RHODOQUINONE AS A CONSTITUENT OF THE DARK ELECTRON-TRANSFER SYSTEM OF *RHODOSPIRILLUM RUBRUM*

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1. Introduction

Since 1962 *Rhodospirillum rubrum* has been known to contain rhodoquinone [1], but until [2] little was known about the specific participation of this redox carrier in the electron-transfer system of the microorganism. We showed in [2] that non-phototrophic strain F11 is a mutant deficient in rhodoquinone and that this benzoquinone is specifically required for some light-dependent redox reactions. Here we present data suggesting that rhodoquinone is also involved in 2 dark redox processes which are catalyzed by membrane vesicles isolated from *R. rubrum*, the reduction of tetrazolium blue by succinate and that of fumarate by NADH. It seems possible that both reactions are mediated by the same catalytic system.

2. Methods

Wild-type *R. rubrum* (strain S1), the non-phototrophic, rhodoquinoneless mutant (strain F11) and the phototrophic revertant of the mutant (strain RF110) were described in [2,3]. Cultures were grown in the dark under low oxygen tension [4] to derepress pigment synthesis [5]. Intracytoplasmic membrane vesicles (chromatophores) were prepared as in [6]. Electron transfer activities were assayed using chromatophores suspended in 45 mM sodium phosphate (pH 7.4). NADH (100 μM) or 5 mM sodium succinate were used as electron donors and 5 mM sodium fumarate, 150 μM ferricytochrome *c*, 50 μM 2,6-dichlorophenol-indophenol, 90 μM tetrazolium blue or saturating oxygen as electron acceptors. The assay mixtures for the reduction of fumarate, tetrazolium blue and cytochrome *c* were made anaerobic with the help of an oxygen scavenger system [4]. Since 2,6-dichlorophenolindophenol was reduced by this auxiliary system, its reduction by NADH or succinate was assayed aerobically in the presence of 10 mM KCN. All assays were done at room temperature, except the reduction of tetrazolium blue by succinate, which was found to be considerably stimulated by higher temperatures and was assayed at 34°C. The reactions were followed spectrophotometrically in 1 cm optical-path cuvettes. The measuring wavelength was 340 nm (NADH) or, when feasible, that corresponding to the most prominent visible band of the oxidized minus reduced spectrum of the acceptor.

3. Results

The intracytoplasmic membrane of *R. rubrum* contains, in addition to the photosynthetic apparatus, an aerobic respiratory chain which is thought to be responsible for some catalytic redox activities which are present in isolated chromatophores [7]. Table 1 shows the specific levels of some of the dark activities in chromatophores obtained from both the wild-type (S1) and the rhodoquinoneless (F11) strains. The activities responsible for the reductions of fumarate by NADH and of tetrazolium blue by succinate, respectively, are considerably decreased in mutant chromatophores, an observation which suggests that rhodoquinone is involved in both of them. This conclusion is reinforced by the additional finding (table 1) that fumarate and tetrazolium blue reductions are catalyzed at wild-type rates by chromatophores isolated from a revertant strain (RF110) which has been selected from the mutant only for restoration

Table 1
Some catalytic activities of isolated *R. rubrum* chromatophores

Donor:acceptor	Specific activities (nmol acceptor reduced . min^{-1} . mg^{-1} protein)		
	Strain S1	Strain F11	Strain RF110
NADH oxygen	28.6	26.9	n.a.[a]
NADH:DCIP[a]	112.5	112.8	n.a.
NADH:fumarate	5.5	1.4	6.0
Succinate:cytochrome *c*	27.4	26.5	n.a.
Succinate:DCIP	39.3	40.1	n.a.
Succinate:tetrazolium blue	1.82	0.15	1.22

[a] DCIP: 2,6-dichlorophenolindophenol; n.a., not assayed

The chromatophore preparations contained 27 (S1), 23 (F11) and 22 (RF110) nmol bacteriochlorophyll/mg protein

of phototrophic competence and which has simultaneously recovered normal rhodoquinone levels [2]. Also, the rest of the assayed activities, some of which have been shown to require ubiquinone but not rhodoquinone [8,9], are not significantly reduced in mutant chromatophores (table 1).

