Restoration of Respiratory Electron-Transport Reactions in Quinone-Depleted Particle Preparations from *Anacystis nidulans*

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Electron transport from H_2 , NADPH, NADH and succinate to O_2 or ferricytochrome *c* in respiratory particles isolated from *Anacystis nidulans* in which hydrogenase had been induced was abolished after extraction of the membranes with *n*-pentane; oxidation of ascorbate plus *NNN'*-tetramethyl-*p*-phenylenediamine remained unaffected. Incorporation of authentic ubiquinone-10, plastoquinone-9, menaquinone-7 and phyloquinone (in order of increasing efficiency) restored the electron-transport reactions. ATP-dependent reversed electron flow from *NNN'*-tetramethyl-*p*-phenylenediamine to $NADP^+$ or, via the membrane-bound hydrogenase, to H^+ was likewise abolished by pentane extraction and restored by incorporation of phyloquinone. Participation of the incorporated quinones in the respiratory electron-transport reactions of reconstituted particles was confirmed by measuring the degree of steady-state reduction of the quinones. Isolation and identification of the quinones present in native *Anacystis* membranes yielded mainly plastoquinone-9 and phyloquinone; neither menaquinone nor α -tocopherolquinone could be detected. Together with the results from reconstitution experiments this suggests that phyloquinone might function as the main respiratory quinone in *Anacystis nidulans*.

Quinones have universally been found to take part as electron-transporting and proton-translocating catalysts in the membrane-bound photosynthetic and respiratory electron-flow chains of living cells (see Bishop, 1961; Barr & Crane, 1972; Crane, 1977; Jones, 1977). Apart from direct spectroscopic evidence (e.g. see Ames, 1964), most information on the quinones involved in electron transport has come from extraction–reconstitution experiments. Such experiments have been carried out on bacterial respiratory systems (Kröger & Dadać, 1969; Kröger *et al.*, 1971; Holländer, 1976), on mitochondria (Szarkowska, 1966; Ernster *et al.*, 1969) and on the photosynthetic electron-transport chains of chloroplasts (Bishop, 1961; Krogmann & Olivero, 1962; Henninger & Crane, 1963) and blue-green algae (cyanobacteria) (Lightbody & Krogmann, 1966). Virtually nothing is known about possible respiratory functions of quinones in blue-green algae (Carr, 1973; Stanier & Cohen-Bazire, 1977).

Under certain growth conditions *Anacystis nidulans* can be induced for O_2 -dependent uptake of

molecular H_2 (Peschek, 1979*b,c*); this reaction appears to be mediated by an 'aerobic uptake hydrogenase' feeding electrons from H_2 into the respiratory chain (Peschek, 1979*b*). A comprehensive study of the effects of inhibitors on respiratory electron transport in particle preparations from *Anacystis* in which hydrogenase had been induced was recently undertaken (Peschek, 1980). The results relevant to an understanding of the present paper may be briefly summarized as follows: (a) NADPH was the preferred electron donor to the respiratory chain (Biggins, 1969; Leach & Carr, 1970; Peschek, 1975, 1979*d*, 1980); (b) H_2 , NADPH, NADH and succinate all entered the same type of respiratory chain (c) by demonstrating respiratory control and by the use of proton ionophores, it was suggested that ATP synthesis was coupled to the oxidation of these substrates, but the phosphorylating capacity appeared to be highly unstable; (d) a cyanide-sensitive branch and a cyanide-resistant branch of the pathway (see White & Sinclair, 1971) could be characterized, possibly sharing a common quinone moiety. Unfortunately, it has not been possible so

far to unravel the molecular structure of the respiratory chains of blue-green algae by using spectroscopic techniques.

The purpose of the present paper is to report the characterization of the respiratory quinone(s) of *Anacystis* as studied in extraction-reconstitution experiments, by use of specific inhibitors and by spectroscopy. The results indicate phyloquinone to be the main respiratory quinone involved in the oxidation of H_2 , NADPH, NADH and succinate in the *Anacystis* membrane preparations.

