.00 ml 0.1 M Tris–HCl buffer (pH 8.0) containing 10% sucrose (Tris–sucrose buffer); 0.20 ml 0.1 M $MgCl_2$; 0.20 ml 1.0 M ascorbate or 5 mM NADH; 0.20 ml 0.05 M ADP; 0.20 ml 0.1 M $^{32}P_i$ (approx. 10^5 counts/min); water to make a total volume of 3.00 ml, and in the side-arm, 0.20 ml of chromatophore suspension (approx. 50 $A_{880\,m\mu}$ units/ml; approx. 1.7 mg in dry weight). In some special cases, 0.5 μmole of ATP, 0.2 mg of hexokinase (ATP: D-hexose-6-phosphotransferase, EC 2.7.1.1) and 20 μmoles of glucose ("ATP–glucose–hexokinase *plus* P_i" system) were added instead of ADP. To attain anaerobic conditions, tubes were evacuated, then filled with argon gas. This procedure was repeated three times. Reactions were started by mixing the contents of both chambers, and were carried out in a vacuum at 30° in the light (approx. 1000 ft-candles); control runs were carried out in the dark.

Reactions for both aerobic and anaerobic photophosphorylations were carried out in the presence of ADP and P_i without addition of hexokinase and glucose ("ADP *plus* P_i" system) unless otherwise stated.

The activities of oxidative phosphorylation were assayed in small test tubes of 1 cm diameter. Standard components of the reaction mixture were as follows: 0.30 ml 0.1 M GTA buffer (equimolar mixture of 3,3-dimethylglutaric acid, Tris, and 2-amino-2-methyl-1,3-propanediol)[18] (pH 7.0) containing 10% sucrose (GTA–sucrose buffer); 0.05 ml 0.1 M $MgCl_2$; 0.10 ml 5 mM ATP; 0.10 ml hexokinase (2 mg per ml); 0.10 ml 0.2 M glucose; 0.10 ml 0.1 M $^{32}P_i$ (approx. 10^6 counts/min); 0.10 ml 5 mM NADH; 0.10 ml of chromatophore suspension (approx. 100 $A_{880\,m\mu}$ units/ml); water to make a total volume of 1.00 ml. Dark conditions were attained using tubes completely wrapped with aluminum foil. Reaction mixtures without chromatophores were preincubated at 30° for 20 min. Reactions were initiated by adding chromatophore suspensions, and were carried out at 30° for 10 min in the air and in the dark. The reactions were terminated by adding 0.30 ml 30% trichloroacetic acid, previously cooled in an ice–water bath.

ATP–P_i exchange reactions were measured with the reaction mixture used for oxidative phosphorylation from which hexokinase and glucose were omitted. Other experimental conditions were the same as for the oxidative phosphorylation, unless otherwise stated.

Oxidation of NADH by molecular O_2 in the dark was assayed with cuvettes of 1 cm optical path. The standard components of the reaction mixture used were the same as for the assay of the oxidative phosphorylation mentioned above. In some cases, ATP, $^{32}P_i$ and hexokinase were omitted. Reactions were started by adding NADH, and followed by measuring the difference in absorbance at 340 mμ between two cuvettes, one with and one without added NADH. In other cases, reactions were stopped by the addition of 1.00 ml of saturated $(NH_4)_2SO_4$ solution (pH 8.0), followed

by centrifugation, the resultant supernatants were assayed to estimate the amount of NADH oxidized. Other experimental conditions were the same as for the oxidative phosphorylation mentioned above.

The nucleotides formed by oxidative and photosynthetic phosphorylation were analyzed according to the method of AVRON[19], OHMURA AND FUKUI[20], and KHYM AND COHN[21]. Identification of ^{32}P-labeled nucleotides was performed as follows: reactions, when carried out in the presence of ADP and $^{32}P_i$ without hexokinase or glucose, were stopped by adding trichloroacetic acid. The resulting solutions were supplemented with 0.1 ml 0.05 M ATP, allowed to stand for 10 min in an ice–water bath, and centrifuged. Each resulting supernatant solution was passed through a column packed with charcoal (0.5 cm diameter, 2 cm long), which had previously been treated with 1 M HCl and then washed with water. The charged column was washed with 0.01 M HCl until the eluate showed no significant radioactivity. The washed charcoal column was then eluted with a 60 % (v/v) ethanol solution containing 1 % (w/v) NH_4OH, and the eluate collected in fractions. Those fractions showing appreciable radioactivities were pooled and concentrated by evaporation. The concentrate was adjusted to 0.05 ml with water, and 0.01 ml of it was chromatographed on filter paper (Toyo-Roshi No. 51A, Toyo Kagaku-Sangyo Co. Ltd., Osaka) at 20° for 12 h by ascending chromatography with a solvent system of *n*-butyric acid and 0.5 M NH_4OH (10:6, v/v). Location of the chromatographed nucleotides was effected by illumination with an ultraviolet lamp. Identification was based on comparison of R_F values referred to those of standard nucleotides, chromatographed simultaneously. Radioactivity on chromatograms was assayed by a chromatogram scanner (Aloka, Model JPC-102, Nihon Musen Irigaku Kenkyusho, Tokyo).

When reactions were carried out in the presence of the ATP–hexokinase–glucose system instead of ADP, identification of ^{32}P-labeled substances was carried out as follows: reactions were terminated by addition of 0.30 ml 30 % cold $HClO_4$. To the solution, 0.1 ml 0.05 M ATP and 0.1 ml 0.05 M glucose 6-phosphate were added. The resulting mixture was adjusted to pH 7 with 20 % KOH solution in an ice–water bath, and then centrifuged. To the supernatant solution, 1 g of a cation-exchange resin, Dowex 50 (H^+ form), was added and the resin was then removed by filtration. The filtrate was adjusted to pH 8.0 with aqueous ammonia. The adjusted solution was passed through a column of an anion-exchange resin, Dowex 1 (Cl^- form, 0.5 cm diameter and 2 cm long). The charged resin was washed with 1 mM NH_4OH and then with a mixture of 25 mM NH_4Cl and 10 mM $K_2B_4O_7$ until the washings were free of significant radioactivity. The washed resin was further washed with water and eluted with 0.05 M HCl. Radioactive eluates were collected and concentrated by evaporation. The samples thus concentrated were chromatographed on filter papers. Glucose 6-phosphate on the chromatogram was visualized by spraying with acid molybdate reagent according to the method of HANES AND ISHERWOOD[22]. Other procedures were the same as in the determination of radioactive nucleotides mentioned above.

