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The requirement of ubiquinone-10 for an ATP-forming system and an ATPase system of chromatophores from *Rhodospirillum rubrum*

Chromatophores from *Rhodospirillum rubrum* show ATPase (EC 3.6.1.3) activity and ATP–P_i exchange activity. It was reported earlier that, in darkness, both activities originate at least in part from the reversibility of the energy-conversion system coupled to photosynthetic or oxidative electron transport[1].

Racker and his associates[2] extracted and purified from mitochondria a protein capable of catalyzing an ATPase activity, and they reported that phosphorylating activity by impaired mitochondria was stimulated by adding the enzyme. However, all attempts so far made in this laboratory for extraction of such an enzyme from chromatophores, by, *e.g.*, sonic disruption[1], treatment with various detergents or illumination with ultraviolet light, have resulted in a nearly parallel loss of photosynthetic ATP-forming activity, and ATPase and ATP–P_i exchange activities in darkness.

It is known that chromatophores contain a considerable amount of ubiquinone-10 (Q-10)[3], and that the level of oxidation–reduction state of quinone(s) present in chromatophores is changed when chromatophores are illuminated[4–6]. Rudney[7,8] found that chromatophores prepared from cells grown in the presence of diphenylamine contained a decreased amount of Q-10 and were less active in photosynthetic ATP formation, and that ATP formation was significantly stimulated by adding Q-10; he concluded that the stimulation was merely caused by adjusting the oxidation–reduction potential of the reaction medium in favor of the maximum coupling[9] of photosynthetic ATP formation.

Chromatophores were prepared from light-grown cells of a blue-green mutant (G-9) of *R. rubrum*, kindly supplied by Dr. J. W. Newton, and ATP-forming activity in the light and activities for ATPase and ATP–P_i exchange in darkness were assayed according to the methods described previously[1,10]. It was found that the rate of most of the ATPase activity and almost all of the ATP–P_i exchange activity was influenced by adding 2,6-dichlorophenolindophenol (DCIP) (Fig. 1). Chromatophores could reduce a part of DCIP without addition of electron donors. The rate of ATP–P_i exchange decreased with increasing concentration of DCIP and was negligible at $6.7 \cdot 10^{-5}$ M. On the other hand, the rate of ATPase increased with increasing concentration of DCIP, reaching a maximum at $6.7 \cdot 10^{-5}$ M, and then decreasing at higher concentrations. The activities of ATPase and ATP–P_i exchange, which had been depressed in the presence of $6.7 \cdot 10^{-4}$ M DCIP, were restored when ascorbate was also added;

Abbreviations: Q-10, ubiquinone-10; DCIP, 2,6-dichlorophenolindophenol.

the maximum restorations were obtained at $6.7 \cdot 10^{-4}$ M for the ATPase activity and at $6.7 \cdot 10^{-3}$ M for the ATP–P_i exchange activity, where the oxidation–reduction potentials of the reaction mixtures were approx. +0.13 V and approx. +0.08 V, respectively. In contrast to the ATPase activity, the ATP–P_i exchange activity was hardly affected at higher concentrations of ascorbate. Experiments with different concentrations of DCIP and ascorbate indicated that the maximum restorations were related to the E_h values of the reaction mixtures but not to the concentrations of DCIP *plus* ascorbate. Essentially the same result was obtained when phenazine methosulfate *plus* ascorbate was used instead. In addition, chromatophores could be repeatedly washed with 0.1 M glycylglycine buffer (pH 8.0) containing 10 % sucrose and DCIP and/or ascorbate without appreciable loss of the activities of ATP formation, ATPase and ATP–P_i exchange. These results indicate that the effect of these redox dyes was due to a reversible reaction. The uncoupler, 2,4-dinitrophenol ($2 \cdot 10^{-3}$ M), inhibited (uncoupled) the ATP–P_i exchange activity in the entire range of E_h values tested, whereas it stimulated the ATPase activity to approx. 80 % of the rate at the optimal E_h value in the range of E_h values more negative than the