Materials and Methods

Growth of the algae

Anacystis nidulans (strain 1402-1; Culture Collection of Algae, Göttingen, West Germany) was grown axenically at 25°C and 10 W/m² fluorescent light under H_2 supplemented with 5% of CO_2 . Details of the growth conditions were described previously (Peschek, 1978).

Preparation of respiratory particles

Freshly harvested cells were suspended in a solution containing 30 mM-Tris/Tricine {N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine} buffer, 2 mM- Na_2HPO_4 , 1 mM- $MgCl_2$ and 10 mM-KCl ('standard medium'; final pH adjusted to 7.5); 0.3 M-mannitol and 1 mM-dithiothreitol were added. Membrane preparations were obtained from this suspension (containing 0.5–2.0 mg of chlorophyll/ml) after lysozyme treatment followed by short sonication and final sedimentation at 144 000 g (r_{av} , 8 cm) for 3 h as previously described (Peschek, 1979a).

Analytical determination of the quinones

The lipids were extracted from freeze-dried batches of isolated membranes with acetone, transferred to light petroleum (b.p. 40–60°C) and finally identified by their spectral properties and behaviour on t.l.c. by the procedures described by Carr *et al.* (1967) (see also Henninger *et al.*, 1965). In several instances chlorophyll and polar carotenoids were separated from non-polar lipids by column chromatography on silicic acid followed by differential elution as described by Henninger *et al.* (1965); the chlorophyll-free and (largely) carotenoid-free crude lipid extract, including the quinones (referred to below as 'native quinones'), was evaporated to dryness, dissolved in n-pentane and immediately used in the reconstitution experiments described below. All the preparative and analytical steps necessary for extraction and identification of the quinones were done, as far as possible, under nitrogen and in dim light ($\lambda > 600$ nm) at 0–4°C, except the evaporation steps, which were carried out at 20°C *in vacuo* for 30–60 min; these precautions were taken so as to minimize possible modification *in situ* of the extrac-

ted quinones as a result of autoxidation and/or photo-oxidation.

U.v. inactivation of the particles

Membrane preparations suspended in standard medium containing 0.3 M-mannitol were stirred in a small beaker (open surface area about 9 cm²) at 4°C under O_2 -free N_2 ; they were then irradiated for 15 min from above with 360 nm light (apparent light-intensity at the surface of the suspension was approx. 35 W/m², as measured with a Yellow Springs Instruments model 65 Radiometer).

'Depleted' and 'reconstituted' particles

Isolated respiratory particles were depleted of quinones by n-pentane extraction after freeze-drying according to established procedures (Szarkowska, 1966; Ernster *et al.*, 1969). This procedure also removed practically all of the membrane-bound chlorophyll and carotenoids; these pigments, together with other lipids, could also effectively be removed by extracting the membranes with methanol or acetone. However, the latter solvents generally and irreversibly damaged the respiratory chain: despite a high quinone content (checked spectrophotometrically), electron-transport rates displayed by methanol- or acetone-extracted particles on quinone incorporation (see below) never exceeded 10% of the rates observed with pentane-extracted particles. For the reconstitution experiments the quinone-depleted particles were gently homogenized for 5 min with a Potter–Elvehjem homogenizer in 3–5 ml of n-pentane containing authentic quinones each at the concentration of 0.1 μ mol/mg of particle protein. Excess quinones were then removed by centrifugation (144 000 g, r_{av} , 8 cm, 1 h), followed by briefly rinsing the pellet with a small volume of pentane. The 'quinone-incorporated' particles were finally dried *in vacuo* at 20°C for 30–60 min. The following quinones were tested: phyloquinone, ubiquinone-10, L- α -tocopherolquinone (all purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.), menaquinone-7 (obtained from Hoffman–La Roche, Basle, Switzerland) and plastoquinone-9; the latter was freshly prepared either from *Anacystis nidulans* or from spinach chloroplasts by published procedures (Crane, 1959; Barr & Crane, 1972); plastoquinone from both sources showed identical behaviour in the assays described in the present paper. All steps of the procedures described (except the evaporation step) were carried out at 0–4°C in dim light ($\lambda > 600$ nm) under N_2 as far as possible in order to minimize possible photo-oxidative damage to membranes and/or lipids.