ATP, ADP, NAD, NADH and hexokinase (type IV) were commercial products of the Sigma Chemical Co., Mo. Antimycin A was purchased from the Kyowa Fermentation Industries Co. Ltd., Tokyo. ^{32}P-Labeled phosphate was a commercial product from the Radiochemical Centre, Amersham, and was purified according to the method of SUELTER *et al.*[23].

RESULTS

Oxidation of NADH by molecular O_2 in the dark with chromatophores

In accordance with the findings of WHITE AND VERNON[24], and GELLER[13,14], chromatophores from *R. rubrum* catalyzed the oxidation of NADH by molecular O_2. It was found that the NADH oxidation in the dark was stimulated markedly in the presence of an energy-trapping system (ADP *plus* P_i, or ATP–hexokinase–glucose *plus* P_i) (Fig. 1). The oxidation proceeded at a maximal rate at approx. pH 6.9 in

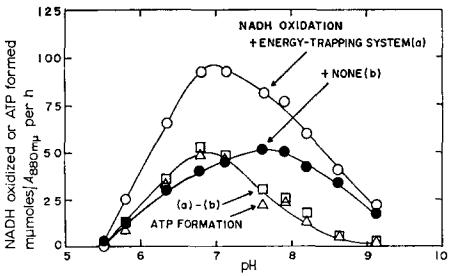


Fig. 1. Effect of pH on NADH oxidation and its coupling with phosphorylation. Experimental conditions were the same as described in the text. ○—○, NaDH oxidation in the presence of the "ATP–hexokinase–glucose *plus* P_i" system (a); ●—●, NADH oxidation in the absence of the energy-trapping system (b); □—□, difference between (a) and (b); △—△, ATP formation coupled with the reaction (a).

its presence, and at pH 7.5 in its absence. The optimal pH of activity of NADH oxidation varied in the rate between 7.4–7.8 from batch to batch of chromatophore preparation. Mg^{2+} appeared to be essential for stimulation of NADH oxidation by the energy-trapping system. Its effect varied with each batch of chromatophore preparation. The rate of NADH oxidation in the absence of the energy-trapping system was unaffected by Mg^{2+}. Stimulation occurred in the presence of 1 mM 2,4-dinitrophenol, added instead of the energy-trapping system. This stimulation by 2,4-dinitrophenol was observed regardless of the presence or absence of Mg^{2+}.

Incorporation of $^{32}P_i$ into organic substances, coupled to NADH oxidation with chromatophores

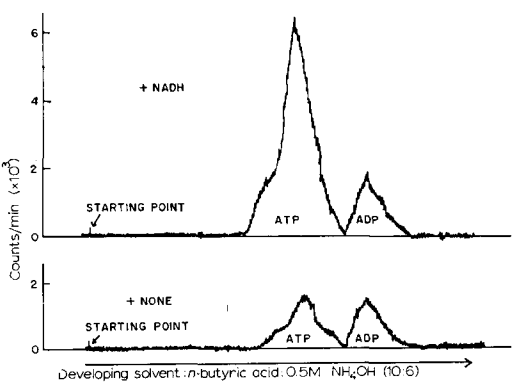
When chromatophores were incubated with 5 mM ADP, 10 mM $^{32}P_i$ and 0.5 mM NADH aerobically in the dark, $^{32}P_i$ was incorporated into the organic fraction (No. 4 in Table I). The incorporation proceeded linearly for more than 20 min. Similar results were obtained when ADP was supplemented with hexokinase–glucose (No. 1), or replaced by ATP–hexokinase–glucose that had been sufficiently incubated to reach a steady state (No. 7). The amount of $^{32}P_i$ incorporated was much less when the reaction mixture did not contain NADH (Nos. 2, 5 and 8), as well as when reactions were carried out anaerobically. When 5 mM ATP, without hexokinase–glucose, instead of ADP was added to the reaction mixture (No. 10), $^{32}P_i$ was incorporated two to three times faster than when the reaction was carried out with ADP (No. 4). This rapid $^{32}P_i$ incorporation with ATP occurred in the absence of NADH under either aerobic or anaerobic conditions (No. 11), and it was 20–30% stimulated in the presence of NADH under aerobic but not under anaerobic conditions (Nos. 10 and 11).

TABLE I

INCORPORATION OF $^{32}P_i$ INTO THE ORGANIC FRACTION DURING REACTIONS WITH AND WITHOUT NADH BY CHROMATOPHORES IN DARK UNDER AEROBIC CONDITIONS

The standard components of the reaction mixture were as follows: 0.30 ml 0.1 M GTA–sucrose buffer (pH 7.2), 0.05 ml 0.1 M $MgCl_2$, 0.10 ml 0.1 M $^{32}P_i$ ($2.5 \cdot 10^7$ counts/min), 0.1 ml chromatophore suspension (50 $A_{880\,m\mu}$ units/ml), and water to make a total volume of 1.00 ml. Additions to the reaction mixture were as indicated. Reactions were carried out at 30° aerobically in dark.

No.	*Reaction time (min)*	*Addition*				*$^{32}P_i$ incorporated ($m\mu$moles/$A_{880\,m\mu}$ unit per h)*
		ADP (5 mM)	*ATP (5 mM)*	*Hexokinase (0.1 mg) plus glucose (10 mM)*	*NADH (0.5 mM)*	
1	10	+	—	+	+	45.3
2	10	+	—	+	—	4.7
3	0	+	—	+	+	2.7
4	10	+	—	—	+	49.1
5	10	+	—	—	—	11.7
6	0	+	—	—	+	2.3
7	10	—	+	+	+	51.6
8	10	—	+	+	—	4.0
9	0	—	+	+	+	2.3
10	10	—	+	—	+	138.5
11	10	—	+	—	—	111.9
12	0	—	+	—	+	2.2



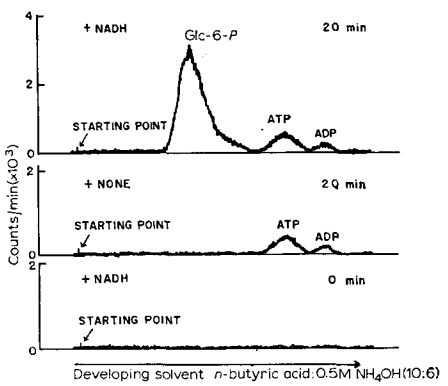


Fig. 2. Chromatogram of reaction mixture of NADH-induced oxidative phosphorylation, in which ADP was used as phosphate acceptor. Details of experimental conditions are described in the text. Reactions were carried out at 30° for 20 min under dark, aerobic conditions with and without NADH. $^{32}P_i$ (10 μmoles, 10^8 counts/min) and 5 μmoles of ADP were substituted for the mixture of ATP, hexokinase and glucose to be added in the standard reaction mixture for oxidative phosphorylation. Chromatography was performed with the indicated solvent mixture from the origin in the direction indicated by the arrow.

