NAPHTHYLMERCAPTOBENZOQUINONE, A NEW INHIBITOR OF PHOTOPHOSPHORYLATION IN RHODOSPIRILLUM RUBRUM CHROMATOPHORES AT THE LEVEL OF UBIQUINONE

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Summary

Naphthylmercaptobenzoquinone (NMBQ) inhibits photophosphorylation in Rhodospirillum rubrum chromatophores. The endogenous unsupplemented system is the most sensitive one being 50% inhibited by 0.5 μM NMBQ, whereas 20 μM are required for 50% inhibition of photophosphorylation in the presence of N-methylphenazonium methosulfate or diaminodurene. The inhibition is less effective under argon, especially in the presence of ascorbate, and is reversed on addition of various naphthoquinones, which can also reverse the inhibition of photophosphorylation by dibromothymoquinone (DBMIB). Another quinone analog, dodecylmercaptonaphthoquinone (DMNQ), inhibits endogenous photophosphorylation even more effectively than NMBQ, but its inhibition is not reversed by the added naphthoquinones. It is concluded that NMBQ and DBMIB, but not DMNQ, inhibit photophosphorylation in chromatophores by acting as antagonists of ubiquinone.

Introduction

The effect of the quinone analog DBMIB (1) on various light-induced reactions has recently been tested in chromatophores of Rhodospirillum rubrum (2, 3) and Rhodopseudomonas capsulata (3). It was found to inhibit the reoxidation of photoreduced cytochrome b (2) indicating that its effect must be located between cyrochromes b and c. Furthermore, its inhibitory effect on photophosphorylation, which was shown to be reversed by 1,2-naphthoquinone, indicated that DBMIB acts as a ubiquinone antagonist (3) and it was, therefore, proposed that the electron transport between cytochromes b and c in chromatophores involves a ubiquinone molecule.

In the present study the effect of two additional quinone analogs, NMBQ and DMNQ was tested in R. rubrum chromatophores. Both were found to inhibit endogenous phosphorylation but, whereas the inhibitory effect of NMBQ was found to be very

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Abbreviations: DAD, diaminodurene = 2,3,5,6-Tetramethyl-p-phenylenediamine; DBMIB, dibromothymoquinone = 2,5-Dibromo-3-methyl-6-isopropyl-p-benzoquinone; DMNQ, dodecylmercaptonaphthoquinone = 3-n-Dodecylmercapto-2-hydroxy-1,4-naphthoquinone; NMBQ, naphthylmercaptobenzoquinone = 6-β-Naphthylmercapto-5-chloro-2,3-dimethoxy-p-benzoquinone; PMS, N-methylphenazonium methosulfate.

similar to that of DBMIB, the inhibition by DMNQ was different, suggesting that their site of action is different.

Methods

R. rubrum strain S_1 cells were grown photosynthetically in the medium of Ormerod et al. (4) as previously described (5). Harvested cells were washed, broken in a Yeda Press and chromatophores were isolated as outlined by Gromet-Elhanan (6, 7). Bacteriochlorophyll was measured using the in vivo extinction coefficient given by Clayton (8).

Photophosphorylation was carried out in an illuminated Warburg bath in Erlenmeyer flasks closed with special stoppers fitted with glass tubing for gassing (9). Reaction mixtures contained, in a final volume of 3 ml: 30 mM Tricine-NaOH, pH 8.0; 3.3 mM $MgCl_2$; 3.3 mM sodium phosphate (containing 2×10^6 counts/min ^{32}P); 1.66 mM ADP; chromatophores containing 25 μg bacteriochlorophyll and, where indicated, 1.6 mM ascorbate; 0.5 mM DAD; 66 μM PMS or 0.1 mM of the various naphthoquinones.

The reactions were allowed to equilibrate under the gas phase indicated for 10 min in the dark and then illuminated for 3 min at 30° by 50,000 lux of white light. ATP formation was assayed according to Avron (10) on aliquotes deproteinized by perchloric acid (3% W/V).