Respiratory electron-transport reactions

H_2 uptake was measured either manometrically or by conventional gas chromatography; O_2 uptake

was determined with a Clark-type oxygen electrode; oxidation of NAD(P)H was followed spectrophotometrically. Detailed descriptions of all these assays were given previously (Peschek, 1978, 1979a,b). The following final concentrations were used: 5 mM-NADPH, 5 mM-NADH, 15 mM-sodium succinate, 0.5 mM horse heart ferricytochrome *c*, 5 mM-sodium ascorbate, 0.1 mM-*NNN'**N'*-tetramethyl-*p*-phenylenediamine. For aerobic assays in the presence of H₂, the suspensions were sparged with 15% O₂ in H₂, corresponding to an initial H₂ concentration of 0.634 mM in the suspension. Temperature of measurements was 35°C throughout. O₂ was never limiting in the Oxygraph experiments (Figs. 2–5).

Steady-state reduction of incorporated quinones

The quinones were measured in a Perkin–Elmer model 200 spectrophotometer at the following wavelengths (see Krogmann & Olivero, 1962; Ames, 1964; Carr *et al.*, 1967): phyloquinone, 244 nm; plastoquinone, 255 nm; ubiquinone, 275 nm. The absorbance of quinone-incorporated particles incubated under assay conditions was measured, at the appropriate wavelength, against a reference cuvette containing the reconstituted membrane preparation oxidized by O₂ or ferricytochrome *c* in the absence of an electron donor; in this way a relative measure (ΔA) for the amount of substrate-reduced quinones was obtained. Thereafter the quinone-containing particles in the assay cuvette were reduced as described by Lester *et al.* (1964) by injecting a saturating amount (< 10 μ l) of concentrated acetate-buffered KBH₄ solution, and absorbance was read again ($\Delta A'$). $\Delta A'$ was taken to reflect full reduction of the quinone and the ΔA values were expressed as percentages of the corresponding $\Delta A'$ ('% reduction'; see Table 4).

Chlorophyll and protein

These were measured as described previously (Peschek, 1978). The protein/chlorophyll ratio of native particles was 10.

Controls

Controls in which either the electron donor or the acceptor was left out were always run in parallel with the assays. In several experiments particles heated at 90°C for 5 min served as additional controls (see Peschek, 1979a,b). Values given in the Figures and Tables are the means for three to five independent representative experiments each starting with a new batch of freshly harvested algae; maximal deviations of single determinations from the corresponding mean were between 10 and 20% of the mean for each given set of experiments.

Results and Discussion

Quinone content of the isolated membranes

Freshly prepared membranes equivalent to 70, 85 and 105 mg of chlorophyll were analysed for phyloquinone, 'polar naphthoquinone', menaquinone, plastoquinone and α -tocopherolquinone (Expts. 1–3, Table 1). The results essentially agree with earlier reports on the quinone content of whole cells of blue–green algae (Carr *et al.*, 1967; Henninger *et al.*, 1965). However, in our hands the so-called 'polar naphthoquinone' (see also Powls & Redfearn, 1969) appeared to show a significantly lower concentration, which also was less reproducible, than reported by Henninger *et al.* (1965) and Carr *et al.* (1967). Since in the present study extraction and identification of the quinones were performed under conditions where O₂ was rigorously excluded, and elevated temperatures and short-wave light (λ < 600 nm) were avoided, it is tempting to suggest that the 'polar naphthoquinone' might sometimes arise as an artifact of autoxidation and/or photo-oxidation of phyloquinone.

Reconstitution of respiratory electron-transport reactions in depleted and in u.v.-inactivated particles

Quinone-depleted (Figs. 1 and 2) or u.v.-inactivated (Table 2) particles were no longer able to catalyse efficient respiratory O₂ uptake supported by NAD(P)H, succinate or H₂, whereas O₂ uptake

Table 1. *Quinone content of isolated Anacystis membranes*

The membranes were prepared from three different batches of algae (Expts. 1–3) as described previously (Peschek, 1979a). Quinones were extracted and identified as described in the Materials and Methods section.