Fig. 3. Chromatogram of reaction mixture of NADH-induced oxidative phosphorylation, in which ATP, hexokinase and glucose were used as phosphate acceptor system. Experimental conditions were the same as for Fig. 2, except for the radioactivity of phosphorus (10^7 counts/min) and the addition of 0.5 μmole of ATP, 0.2 mg of hexokinase and 20 μmoles of glucose in place of ATP.

The ^{32}P-labeled substances thus formed were analyzed (Figs. 2 and 3). When reactions were carried out in the presence of ADP aerobically in the dark, in the absence of NADH, a small amount of $^{32}P_i$ was incorporated into both the ATP and

ADP when using chromatophores prepared by the sonication of cells. The ratio of the activities of ATP and ADP was approx. 1 or lower. With chromatophores prepared by grinding cells[25] instead of sonication, a much smaller amount of $^{32}P_i$ was incorporated into ATP, while the formation of radioactive ADP was not detected. With either preparation, the incorporation of $^{32}P_i$ into ATP was markedly stimulated in the presence of NADH. On the other hand, the incorporation of $^{32}P_i$ into ADP was much less, even with chromatophores prepared by sonication. It appeared, therefore, that the difference in the values, No. 1 *minus* No. 2 in Table I, represents the net amount of [^{32}P]ATP formed by a reaction due to NADH oxidation.

FRENKEL[1] has reported that chromatophore preparations from *R. rubrum* show an adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) activity. In addition, it has been found that chromatophores show an ATP–P_i exchange activity, and that the activity increases markedly under illumination[25]. The question arises, therefore, whether the [^{32}P]ATP formation ascribed to NADH oxidation occurring aerobically and in the dark might result from a stimulation by NADH, as by light, of the ATP–P_i exchange activity with the ATP which could be formed from ADP by the adenylate kinase.

This possibility was ruled out in the following manner. First, in the presence of ATP–hexokinase–glucose system, glucose 6-[^{32}P]phosphate was synthesized when reactions were carried out in the presence, but not in the absence, of NADH, indicating that the amount of ATP, whether radioactive or not, really increased as NADH was oxidized (Fig. 3). Secondly, regardless of the presence and absence of NADH, the ATP–P_i exchange activity in the dark was negligible if the concentration of ATP present was 33 μM or lower; at higher concentrations, it proceeded at a detectable rate, and was somewhat stimulated in the presence of NADH under aerobic con-

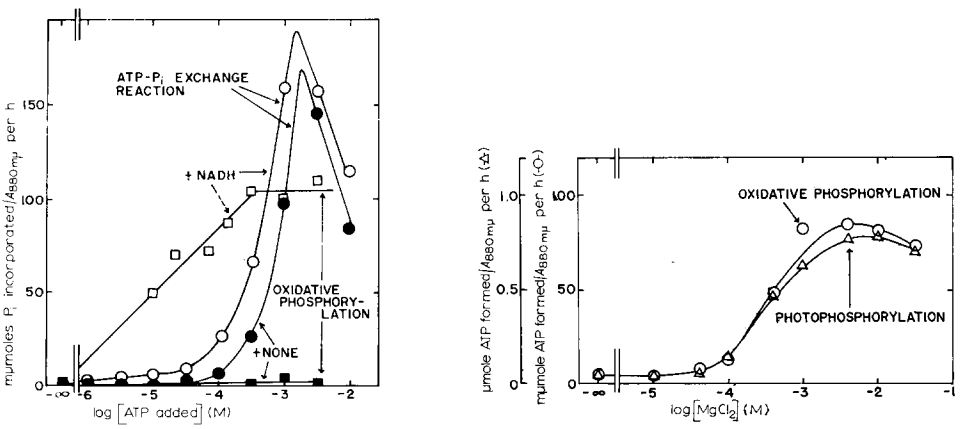


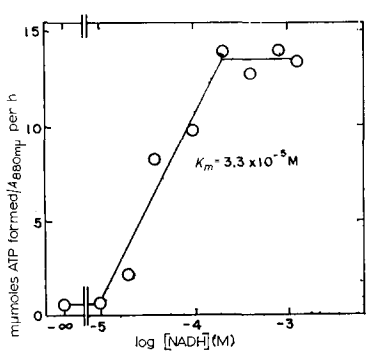
Fig. 4. Effect of ATP on oxidative phosphorylation and ATP–P_i exchange reaction in the presence and absence of NADH. Experimental conditions were the same as described in the text. Abscissae show concentration of ATP added to the reaction mixture prior to incubation with hexokinase and glucose in the case of oxidative phosphorylation. □—□ and ■—■, oxidative phosphorylation; ○—○ and ●—●, ATP–P_i exchange reaction. Open and closed symbols represent the presence and absence of NADH, respectively.

Fig. 5. Effect of Mg^{2+} on photophosphorylation and oxidative phosphorylation induced with NADH. Experimental conditions were the same as described in the text. △—△, photophosphorylation; ○—○, oxidative phosphorylation.

ditions (Fig. 4). ROBBINS AND BOYER[26] have found that the equilibrium constant of the phosphate-transferring reaction of hexokinase is approx. $2 \cdot 10^3$. This value suggests that after sufficient incubation of the ATP–hexokinase–glucose system, the concentration of ATP initially added, when 0.5 mM or less, is lowered to less than 33 μM, which is practically the minimal concentration of ATP needed to evoke the ATP–P_i exchange activity. This implies that under the experimental conditions used (5 mM ADP or ATP), the hexokinase–glucose system could maintain ATP concentration at a sufficiently low level so that appreciable ATP–P_i exchange did not occur. Also when reactions were carried out in the presence of the hexokinase–glucose system, the difference in amounts of $^{32}P_i$ incorporated between the reaction with and without added NADH was a measure of the quantities of ATP formed from ADP and P_i by NADH oxidation. Hence, the hexokinase–glucose system was subsequently used for the assay of oxidative phosphorylation with NADH, unless otherwise stated.

Effects of concentrations of Mg^{2+}, NADH, P_i, and ADP on phosphorylation coupled to NADH oxidation with chromatophores

The rate of chromatophore ATP formation, coupled to NADH oxidation by molecular O_2 in the dark, was stimulated in the presence of Mg^{2+}; it was maximal at 6.7 mM. With some of the chromatophore preparations, formation of ATP depended on the addition of appropriate concentrations of Mg^{2+}; the influence of Mg^{2+} concentration was almost the same for oxidative phosphorylation with NADH, as for NADH-induced photophosphorylation (Fig. 5). Oxidative phosphorylation with NADH increased in rate with increasing concentration of P_i, and NADH (Figs. 6 and 7): the values of K_m were 33 μM for NADH and 1.3 mM for P_i. These values are the same as for a NADH dehydrogenase purified from light-grown cells of *R. rubrum*[7] and for photophosphorylation[27].