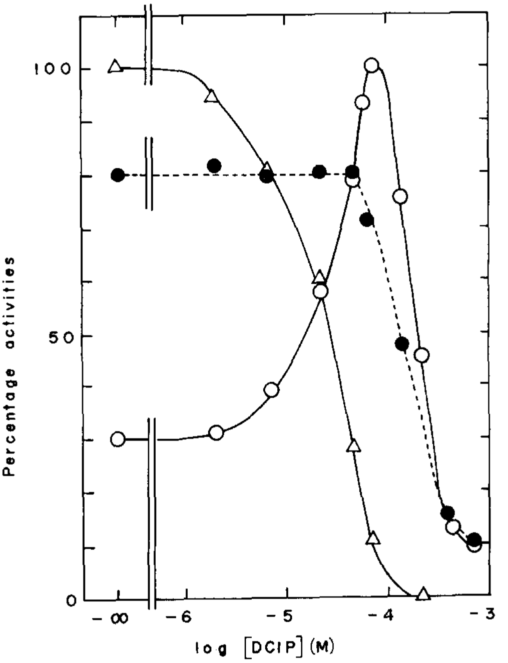

Fig. 1. Effect of DCIP on ATPase activity and ATP–P_i exchange activity by chromatophores in darkness. The components of the reaction mixture for assay of ATPase activity were as follows: 0.50 ml 0.2 M glycylglycine–NaOH buffer (pH 8.0) containing 10 % sucrose; 0.10 ml 0.1 M $MgCl_2$; 0.10 ml $3.0 \cdot 10^{-3}$ M [γ-^{32}P]ATP (approx. 10^6 counts/min); 0.10 ml chromatophore suspension ($A_{873\,m\mu}$/ml = 50); 0.10 ml various concentration of DCIP; water to make the total volume 1.50 ml. In some cases, 0.10 ml $3.0 \cdot 10^{-2}$ M 2,4-dinitrophenol was added. For assay of ATP–P_i exchange activity, 0.10 ml 0.05 M ATP and 0.10 ml 0.05 M $^{32}P_i$ (approx. 10^7 counts/min) were added instead of the radioactive ATP. The reactions were carried out at 30° for 4 min in darkness. It was determined that the oxidation–reduction potential of the reaction medium became more positive with increasing concentration of DCIP. The activity for photosynthetic ATP formation decreased in parallel with the ATP–P_i exchange activity in darkness. ○—○, ATPase activity; ●---●, ATPase activity in the presence of 2,4-dinitrophenol; △—△, ATP–P_i exchange activity.

optimal, but not in the other range. One of possible explanations for these findings may be as follows. Two different oxidation–reduction components adjacent on the electron transport system are required for one of the coupling sites leading to ATP formation. The ATPase activity for the site appears when the two components are in the oxidized form and the reduced form, respectively, whereas the ATP–P_i exchange activity for the site requires one of the two components in the reduced form. 2,4-Dinitrophenol decomposes an energized intermediate formed in the ATP–P_i exchange reaction.

Chromatophores were washed with water, lyophilized, suspended in a volume of isooctane such that they would show $A_{873\,m\mu}/ml = 5$ if suspended in the same volume of water and shaken at 4° for 80 min, followed by centrifugation. The resulting precipitate (extracted chromatophores) was dried under vacuum. It was determined that the quinones present in chromatophores were almost all extracted by this treatment; chromatophores possessed approx. 4 mμmoles of Q-10 and approx. 0.8 mμmole of rhodoquinone (RQ) per $A_{873\,m\mu}$ (approx. 7 mμmoles of bacteriochlorophyll). Other quinones were not present in appreciable amounts. The extracted chromatophores were suspended in a solution containing a pure quinone in isooctane and the suspension was then dried under vacuum. By extraction of the quinones, the rate of photosynthetic ATP formation, which was assayed in the presence of the optimal concentration ($6.7 \cdot 10^{-2}$ M) of ascorbate, and the rates of ATPase activities in darkness, which were assayed in the presence and absence of DCIP and 2,4-dinitrophenol, markedly decreased (Table I). These rates were significantly restored by adding Q-10, and the restoration reached the maximum at approx. 4 mμmoles/$A_{873\,m\mu}$ of Q-10, the same amount as was originally present. For the restoration of photosynthetic ATP-forming activity, Q-6 was as effective as Q-10, whereas rhodoquinone, phylloquinone and menadione hardly reactivated the activity. A part of the ATPase activity remained after the quinone extraction. The majority of the remaining ATPase activity was not influenced by oligomycin (3.3 μg/ml), whereas the ATPase activity before