As shown in fig.1, the catalytic activities which are altered in mutant chromatophores are inactivated on exposure of wild-type chromatophores to ultraviolet light. Although this experiment does not prove by itself the participation of rhodoquinone in the endogenous electron-transfer systems which mediate the reactions, the results are consistent with such a participation because quinones are irreversibly altered by ultraviolet irradiation [10]. Photoinactivation follows apparent first-order kinetics and, in the case of tetrazolium blue reduction, the time of half-inactivation is about the double of that for fumarate reduction. This observation may be relevant with regard to the mechanism of the reactions (see section 4).

To obtain further evidence in support of the involvement of rhodoquinone in both fumarate and tetrazolium blue reductions, we have investigated the effect of the addition of quinones on the activities of lyophilized mutant chromatophores. Tetrazolium blue reduction was clearly stimulated by rhodoquinone but not by ubiquinone (fig.2). Ubiquinone also failed to stimulate when added together with rhodoquinone (not shown). Moreover, similar results were obtained with wild-type chromatophores which had been pre-extracted with isooctane to remove most of rhodoquinone and part of ubiquinone (not shown). Thus, these data support the conclusion that rhodoquinone is a constituent of the chromatophore system which mediates the reduction of tetrazolium blue by succinate. It should be remarked that, although the stimulatory effect of rhodoquinone addition is clear, the reconstituted activity (fig.2) amounts only

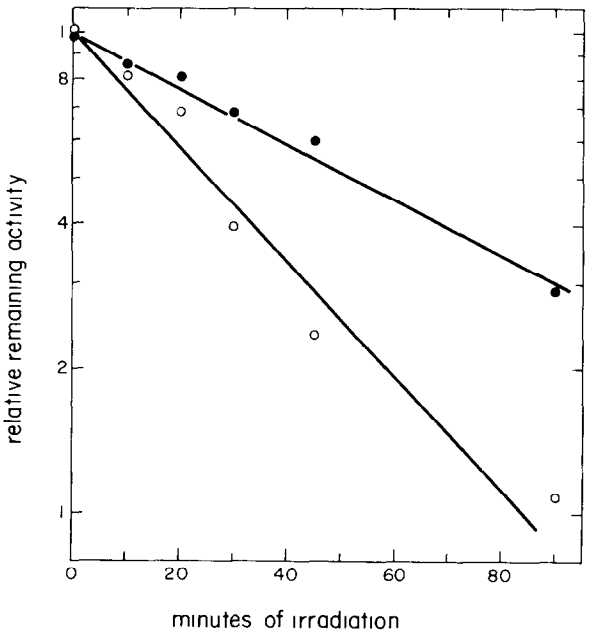

Fig.1. Effect of ultraviolet light on the ability of wild-type chromatophores to catalyze the reduction of tetrazolium blue by succinate (●) and that of fumarate by NADH (○). A 5 mm layer of a chromatophore suspension (1.3 mg protein/ml, 17 nmol bacteriochlorophyll/mg protein) was kept near 0°C and illuminated through a wide-band color filter (254 nm). Incident light intensity was 2 W/m^2.

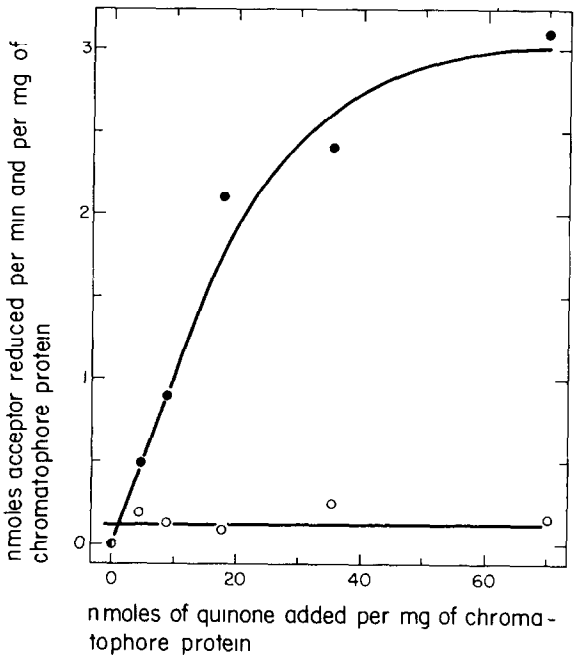

Fig.2. Effect of added quinones on the ability of mutant chromatophores to catalyze the reduction of tetrazolium blue by succinate. Ubiquinone-10 (○) and rhodoquinone-10 (●) were purified from *R. rubrum* cells [15] and added in isooctane solution to lyophilized chromatophores as in [16].