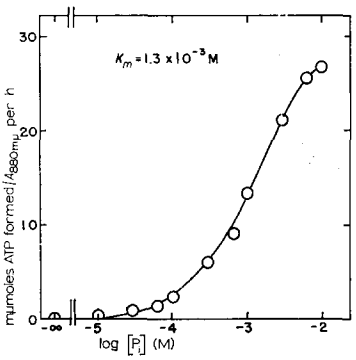


Fig. 6. Effect of NADH on oxidative phosphorylation. Experimental conditions were the same as described in the text.

Fig. 7. Effect of P_i on oxidative phosphorylation. Experimental conditions were the same as described in the text.

The effect of changes in concentration of ADP on oxidative phosphorylation with NADH was examined as follows. Reaction mixtures containing hexokinase, glucose and various concentrations of ATP were incubated for 20 min at 30°, and reactions were then initiated by adding chromatophores. The effective concentrations of ADP present during the reactions were assumed to be essentially that of the ATP

added. The K_m for ADP thus obtained was 13 μM (Fig. 4). This value is almost the same as that for photophosphorylation[27].

Effects of pH on NADH oxidation and on its coupling phosphorylation

Chromatophore NADH oxidations by molecular O_2 in the dark in the presence of the energy-trapping system and its coupled phosphorylation were maximal in rate at pH 6.9 and 6.8, respectively (Fig. 1). At pH 6.8, the ratio P/O for oxidative phosphorylation with NADH was as great as 0.5 (Fig. 1). When assayed under experimental conditions as similar as possible, NADH-induced and ascorbate-induced photophosphorylations were maximal in rate at pH 7.5 and at pH 8, respectively.

Effect of o-phenanthroline on various activities with chromatophores

In a preceding paper[8], it has been shown that *o*-phenanthroline inhibition of various reactions with chromatophores may be differentiated into three types; 50 % inhibition occurs at approx. 2 μM (Type I), at approx. 50 μM (Type II) and at approx. 2 mM (Type III). Photophosphorylation induced by NADH was found to be of Type II (Fig. 8), even if induced by the addition of both ascorbate and NADH. This same result was observed at the various concentrations of ascorbate tested, 0–67 mM. Variation in the reaction system markedly influenced the inhibitory effect of *o*-phenanthroline on photophosphorylations induced by NADH or ascorbate. *o*-Phenanthroline inhibited NADH-induced photophosphorylation at pH 8 more than at pH 7, whereas this reagent inhibited ascorbate-induced photophosphorylation more when ADP and P_i were used for the high-energy acceptor than when ATP, P_i,

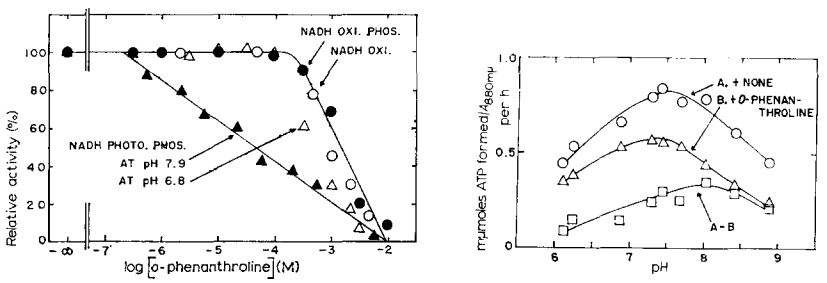


Fig. 8. Effect of *o*-phenanthroline on various NADH-induced reactions with chromatophores. Experimental conditions were the same as described in the text. ▲—▲, NADH-induced photophosphorylation at pH 7.9; △—△, NADH-induced photophosphorylation at pH 6.8; ●—●, NADH-induced oxidative phosphorylation; ○—○, NADH-induced oxidation in the presence of the 'ATP–hexokinase–glucose *plus* P_i' system.

Fig. 9. Effect of *o*-phenanthroline on NADH-induced photophosphorylation at various pH values. Experimental conditions were the same as described in the text. The pH of the reaction mixture was controlled by the addition of GTA–sucrose buffer at various pH. ○—○, NADH-induced photophosphorylation in the absence of *o*-phenanthroline (A); △—△, NADH-induced photophosphorylation in the presence of 0.3 mM *o*-phenanthroline (B); □—□, derived values: (A) *minus* (B).

hexokinase and glucose were used (Figs. 9 and 10). Phosphorylation coupled to NADH oxidation was Type III, as were phenazine methosulfate (PMS)-induced photophosphorylations in the presence and absence of antimycin A. The *o*-phenanthroline inhibition of NADH oxidation was not influenced by the addition of the energy-trapping system nor affected by alterations in the pH of the reaction medium.

Effects of other metal-chelating reagents on ascorbate- and PMS-induced photophosphorylations

Like *o*-phenanthroline inhibition of photophosphorylation, α,α'-dipyridyl also significantly inhibited ascorbate-induced photophosphorylation, while it did not inhibit PMS-induced photophosphorylation. Tiron (disodium 1,2-dihydroxybenzene-3,5-disulfonate) had no effect on either ascorbate- or PMS-induced photophosphorylation.

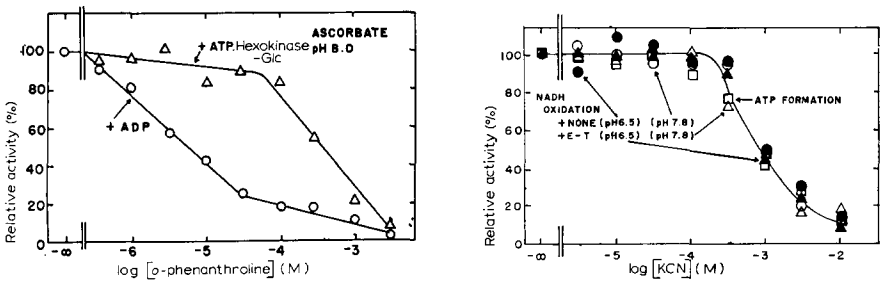


Fig. 10. Effect of *o*-phenanthroline on ascorbate-induced photophosphorylation in the presence of two types of energy-trapping system. Experimental conditions were the same as described in the text. ○—○, in the presence of the 'ADP *plus* P_i' system; △—△, in the presence of the 'ATP–hexokinase–glucose *plus* P_i' system.