TABLE I

EFFECT OF BACK-ADDITION OF VARIOUS QUINONES ON ATP-FORMING ACTIVITY IN LIGHT AND ATPase ACTIVITY IN DARKNESS OF ISOOCTANE-EXTRACTED CHROMATOPHORES

The conditions for reactions and the components of the reaction mixtures were the same as those described in Fig. 1, except that for the assay of photosynthetic ATP formation, 0.10 ml 0.1 M $^{32}P_i$ (approx. 10^6 counts/min), 0.10 ml 0.1 M ADP and 0.10 ml 1.0 M ascorbate were added instead of the radioactive ATP of the reaction mixture for ATPase activity assay.

Chromatophores	*Additions (4 mμmoles/$A_{873\,m\mu}$)*	*Photosynthetic ATP formation (μmoles/$A_{873\,m\mu}$ per h)*	*ATPase activity in darkness (mμmoles/$A_{873\,m\mu}$ per h)*		
			None	*+DCIP ($6.7 \cdot 10^{-5}$ M)*	*+2,4-Dinitrophenol ($2 \cdot 10^{-3}$ M)*
Non-treated	None	2.160	304	511	453
Lyophilized	None	0.580	138	162	199
Extracted	None	0.000	60	59	118
	Q-10	0.560	115	145	228
	Q-6	0.587	99	161	200
	Rhodoquinone	0.039	90	150	178
	Menadione	0.000	104	104	166

the quinone extraction and that reactivated by adding Q-10 were inhibited to the level of the remaining activity. For the restoration of the ATPase activity, all quinones mentioned above were as effective as Q-10, in contrast to the case of photosynthetic ATP-forming activity. On the contrary, the ATP–P_i exchange activity in darkness was not affected by the quinone extraction.

Probably, ubiquinone can serve as one of the oxidation–reduction components in the photosynthetic electron flow, whereas all quinones tested can function equally well as one of the constituents for the ATPase activity at one of the coupling sites.

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Division of Enzymology,
Institute for Protein Research,
Osaka University,
Osaka (Japan)

TAKEKAZU HORIO
KATSUZO NISHIKAWA
SHIGEKI OKAYAMA
YOSIFUMI HORIUTI
NOBORU YAMAMOTO
YOSHIKO KAKUTANI

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Mitochondrial citrulline synthesis with exogenous ATP

It is generally accepted that the movement of adenine nucleotides through the mitochondrial membrane system is controlled by a specific translocase associated with the inner membrane[1-3]. As first shown by KLINGENBERG AND PFAFF[1] and PFAFF, KLINGENBERG AND HELDT[3] this process has an enzymic character. Strong support for the idea of an enzyme-regulated adenine nucleotide translocation originally came from the studies with atractyloside, a specific inhibitor of this process. It has been shown that several adenine nucleotide-requiring systems are inhibited by atractyloside when they are dependent on extramitochondrial adenine nucleotides[1-10].

We have reported that exogenous ATP is a very ineffective energy donor for the synthesis of citrulline from ornithine, NH_3 and CO_2 in isolated rat-liver mitochondria[9]. We interpreted these results by assuming that the adenine nucleotide