	Quinone content (nmol/mg of protein)		
	Expt. 1	Expt. 2	Expt. 3
Phyloquinone	1.5	1.9	1.2
'Polar naphthoquinone'	0.2	1.1	<0.05
Plastoquinone-9	30	32	28
Ubiquinone	<0.05	<0.05	<0.05
α -Tocopherolquinone	<0.05	<0.05	<0.05

Fig. 1. Activity of the NADPH- or H_2 -dependent ferricytochrome *c* reductase in untreated membranes (5), in 'depleted' membranes (1) and in 'depleted' membranes reconstituted with ubiquinone (2), plastoquinone (3) and phylloquinone (4)

Absorbance difference between sample and reference cuvette was recorded spectrophotometrically at 550 nm in anaerobic particle suspensions as described in the Materials and Methods section. Injection of particles is marked by the arrow. Electron-transport rates in untreated preparations corresponded to 28 (NADPH; 0.3 mg of protein/ml) and 31 (H_2 ; 0.2 mg of protein/ml) nmol of ferricytochrome *c* reduced/min per mg of protein respectively.

Fig. 2. Effects of NADPH, *NNN'N'*-tetramethyl-*p*-phenylenediamine and KCN on O_2 uptake in 'depleted' particles

The polarographic recorder trace was taken under the conditions described in the Materials and Methods section. Numbers below the curve give O_2 -uptake rates expressed as nmol/min per mg of protein. Abbreviation: TMPD, *NNN'N'*-tetramethyl-*p*-phenylenediamine.

dependent on *NNN'N'*-tetramethyl-*p*-phenylenediamine remained almost unaffected. In addition, quinone-depleted particles were deficient in the anaerobic reduction of ferricytochrome *c* by NADPH or H_2 . When certain authentic quinones were incorporated into the depleted or inactivated particles, electron-transport competence was regained (Fig. 1 and

Table 2). Restoration efficiencies depended on the quinone employed: phylloquinone (vitamin K_1) permitted electron-transport rates almost equal to those observed in untreated membranes, followed by plastoquinone and, with rather low activity, ubiquinone. The efficiency pattern of the quinones was the same in depleted and in u.v.-inactivated particles. Inactivation by near-u.v. irradiation mimics pentane extraction in that the quinones contained within biological material have been shown to be selectively damaged by u.v. light (Kashket & Brodie, 1962; see Pflitzner, 1972). In addition, u.v. light apparently affected hydrogenase activity directly (Table 2; Peschek, 1979*a,b*). Fig. 1 shows the effect of the quinones in restoring the anaerobic respiratory electron transport from NADPH and H_2 to ferricytochrome *c*. Similar results were obtained with NADH or succinate as electron donors (results not shown). The same order of efficiency of the quinones was found when the substrate-dependent O_2 uptake by 'incorporated' (i.e. reconstituted) particles was measured (results not shown).

The effect of inhibitors on aerobic electron-transport reactions in phylloquinone-reconstituted particles is shown in Fig. 3. Both qualitatively and quantitatively the response of the 'incorporated' particles towards inhibitors and artificial electron donors or acceptors compared well with that of native particles (Peschek, 1980). The pronounced differential effect of ubiquinone, plastoquinone and phylloquinone (Fig. 1 and Table 2) indicated that specific requirements for the functional interaction of incorporated quinones with the electron-transport system had to be fulfilled. This agreed with the result that incorporation of 'native quinones' previously

Table 2. Restoration of respiratory O_2 uptake in u.v.-irradiated particles

The particles were irradiated with 360nm light and then subjected to incorporation treatment with the quinones as described in the Materials and Methods section. O_2 -uptake rates are expressed as nmol/min per mg of protein. Abbreviation: TMPD, *NNN'*-tetramethyl-*p*-phenylenediamine.