Fig. 11. Effect of cyanide on various reactions induced with NADH. Experimental conditions were the same as described in the text. ●—●, NADH oxidation at pH 6.5; ○—○, NADH oxidation at pH 7.8; ▲—▲, NADH oxidation at pH 6.5 in the presence of the 'ATP–hexokinase–glucose *plus* P_i' system; △—△, NADH oxidation at pH 7.8 in the presence of the energy-trapping (E-T) system; □—□, NADH-induced oxidative phosphorylation at pH 7.2.

Effect of cyanide on NADH oxidation and its coupled phosphorylation

As already found by many investigators[6,12,13] 0.01 M cyanide inhibited completely NADH oxidation and its coupled phosphorylation with chromatophores (Fig. 11). These effects were very similar to those observed for the respiration of the whole cell of *R. rubrum* by HORIO AND KAMEN[6]. The addition of the energy-trapping system or the alteration of pH of the reaction medium did not change the response to cyanide, as in the case of *o*-phenanthroline inhibition.

Effect of preillumination of chromatophores with ultraviolet irradiation on NADH oxidative phosphorylation

In a preceding paper[8], it was found that the ultraviolet irradiation inhibited both ascorbate- and PMS-induced photophosphorylations of chromatophores. This treatment also inhibited the activity of NADH-induced oxidative phosphorylation in the same manner. The addition of 1 mM FAD, FMN and riboflavin could not restore the activity of oxidative phosphorylation. Restoration of activity with quinone was not tested.

Effect of heating on various chromatophores reactions with NADH

The heat stability of chromatophores was examined with respect to the three kinds of reactions of NADH by comparison with their ascorbate-induced and PMS-induced photophosphorylations (Table II). Heating inactivated NADH-induced photophosphorylation in parallel with NADH oxidation and its coupled phosphory-

TABLE II

EFFECTS OF HEATING OF CHROMATOPHORES ON VARIOUS ACTIVITIES

A chromatophore suspension in 0.05 M Tris–sucrose buffer (pH 8) (50 $A_{880\ m\mu}$ units/ml) was heated for 3 min at temperatures as indicated. Other experimental conditions were as described in the text. Rates of ATP formation and NADH oxidation are in parentheses, in μmoles of ATP formed per $A_{880\ m\mu}$ unit per h for photophosphorylations, and in mμmoles of ATP formed or NADH oxidized per $A_{880\ m\mu}$ unit per h for oxidative phosphorylation coupled to NADH oxidation and NADH oxidation.

Temp.	*Photophosphorylation induced by*				*Oxidative phosphorylation with NADH*	*Oxidation of NADH*		
	None at pH 7.9 (%)	*Ascorbate at pH 7.9* (%)	*PMS at pH 7.9* (%)	*NADH at pH 7.5* (%)	*at pH 6.6* (%)	*at pH 6.0* (%)	*at pH 6.6* (%)	*at pH 7.7* (%)
—	100 (0.10)	100 (1.10)	100 (2.4)	100 (0.72)	100 (40)	100 (41)	100 (79)	100 (50)
40°	97 (0.10)	98 (1.10)	101 (2.5)	47 (0.34)	25 (10)	24 (10)	28 (26)	42 (21)
45°	87 (0.09)	86 (0.96)	92 (2.2)	24 (0.17)	4 (2)	2 (1)	13 (10)	16 (8)
50°	67 (0.07)	77 (0.88)	84 (2.0)	21 (0.15)	6 (2)	5 (2)	3 (2)	10 (5)
55°	54 (0.05)	75 (0.84)	75 (1.8)	12 (0.09)	4 (2)	5 (2)	3 (2)	4 (2)
60°	21 (0.02)	39 (0.44)	33 (0.8)	6 (0.05)	2 (1)	—	—	—

lation; all were markedly more labile than ascorbate-induced and PMS-induced photophosphorylations. When the activity of NADH oxidation with heated chromatophores was assayed at different values of pH in the presence of the energy-trapping system, the activity was more stable at pH 7.7 than at pH 6.6 and pH 6.0.

Effect of antimycin A on NADH-induced photophosphorylation with chromatophores

It is known that antimycin A is a potent inhibitor of some types of photophosphorylation with chromatophores. Thus, ascorbate-induced photophosphorylation is completely inhibited in the presence of a concentration less than 1 μg/ml of antimycin A, but not PMS-induced photophosphorylation, with all batches of chromatophores preparations examined[8]. Effects of antimycin A on reduction of cytochrome c_2 by NADH and by succinate, on the other hand, varied with the batch of chromatophore preparations. With most batches, antimycin A inhibited the reductions; the inhibition at 1 μg/ml and at pH 6.5 was 70–85 % in most cases and 96 % at the most. It was found that under anaerobic conditions, antimycin A failed to completely inhibit NADH-induced photophosphorylation, while it completely inhibited ascorbate-induced photophosphorylation (Fig. 12). At 0.3 μg/ml, or higher concentrations, of antimycin A, the anaerobic NADH-induced photophosphorylation was not inhibited

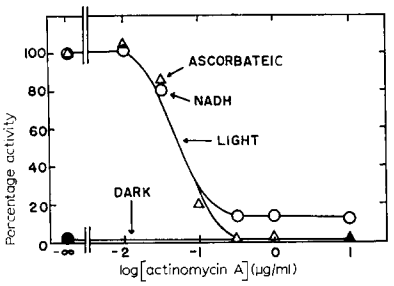
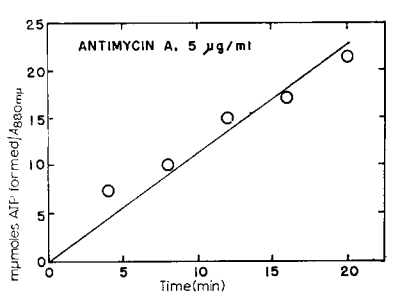


Fig. 12. Effect of antimycin A on anaerobic photophosphorylations induced by ascorbate and NADH. Experimental conditions were the same as described in the text. △—△, ascorbate-induced photophosphorylation; ○—○, NADH-induced photophosphorylation; open symbols, in the light; closed symbols, in the dark.



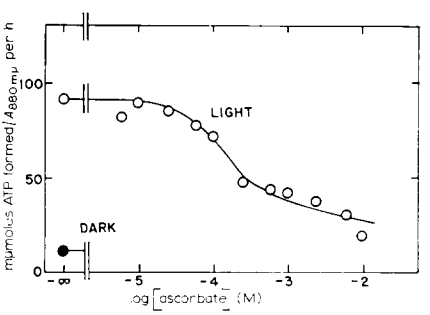


Fig. 13. Time course of NADH-induced photophosphorylation in the presence of 5 μg/ml of antimycin A under anaerobic conditions (light *minus* dark). Experimental conditions were the same as for Fig. 12.