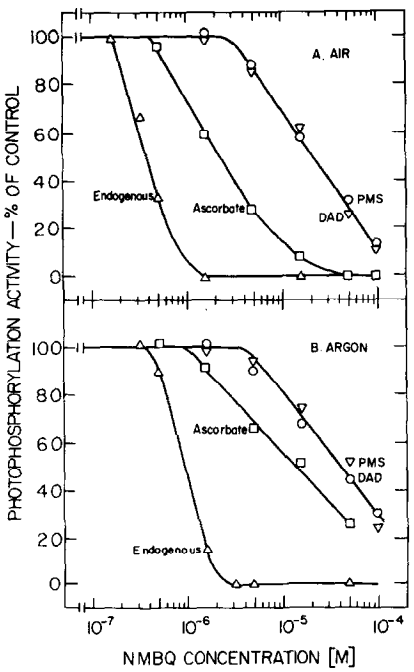

Fig. 1 - Effect of naphthylmercaptobenzoquinone (NMBQ) on photophosphorylation activities of R. rubrum chromatophores under air or argon. Control activities expressed in μmoles ATP formed per mg bacteriochlorophyll per hr were:
under air: Δ - 408; □ - 453; ▽ - 706; 0 - 944.
under argon: Δ - 623; □ - 753; ▽ - 801; 0 - 890

Table 1

Influence of added 1,2-naphthoquinone on the inhibition of endogenous photophosphorylation under air by naphthylmercaptobenzoquinone (NMBQ) and dodecylmercaptonaphthoquinone (DMNQ)

Inhibitor added (M)	Photophosphorylation with system tested: endogenous specific activity[a]	endogenous % of control	+1,2-naphthoquinone specific activity[a]	+1,2-naphthoquinone % of control
None	484	100	206	100
NMBQ, 5×10^{-7}	256	53	206	100
" 2×10^{-6}	5	1	185	90
" 5×10^{-6}	0	0	86	42
DMNQ, 5×10^{-8}	411	85	199	97
" 2×10^{-7}	261	54	101	49
" 5×10^{-7}	87	18	45	22

[a]Specific acitivty is expressed in μmoles ATP formed/hr/mg bacteriochlorophyll

Results

Photophosphorylation in R. rubrum chromatophores is strongly inhibited by NMBQ (Fig. 1). The endogenous system is the most sensitive one: 0.5-1.0 μM NMBQ are required for 50% inhibition of this system under air or argon respectively, whereas in the presence of the artificial electron carriers, PMS or DAD, 20-40 μM are respectively required. Addition of ascorbate leads also to a partial protection against NMBQ inhibition, and this protection is especially pronounced under argon (Fig. 1AB). An even more effective protection by ascorbate was observed in another photosynthetic bacterium Rps. capsulata, and there the degree of protection increased with increasing ascorbate concentrations (11).

NMBQ, like DBMIB, is a quinone analog and the pattern of its inhibitory effect on various photophosphorylation systems in R. rubrum chromatophores (Fig. 1) is very similar to that reported with DBMIB in R. rubrum as well as in Rps. capsulata (3). The inhibition of endogenous phosphorylation by DBMIB was reversed in both photosynthetic bacterial systems by the addition of 1,2-naphthoquinone. 1,4-naphthoquinone gave some reversal but was by itself a strong inhibitor of photophosphorylation when tested under air (3).