to a fraction of the original activity present in wild-type chromatophores (table 1). That is likely to be the result of some side effect of the organic solvent on other endogenous constituents which may participate in the catalytic activity.

At variance with the above results, fumarate reduction by NADH was not enhanced when purified rhodoquinone was added to rhodoquinoneless chromatophores (not shown). Since suspension of lyophilized chromatophores in isooctane or other organic solvents resulted always in the complete loss of this activity, even when the solvents contained rhodoquinone and/or ubiquinone, we feel that these results are also the consequence of the inactivation of additional endogenous factors required for the activity by the organic solvents used to attempt reconstitution.

4. Discussion

The catalytic system which mediates the reduction of fumarate by NADH in *R. rubrum* chromatophores is a new example of the relatively numerous membrane-linked, fumarate-reducing systems which require a quinone different from ubiquinone [11,12]. The quinone, which connects a substrate dehydrogenase to a fumarate reductase, is usually a naftoquinone (menaquinone or desmethylmenaquinone) in bacteria and rhodoquinone in mitochondria. Thus the *R. rubrum* system is peculiar because it is, at least to our knowledge, the first non-mitochondrial system of fumarate reduction which has been reported to require rhodoquinone.

The other reaction reported here to depend on rhodoquinone, the reduction of tetrazolium blue by succinate, may be mediated by the same quinol: fumarate reductase which is expected to participate in fumarate reduction. If this were so, the different sensitivity of the two catalytic activities to organic solvents and to ultraviolet light might be due to the additional participation of a NADH dehydrogenase in the transfer of electrons from NADH to fumarate. Thus, if rhodoquinone reacted with two membrane enzymes during fumarate reduction and with only one during tetrazolium blue reduction, the dependence of each activity on the rhodoquinone level in the membrane would be quadratic and linear, respectively. This is consistent with the relative length of the apparent times of half photoinactivation shown by the catalytic activities (fig.1). At any rate, further work is needed to characterize unequivocally the enzymic entities involved in the rhodoquinone-dependent processes.

In other organisms the membrane-linked reduction of fumarate by NADH or by other metabolic reductants may be used as an anaerobic type of respiration which drives ATP synthesis and other endergonic processes [11,12]. The *R. rubrum* system could, in principle, serve a similar physiological function. Alternatively, the *R. rubrum* system could mediate the reduction of NAD^+ by succinate, an energy-requiring process of reverse electron transfer which appears to be responsible in *R. rubrum* for the generation of NADH during phototrophic growth [13]. However, in both cases the reduction of fumarate by NADH should consist of some energy-conserving step(s), and that could not be demonstrated in [14]. We have been unable to find also any evidence favouring either or both processes as the function of the *R. rubrum* system in vivo. Although these negative results may not be considered as definitive, they suggest a different physiological role.

We have observed that, under a variety of growth conditions, the cytoplasmic NADH/NAD^+ ratios are considerably higher in the rhodoquinoneless mutant than in the wild-type strain. Since a high NADH/NAD^+ ratio inhibits cyclic photophosphorylation both in vivo and in vitro [6], it appears that the physiological function of the fumarate-reducing system might be to maintain the intracellular levels of NADH at a low relative value in order to avoid the interference of this metabolic reductant with photosynthesis. As the rhodoquinoneless mutant is non-phototrophic and shows no apparent defects in aerobic growth [3], it seems possible that both the light-elicited [2] and the dark electron-transfer steps which depend on rhodoquinone may be related to the phototrophic metabolism of *R. rubrum.*

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