Fig. 14. Effect of ascorbate on NADH-induced photophosphorylation in the presence of 5 μg/ml of antimycin A under anaerobic conditions. Experimental conditions were the same as for Fig. 13. Open symbols, in the light; closed symbols, in the dark.

beyond 85 %, and the residual activity was as much as 100 mμmoles of ATP formed per $A_{880\,m\mu}$ unit per h. Furthermore, this anaerobic NADH-induced photophosphorylation in the presence of the high concentration of antimycin A (anaerobic, antimycin A-residual NADH-induced photophosphorylation) proceeded at a fairly linear rate, at least over the reaction time tested (Fig. 13). This photophosphorylation was further inhibited when ascorbate was added; the inhibition increased with increasing concentrations of ascorbate (Fig. 14). At 0.2 mM ascorbate, the photophosphorylation was 50 % inhibited. This concentration (0.2 mM) of ascorbate corresponds to an E_{m1d} value of approx. 0.2 V, referred to the titration curves of cytochrome *c*, and RHP by ascorbate[6].

DISCUSSION

R. rubrum is a photoheterotroph. Semi-aerobically in the dark, cells synthesize no chromatophores, but a minimal photochemical apparatus composed mainly of bacteriochlorophyll and carotenoids[23,29]. Anaerobically in the light, they synthesize bacteriochlorophyll and carotenoids to a much greater extent than they do aerobically in the dark and exhibit chromatophores which contain the photochemical apparatus. Accordingly, it is reasonable to suppose that whereas light-grown cells certainly utilize light energy, dark-grown cells grow only by use of energy from either, or both, glycolytic and oxidative dissimilation of supplied substrates. Spectrophotometric studies with light-grown cells have revealed that the changes in the absorption spectrum caused by light are similar to those induced by molecular O_2, suggesting that the system for the O_2 respiration and photosynthetic electron transport are similar to each other, at least with respect to their oxidation–reduction components[30–35].

SMITH AND BALTSCHEFFSKY[12], and GELLER[13,14], found that extracts both from dark-grown and light-grown cells catalyzed the formation of ATP from ADP and P_i in the presence of various substrates aerobically in the dark, and that the oxidative ATP-forming activity was associated with cell fragments or chromatophores.

Likewise, we were able to prepare chromatophores which effected phosphorylation coupled to oxidation of NADH by molecular O_2 from light-grown cells of *R. rubrum*. Using such chromatophores we have found that under aerobic conditions oxidative phosphorylation with NADH occurs in the dark. ADP, P_i and Mg^{2+} each appeared requisite to the same extent for phosphorylation coupled to NADH oxidation and ascorbate-induced photophosphorylation[15] (Figs. 5–7). The pH optimum for oxidative phosphorylation was 6.8, for NADH-induced photophosphorylation 7.5, and for ascorbate-induced and PMS-induced photophosphorylations 8.0 (refs. 8, 15) (Fig. 1). Hence, it appeared that there were some differences in their electron-transport and energy-conversion systems. The reactions tested with chromatophores—NADH oxidation, phosphorylation coupled thereto, and NADH-induced photophosphorylation—were inactivated in parallel to each other when chromatophores were heated, and all three activities were much less stable to heating than ascorbate-induced and PMS-induced photophosphorylations (Table II). It seems probable, therefore, that the reactions related to NADH depend upon an electron-transport system that includes a common heat-labile part, which ascorbate-induced and PMS-induced photophosphorylations, also coupled to an electron-transport system, do not

contain. It has been found that NADH dehydrogenase purified from light-grown cells of *R. rubrum* show similarly a heat-lability[7].

Under our experimental conditions, antimycin A could completely inhibit ascorbate-induced photophosphorylation, whereas it inhibited NADH-induced photophosphorylation by approx. 85 %, NADH- and succinate–cytochrome c_2 reductions by 70–85% (in most cases), and PMS-induced photophosphorylation, NADH oxidation, or phosphorylation coupled to NADH oxidation, not at all. It is probable that electrons from NADH and from succinate are transferred to cytochrome c_2 through two different types of transport systems, one of which is antimycin A-insensitive. Because NADH-induced photophosphorylation under anaerobic conditions was inhibited no more than 85 % even in the presence of 10 μg/ml of antimycin A (more than thirty times as much as the minimal concentration required for a complete inhibition of ascorbate-induced photophosphorylation), it is suggested that under these conditions electrons from NADH are transferred as follows:

NADH → FP(flavoprotein enzyme) → antimycin A-insensitive site → cytochrome c_2

Further, this type of photophosphorylation was found to be inhibited by the addition of ascorbate (Fig. 14). The concentration of ascorbate required for a 50 % inhibition of the photophosphorylation was 0.2 mM, equivalent to an E_{mid} value of approx. +0.2 V (ref. 6). It may be that chromatophores possess an oxidation–reduction component ("E_{mid} = + 0.2 V Comp." in Fig. 15), having an E_{mid} value of approx. +0.2 V which in its reduced form combines with antimycin A more easily than in

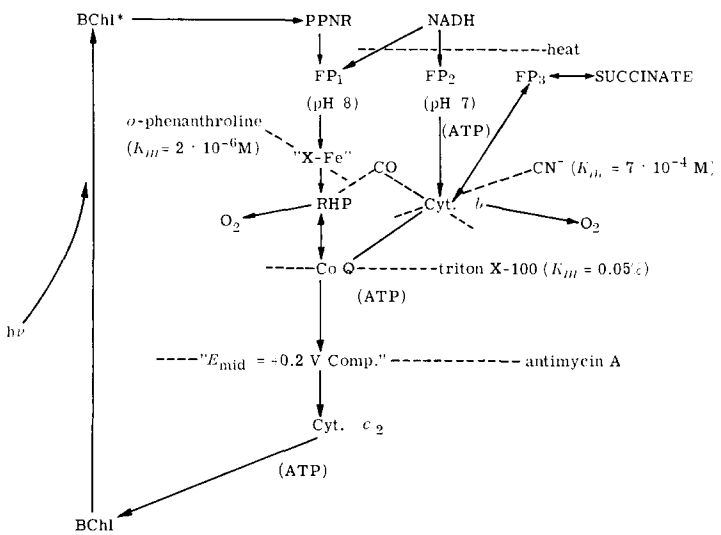


Fig. 15. Hypothetical scheme for electron-transport system of chromatophores from *R. rubrum*. Full lines represent the pathway of electron (or hydrogen) transport and dotted lines the site of action of inhibitor as indicated. The two sites are for the components capable of binding CO, RHP and cytochrome *b*. O_2 uptake due to autoxidation of the former is slower than the latter, in particular with dark-grown cells which do not contain RHP to an easily detectable extent. Action spectrum for the dark-grown cells with respect to restoration of their CO-inhibited O_2 uptake by the light, which is much the same as the absorption spectrum of CO-binding RHP in its reduced form, but which is completely different from the absorption spectrum of CO-binding component (cytochrome *b*), results from the RHP pathway, perhaps because of the property of RHP that its CO-binding state dissociates CO in the light more easily than the CO-binding state of cytochrome *b*.

its oxidized form. Thus antimycin A, if added to an aqueous reaction mixture as a small volume in methanolic solution, fails to inhibit completely the electron-transport system because this component is partially in its oxidized form.