As shown in Table 1 addition of 1,2-naphthoquinone does also reverse the inhibitory effect of NMBQ. Moreover, when tested under argon, 1,4-naphthoquinone

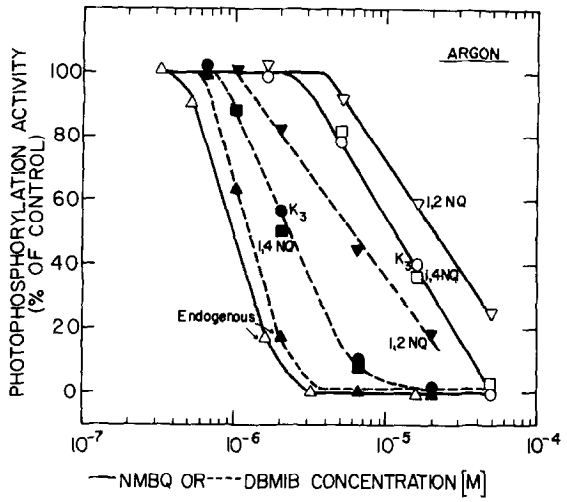

Fig. 2 - Reversal of DBMIB and NMBQ inhibition of endogenous phosphorylation under argon by various naphthoquinones. Control activities in the absence of the inhibitors were: endogenous △, ▲ - 756; menadione (K_3) 0, ● - 806; 1,4-naphthoquinone (1,4 NQ) □, ■ - 492; 1,2-naphthoquinone (1,2 NQ) ▽, ▼- 536.

and menadione as well as 1,2-naphthoquinone could reverse the inhibition of photophosphorylation by both NMBQ and DBMIB (Fig. 2), without exerting the inhibitory effect observed in their presence under air. All three naphthoquinones tested were more efficient in reversing the inhibitory effect of NMBQ than that of DBMIB (Fig. 2).

Another quinone analog DMNQ, which was reported by Porter et al. (12) to inhibit both NADH and succinoxidase mitochondrial CoQ-enzyme systems, was found to inhibit endogenous photophosphorylation even more effectively than NMBQ. But its inhibition, unlike that of NMBQ, was not relieved by 1,2-naphthoquinone (Table 1), indicating that the site of action of these two quinone analogs must be different.

Discussion

The inhibition of photophosphorylation in bacterial chromatophores by NMBQ and its reversal by various added naphthoquinones suggests that this quinone analog can act in chromatophores as an antagonist of ubiquinone.

Ubiquinones have been found in large amounts in photosynthetic bacteria and are assumed to participate in the light-induced cyclic electron transport. The exact location of ubiquinone in this electron transport is, however, unclear. It is commonly accepted to play a role as primary or secondary acceptor (13, 14), but recent reports

indicate that additional ubiquinone molecules might participate in latter steps of the cyclic electron transport in chromatophores. Thus, Dutton and Jackson (15) suggested a role for ubiquinone between two b-type cytochromes, whereas Baltscheffsky (2) and Gromet-Elhanan and Gest (3) proposed that it participates in the electron transport between cytochromes b and c.

The proposed role of ubiquinone between cytochromes b and c was based on results obtained with the quinone analog DBMIB (2, 3). This compound was first introduced by Trebst et al. (1) as an inhibitor of photosynthetic electron transport in chloroplasts, and was shown to act as an antagonist of plastoquinone (16). It was also found to act as an antagonist of ubiquinone in chromatophores (3) and was suggested to inhibit the cyclic electron transport between ubiquinone and cytochrome c.

The pattern of inhibition of phosphorylation in R. rubrum chromatophores by DBMIB (3) and NMBQ (Fig. 1) is very similar: the endogenous phosphorylation system is the most sensitive one and the PMS and DAD supplemented systems are the most resistant ones to both inhibitors. Also, in contrast to the inhibition of phosphorylation by antimycin A (3), the inhibition by both benzoquinone derivatives is partially overcome by ascorbate (Fig. 1 and references 3 and 11) and is reversed by added naphthoquinones (Fig. 2). It is, therefore, concluded that NMBQ and DBMIB inhibit the same site of the cyclic electron transport in chromatophores. On the other hand the finding that inhibition of photophosphorylatic by the other quinone analog tested in this study, DMNQ, was not reversed by added naphthoquinones indicates that its mode of action must be different from that of DBMIB or NMBQ.

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