Electron donor	Untreated particles	U.v.-irradiated particles re-activated with			
		No quinone	Phylloquinone	Plastoquinone	Ubiquinone
NADPH	15.0	1.8	10.0	4.8	2.0
Succinate	2.7	0.4	1.6	0.8	0.5
H_2	27.5	2.1	2.9	2.5	2.1
TMPD + ascorbate	72.3	70.0	71.9	70.5	72.0

Fig. 3. Aerobic NADPH oxidation (a) and H_2 -dependent O_2 uptake (b) in phylloquinone-reconstituted particles. The spectrophotometric (a) or polarographic (b) recorder traces were taken in suspensions saturated with 5% O_2 in N_2 (a) and H_2 (b) respectively, as described previously (Peschek, 1979a,c). For other details see the Materials and Methods section. Numbers below the curves give rates of NADPH oxidation (a) or of O_2 uptake (b) expressed as nmol/min per mg of protein. Abbreviations: HOQNO, 2-n-heptyl-8-hydroxyquinoline *N*-oxide; TMPD, *NNN'*-tetramethyl-*p*-phenylenediamine.

extracted from *Anacystis* (see the Materials and Methods section) and of authentic menaquinone-7 into the depleted particles yielded electron-transport rates of 95 and 60% respectively of the rates observed with phylloquinone-reconstituted particles. Particles reconstituted with authentic α -tocopherol-quinone were found to be virtually inactive (results not shown). The slightly smaller effect of the 'native quinones' when compared with authentic phylloquinone may have been due to some inactivation during the isolation procedure.

Phylloquinone reconstitution not only resulted in respiratory electron-transport patterns almost indistinguishable from those of untreated membranes but also permitted restoration of the energy-dependent reversed electron flow. Table 3 gives results from experiments with two independently prepared batches of particles that had been shown to display respiratory control with NADPH and H_2 as sub-

strates, indicating their capacity for catalysing oxidative phosphorylation (G. A. Peschek, unpublished work). In both native and reconstituted particles ATP brought about reversed electron flow from *NNN'*-tetramethyl-*p*-phenylenediamine to $NADP^+$ (yielding NADPH) and to H^+ (yielding molecular H_2), and this energy-linked electron transport was inhibited by low concentrations of the inhibitor *NN'*-dicyclohexylcarbodi-imide, which is supposed to affect the proton-translocating ATPase.

Steady-state reduction of the incorporated quinones

Table 4 shows the percentages of steady-state reduction (full reduction = 100%) of phylloquinone, plastoquinone and ubiquinone incorporated into 'depleted' particles by using NADPH, succinate or H_2 as electron donors and oxygen ('aerobic') or ferricytochrome *c* as electron acceptors, or without

Table 3. *Reconstitution of energy-linked reversed electron flow from NNN'N'-tetramethyl-p-phenylenediamine + ascorbate to NADP⁺ and to hydrogenase in 'depleted' particles*

The particles were incubated anaerobically in 'standard medium' including 0.3 M-mannitol in the presence of 0.1 mM-NNN'N'-tetramethyl-p-phenylenediamine + 5 mM-ascorbate, and of 3 mM-NADP⁺ in the NADPH assay. The reactions were started by injecting 3 mM-ATP together with 2 mM-KH₂PO₄ and 0.5 mM-MgCl₂ (adjusted to pH 7.5 with 1 M-NaOH), and formation of NADPH or of H₂ was measured as described previously (Peschek, 1979a). NN'-Dicyclohexylcarbodi-imide was added at a final concentration of 50 μM. Identity of H₂ was confirmed by conventional gas chromatography and by adsorption on activated palladium (see Peschek, 1979a). No formation of NADPH or of H₂ was detected in appropriate controls devoid of NNN'N'-tetramethyl-p-phenylenediamine. For further experimental details see the Materials and Methods section. Results, expressed as nmol of NADPH or H₂ formed/min per mg of membrane protein, are given in the Table for particles isolated from two different algal batches (Expts. 1 and 2). Abbreviation: DCCD, NN'-dicyclohexylcarbodi-imide.

Conditions	NADPH formed (nmol/min per mg)		H ₂ formed (nmol/min per mg)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Untreated membranes	0.08	0.02	0*	0*
+ ATP†	2.8	2.0	0.5	1.0
+ ATP† + DCCD	0.2	0.1	0*	0*
'Depleted' membranes	0*	0*	0*	0*
+ ATP†	0*	0*	0*	0*
Phylloquinone-reconstituted membranes	0*	0*	0*	0*
+ ATP†	1.9	1.2	0.3	0.3
+ ATP† + DCCD	0.1	0.1	0*	0*

* Rates lower than 0.001 nmol of NADPH or 0.05 nmol of H₂/min per mg of protein.