Inhibitions by *o*-phenanthroline of various reactions with chromatophores have been distinguished as Type I, II and III (ref. 8) (Fig. 8); in the Type-I inhibition, a low concentration of *o*-phenanthroline (K_m approx. 2 μM) inhibits the cyclic electron-transport system for ascorbate-induced photophosphorylation, and in the Type-III inhibition, a high concentration of *o*-phenanthroline (K_m approx. 2 mM) inhibits oxidative phosphorylation with NADH as well as PMS-induced photophosphorylation[8]. It was found that photophosphorylation, if induced in the presence of both ascorbate and NADH, was of Type II (K_m approx. 50 μM), as well as that induced by NADH alone. Because NADH-induced and ascorbate-induced photophosphorylation proceeded at a comparable rate, and because the rates were approx. 10-fold greater than those of oxidative phosphorylation with NADH (Table II and Figs. 1 and 5), it seemed unlikely that the Type-II inhibition of (ascorbate *plus* NADH)-induced photophosphorylation could be a summation of inhibitions by *o*-phenanthroline on ascorbate-induced and NADH-induced photophosphorylation (Type I and II, respectively) and oxidative phosphorylation with NADH (Type III). It was more probable that the Type-II inhibition was a mixture of Type-I and Type-III inhibitions. Thus, regardless of phosphorylating inducers, NADH alone or NADH *plus* ascorbate, the photophosphorylation with NADH(if carried out at pH 7.9) was affected by *o*-phenanthroline first as a Type-I inhibition, and subsequently as a Type-III inhibition. In other words, photophosphorylation, if induced in the presence of NADH at pH 7.9, belonged 50–60 % to the electron-transport system for ascorbate-induced photophosphorylation (Type I) and 50–40 % to that for the NADH oxidation accompanied by phosphorylation (Type III). The ratio of sharing between an electron-transport system for ascorbate-induced photophosphorylation and one for NADH oxidation can be expected to vary with alteration of the pH of reaction medium; for example, making the reaction medium more alkaline might favor the electron-transport system for ascorbate-induced photophosphorylation.

Ascorbate-induced photophosphorylation could also use two systems for electron transport, one inhibited with *o*-phenanthroline as in Type III. Contrary to the *o*-phenanthroline inhibition of NADH-induced photophosphorylation, the type of inhibition of ascorbate-induced photophosphorylation varied with the change of the energy-trapping system added to the reaction mixture. Since Tiron did not influence the activities of ascorbate- and PMS-induced photophosphorylation, it seemed that Type-III *o*-phenanthroline inhibition was due to the action of *o*-phenanthroline itself.

Light-grown cells of *R. rubrum* consume molecular O_2 in the presence of substrates. It was previously found that the rate of O_2 uptake was markedly reduced under illumination (light respiration), and, upon cessation of light, the rate returned to the same level as that in the dark (dark respiration)[7]. The optimum pH was 8 for the "light" respiration (the O_2 uptake not appreciably influenced by the light or "light-insensitive" respiration), and 7.5 for the "dark *minus* light" respiration (the O_2 uptake influenced by the light for "light-sensitive" respiration), whilst the dark respiration (the total O_2 uptake, "light-sensitive" *plus* "light-insensitive") showed an optimum at pH 7.7. The respiratory coefficient (Q_{O_2}) for the dark respi-

ration at 30° and pH 7 in the presence of the growth medium was 10–20 mm^3/h per mg dry weight, considerably lower than those typical of aerobic microorganisms with *a*-type cytochrome oxidases[7]. In addition, narcotics such as *n*-butanol preferentially inhibited the light-sensitive respiration, and cyanide inhibited the light-insensitive respiration more than the light-sensitive respiration[7]. These facts indicate that the cells grown anaerobically in the light may have at least two different systems for O_2 uptake.

R. rubrum, grown aerobically in the dark, exhibits O_2 uptake which is not influenced by the light. HORIO AND TAYLOR[10,11] found that this O_2 uptake was influenced by CO and that the inhibition was removed by light. They showed that the action spectrum measured based on the restoration of CO-inhibited respiration by the light resembled that for so-called cytochrome *o* (ref. 36), and the absorption spectrum of CO-binding RHP in its reduced form[7,37]. ORLANDO, LEVINE AND KAMEN (unpublished results, 1960), and also TANIGUCHI AND KAMEN[38] found immunologically that heterotrophs, if grown aerobically in the dark, did not possess a detectable amount of RHP, and that they produce RHP in parallel with synthesis of the photochemical apparatus. GELLER[13] made a similar finding with *R. rubrum*. In addition, TANIGUCHI AND KAMEN[38] found that cells of *R. rubrum* if cultured successively in the dark under aerobic conditions, showed a much faster O_2 uptake (more than 5-fold increase in Q_{O_2}) than light-grown cells; however, they exhibited an action spectrum that was much the same as that previously determined by HORIO AND TAYLOR[10,11]. According to TANIGUCHI AND KAMEN, these results might suggest that one of the systems for O_2 uptake in light-grown cells functions *via* cytochrome *o*, without involvement of RHP. TANIGUCHI AND KAMEN[38], with their dark-grown cells, could demonstrate the existence of neither soluble nor insoluble components showing an absorption spectrum in its CO-binding state similar to the action spectrum. In particular with respect to the ratio of α/β peaks; the ratios were 0.93–0.96 for the action spectrum and CO-bound RHP, but higher than 2 for their CO-binding pigment. SUZUKI AND IWASAKI[39] isolated RHP (crytocytochrome *c*) from a non-photosynthetic denitrifing bacterium, which was weakly autoxidizable and its reduced form combined with CO. However, these facts do not exclude the possibility that RHP functions as a component in the slow O_2 uptake found with either light-grown or dark-grown cells of *R. rubrum*; it seems more likely to us that the component for the action spectrum, and KAMEN AND TANIGUCHI's CO-binding pigment which was spectrophotometrically detected, are different, and that in their dark-grown cells the former is present to a lesser extent than the latter. GIBSON AND KAMEN[54] have found that RHP, if allowed to stand aerobically with a high concentration of ascorbate, becomes much more autoxidizable, perhaps because of peroxides formed by ascorbate and molecular O_2, although the absorption spectrum remains unaltered by such treatment. Finally, BARTSCH AND KAMEN[40] isolated RHP from the obligate anaerobe, *Chromatium*. This suggests that RHP *in vivo* in *Chromatium* possesses a function other than that of O_2 uptake. Perhaps, in photosynthetic bacteria, RHP is one of the essential constituents of the cyclic electron-transport system[41].