† ATP + P_i + Mg²⁺ (see the legend).

Table 4. *Steady-state reduction of quinones incorporated into 'depleted' particles*

Experimental procedures were as described in the Materials and Methods section. Absorbances read after addition of KBH₄ were taken as '100% reduction', and the absorbances read in the same cuvettes in the absence of KBH₄ were converted into percentages of these maximum values (given as '% reduction' in the Table). KCN (0.5 mM), chloroquine (1.0 mM) and thenoyltrifluoroacetone (5.0 mM) were added before, and ferricytochrome *c* (0.5 mM) after, the electron donors. Abbreviation: TTFA, thenoyltrifluoroacetone.

Conditions	Reduction of quinone (%)		
	Phylloquinone	Plastoquinone	Ubiquinone
No substrate (anaerobic)	3	2	0
H ₂ , NADPH or succinate, + KBH ₄	100	100	100
H ₂ (anaerobic)	83	32	15
H ₂ (aerobic)	22	18	10
H ₂ (aerobic) + KCN	27	28	15
H ₂ (aerobic) + KCN + ferricytochrome <i>c</i>	19	21	6
H ₂ (anaerobic) + ferricytochrome <i>c</i>	58	24	12
NADPH (anaerobic)	96	38	14
NADPH (anaerobic) + chloroquine	19	7	3
NADPH (anaerobic) + ferricytochrome <i>c</i>	72	24	6
NADPH (aerobic)	24	21	11
NADPH (aerobic) + KCN	30	34	15
Succinate (anaerobic)	75	29	12
Succinate (anaerobic) + TTFA	6	2	1
Succinate (aerobic)	20	16	8
Succinate (aerobic) + KCN	28	14	12

acceptor ('anaerobic'). Highest degrees of reduction were obtained in acceptor-free samples incubated anaerobically. Under otherwise identical conditions phylloquinone always showed the highest, and ubi-

quinone showed the smallest, degree of reduction, plastoquinone being intermediate, thus paralleling the differential efficiencies of the quinones in restoring the respiratory electron transport (see Fig.

1). Aerobic conditions, as well as ferricytochrome *c* in anaerobiosis, effectively decreased the amount of quinone reduced, indicating that electrons were drained from the quinone towards O_2 and ferricytochrome *c*. Addition of cyanide to aerobic particles led to enhanced quinone reduction, though the value under anaerobic conditions was not reached for plastoquinone and, especially, phyloquinone (Table 4); this suggested that the incorporated quinones remained accessible, in part, to O_2 , which thereby directly re-oxidized some of the substrate-reduced plastoquinone or phyloquinone. The extent of anaerobic quinone reduction in the presence of NADPH and succinate was greatly diminished if the reconstituted particles had previously been treated with chloroquine or thenoyltrifluoroacetone respectively (Table 4). In parallel with this finding, it is shown below (see Fig. 5) that chloroquine and thenoyltrifluoroacetone also inhibited the rate of reduction and, hence, autoxidation of menadione in depleted particles incubated with NADPH and succinate respectively.

Effect of low-molecular-weight quinone analogues

Several low-potential quinones devoid of sufficiently long aliphatic side chains, e.g. menadione (vitamin K_3 ; 2,3-dimethyl-1,4-naphthoquinone), dibromothymoquinone (2,5-dibromo-6-isopropyl-3-methyl-*p*-benzoquinone) and juglone (5-hydroxy-1,4-naphthoquinone), were found to be unable to restore the anaerobic NADPH- or succinate-ferricytochrome *c* oxidoreductase reaction in depleted membranes (results not shown). Therefore, quite apart from thermodynamical considerations and from different specificities of the quinones towards

their immediate reactants in the respiratory chain, the C_{20} , C_{45} and C_{35} aliphatic residues of phyloquinone, plastoquinone and menaquinone respectively appear to be a prerequisite for the restoration of the complete respiratory electron flow in depleted particles. A similar phenomenon was noted with plastoquinones of different side-chain length incorporated into heptane-extracted chloroplasts in order to reactivate photophosphorylation and Hill reactions (Krogmann & Olivero, 1962).

However, rapid oxidation of NAD(P)H, succinate and H_2 by menadione, dibromothymoquinone or juglone was observed in the depleted membranes (results not shown); in aerobic conditions the quinones thus reduced were immediately re-oxidized by O_2 , owing to the strongly negative redox potential of the quinones (Fig. 4; only results with NADPH and H_2 shown). However, the reduced quinones could not donate electrons to the terminal section of the respiratory chain. The differential effects of juglone in NADPH and H_2 oxidation respectively indicated that menadione and dibromothymoquinone were capable of accepting electrons either from the flavoproteins involved in the dehydrogenation of NAD(P)H or succinate (Fig. 4a) or directly from the hydrogenase (Fig. 4b; see Peschek, 1979a,b), as flavoproteins were shown not to be involved in the respiratory H_2 oxidation (Peschek, 1980); juglone apparently could not accept electrons directly from the hydrogenase (Fig. 4b).

The data in Fig. 5 show that the fast O_2 uptake due to autoxidation of substrate-reduced menadione was further enhanced by 2-n-heptyl-8-hydroxyquinoline *N*-oxide or KCN. Also, the reduction of

Fig. 4. O_2 uptake due to autoxidation of menadione (1), juglone (2) and dibromothymoquinone (3) reduced by NADPH or H_2 in untreated particles

Numbers below the curves give rates of O_2 uptake expressed as nmol/min per mg of protein. Final protein concentration was 1.3 and 0.6 mg/ml with NADPH and H_2 respectively. For experimental details see the Materials and Methods section. Similar effects were found with 'depleted' or phyloquinone-reconstituted particles and with NADH or succinate as electron donors (results not shown).

Fig. 5. Patterns of (a) NADPH- and (b) succinate-dependent O_2 uptake in phylloquinone-reconstituted particles. Polarographic recorder traces were taken from suspensions previously equilibrated with 5% O_2 in N_2 , so as to minimize autooxidation of the phylloquinone incorporated into the 'depleted' membranes. Numbers below the curves indicate O_2 -uptake rates expressed as nmol/min per mg of protein. Experimental details are given in the Materials and Methods section; see also the text. Abbreviations: HOQNO, 2-n-heptyl-8-hydroxyquinoline *N*-oxide; TTFA, thenoyltrifluoroacetone.

menadione by NADPH and succinate was impaired by chloroquine and thenoyltrifluoroacetone respectively. As with mitochondria (see Crane, 1977), in *Anacystis*, chloroquine and thenoyltrifluoroacetone appear to affect reduction of the respiratory quinone by NAD(P)H and succinate respectively; 2-n-heptyl-8-hydroxyquinoline *N*-oxide and KCN, on the other hand, by inhibiting the terminal segments of the respiratory chain, promote reduction of the quinone. This view is fully consistent with the experiments on the steady-state reduction of incorporated quinones (Table 4).

Concluding remarks

Both preparative and functional evidence discussed in the previous section suggest phylloquinone to be the main respiratory quinone of *Anacystis nidulans*. The strongest argument in favour of this came from extraction-reconstitution experiments, in which phylloquinone gave by far the largest effect. In all such experiments carried out so far the naturally occurring quinones yielded the best results (see Crane, 1977); therefore it appears justified to rule out purely non-specific interaction for *Anacystis* also. A respiratory function of phylloquinone was previously invoked for *Anabaena variabilis*, evidence being derived from kinetic measurements of respiratory electron-transport reactions (Leach & Carr, 1970; see Carr, 1973).

Besides phylloquinone, plastoquinone also showed significant activity in the reconstitution experiments.

For comparison, the non-specific effect of plastoquinone as a substitute for ubiquinone-10 in iso-octane-extracted bovine heart succinate oxidase preparations (Crane, 1959) was much smaller. Thus the intriguing possibility remains that in *Anacystis* plastoquinone, apart from its role in photosynthesis (Krogmann, 1973), might also fulfil respiratory functions *in vivo*. However, it would be premature to regard plastoquinone as the 'common link' between photosynthesis and respiration in blue-green algae (Eisbrenner & Bothe, 1979; see also Jones & Myers, 1963; Peschek & Schmetterer, 1978; Peschek, 1980).

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