BARTSCH, HORIO AND KAMEN[42] have recently succeeded in isolating a highly purified green-colored protein from light-grown cells of *R. rubrum*. They have found that this protein has the same kind of activity as that of "photosynthetic pyridine nucleotide reductase (PPNR)" isolated from spinach leaves by SAN PIETRO AND

LAND[43], when assayed in the photosynthetic reduction of NADP with chloroplasts from spinach. In addition, HORIO, BARTSCH AND KAMEN[44] have purified two different kinds of NADH dehydrogenases, a NADH : haemoprotein reductase[7] and a NADH : dye reductase, from light-grown cells of *R. rubrum*. These are tentatively designated PPNR, FP_1 and FP_2.

We have shown that NADH oxidation is markedly stimulated in the presence of the energy-trapping system; it is maximal in rate at pH 7.8 in the absence of the energy-trapping system and at pH 6.9 in its presence (Fig. 1). The pH–activity difference curve for NADH oxidation, "*plus* energy-trapping system" *minus* "*plus* none", has a maximal value at pH 6.8 and is quite similar to the pH–activity curve for the coupled phosphorylation. In addition, the pH–activity curve of the NADH oxidation in the absence of the energy-trapping system is similar to that of ascorbate-induced or PMS-induced photophosphorylation, maximal at pH 8 (ref. 8). It is plausible therefore to suppose that NADH oxidation is catalyzed by two different systems which may be termed, "pH-8 system" and "pH-7 system". Presumably, ascorbate-induced and PMS-induced photophosphorylations are catalyzed by the electron-transport chain including the pH-8 system mechanism; at least one of its constituents gives an optimum response at pH 8, so that ascorbate-induced and PMS-induced photophosphorylations are maximal in rate at pH 8 (ref. 8). The heat-labile properties of these systems could be traced to that of the NADH dehydrogenase protein moiety[45].

In a preceding paper[15], it was suggested that the oxidation–reduction reaction of NAD, if assayed at pH 8, was not appreciable in the cyclic electron-transport system for photophosphorylation. Possibly, the pH-8 system is involved in the cyclic electron-transport system, which does not contain NAD. Previous findings on inhibitory effects of Triton X-100 (a non-ionic detergent) on ascorbate-induced and PMS-induced photophosphorylations[8] have suggested that there are at least two coupling sites in PMS-induced photophosphorylation. In addition, photooxidation of reduced *c*-type cytochromes (cytochrome c_2 and cytochrome *c*) could be coupled to phosphorylation[8]. Furthermore, it was proposed that the system for reduction of fumarate to succinate by NADH with chromatophores in the dark under anaerobic conditions was regulated by an energy-conservation mechanism[15]. It is possible that the energy-conservation mechanism is common to the NADH–fumarate system and the pH-7 system.

A scheme to account for the electron-transport system of *R. rubrum* which includes previous proposals[6,41,45] is shown in Fig. 15. NADH is oxidized by molecular O_2 through both the pH-8 system (FP_1) and the pH-7 system (FP_2) in chromatophores from *R. rubrum*. These two systems are also included in the photosynthetic electron-transport systems for ascorbate-, NADH- and PMS-induced photophosphorylation. The liberated electrons are transferred to molecular O_2 *via* two systems; one is a chain:

$$NADH \rightarrow FP_1 \rightarrow (o\text{-phenanthroline-sensitive non-heme iron, 'X-Fe'}) \rightarrow RHP \rightarrow CoQ \rightarrow \text{cyt. } b \rightarrow O_2 \text{ (or } RHP \rightarrow O_2)$$

and another:

$$NADH \rightarrow FP_2 \rightarrow CoQ \rightarrow \text{cyt. } b \rightarrow O_2 \text{ (or cyt. } b \rightarrow CoQ \rightarrow O_2)$$

It is probable that the action spectrum is due to the difference in CO-binding proper-

ties between RHP and cytochrome *b*; CO-binding RHP is more photodissociable than CO-binding cytochrome *b*.

ORLANDO, LEVINE AND KAMEN[46] found that RHP did not originate from the cytoplasmic membrane of light-grown cells but was associated with chromatophores, and, as noted above, that bacteriochlorophyll and RHP appeared to be synthesized in parallel when growth conditions were shifted from dark to light. Chloroplasts from green leaves appear to lack RHP, FP_2 and FP_3 (succinate dehydrogenase), while possessing photochemical ability for the reduction of *b*-type cytochrome (cytochrome b_6) and simultaneous evolution of molecular O_2; thus, they do not appear to perform cyclic electron transport under physiological conditions[47–53].

All attempts have so far failed to demonstrate phosphorylation coupled to the anaerobic oxidation of NADH by fumarate in the dark[15]. Hence, there is no evidence to indicate that the oxidation–reduction system from NADH to FP_1 or FP_2 involves a coupling site. Exact locations of coupling sites are still uncertain, although they are indicated at the FP–cytochrome *b* level, at the coenzyme Q (CoQ) level and at the cytochrome c_2 level in the scheme shown. Cyclic electron transport through both pH-8 system and pH-7 system couples phosphorylation. However, the efficiency of coupling of phosphorylation seems different in the two systems. In other words, in the pH-8 system the reaction inclines toward the ATP formation, and in the pH-7 system the reaction does not so, requiring the hexokinase–glucose system in order to exhibit electron transport. The inhibition caused by light on O_2 uptake with the light-grown cells[7] possibly results from the inhibition of the pH-7 system by the pH-8 system, producing ATP photosynthetically and more easily.

The P/O ratio for the phosphorylation coupled to the NADH oxidation through the pH-7 system appears to be nearly 1. The NADH oxidation through the pH-7 system proceeds only when the energy-trapping system, or 2,4-dinitrophenol, is present. It is probable that 2,4-dinitrophenol uncouples the NADH oxidation from its own energy-transport mechanism through the pH-7 system, in the same manner as in the typical oxidative phosphorylation with mitochondria. As neither ADP, P_i nor Mg^{2+} are required for 2,4-dinitrophenol to uncouple the pH-7 system from the energy-transport system, the uncoupling takes place at a site on the energy-transport system at which ADP, P_i and Mg^{2+} are not present.

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