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QUINONES AS MEDIATORS OF BOTH ARTIFICIAL AND CYCLIC PHOSPHORYLATION IN SPINACH CHLOROPLASTS

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Summary

A new method to prereduce mediators catalyzing cyclic electron transfer in washed, spinach thylakoid membranes was developed. Hydrophilic and lipophilic quinones were tested for their ability to catalyze phosphorylation in both cyclic electron transfer and electron transfer in an artificial transmembrane redox reaction. Quinones varied widely in their ability to catalyze cyclic photophosphorylation, but cyclic phosphorylation in all cases was inhibited by the plastoquinone antagonist dibromothymoquinone. Many of the quinones also catalyzed transmembrane electron transfer to ferricyanide trapped internally within the thylakoid vesicles. In this system, phosphorylation catalyzed by hydrophilic quinones was inhibited by dibromothymoquinone, whereas phosphorylation catalyzed by lipophilic quinones was dibromothymoquinone-insensitive. This is taken as evidence that transmembrane electron transfer catalyzed by hydrophilic quinones is mediated by the endogenous plastoquinone pool within the thylakoid membrane.

Introduction

Several segments of the native electron transfer chain in chloroplast thylakoid membranes have been localized and studied (for review, see Ref. [1]). This has chiefly been accomplished by judiciously employing various electron donors, acceptors and electron transfer inhibitors in such a way that only a

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Abbreviations: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethyl urea; Chl, chlorophyll.

selected portion of the electron transfer chain is operative. Several artificial electron transfer mediators have been employed to catalyze cyclic electron transfer and photophosphorylation [2—4]. Phenazine methosulfate, for example, is one such compound that catalyzes cyclic phosphorylation, using only a very short sequence of the chloroplast electron transfer chain containing Photosystem I [5]. Ferredoxin, on the other hand, catalyses in vivo cyclic electron transfer and phosphorylation using a larger segment of the electron transfer chain, including dibromothymoquinone and Antimycin A sensitive sites [6—8]. Both mediators, however, are presumed to function in ATP synthesis by catalyzing the simultaneous transmembrane transport of both electrons and protons [3]. This in turn produces a proton gradient, which is the driving force of phosphorylation [9].

Several methods have been developed to separate ATP synthesis from electron transfer in thylakoids. This is necessary in order to study the mechanism of ATP synthesis without the complicating effects of other thylakoid reactions. The first such method to demonstrate ATP synthesis in the complete absence of light was developed by Uribe and Jagendorf [10]. 'Acid-base phosphorylation' was instrumental in showing that a proton gradient is probably an intermediate in energy transduction in thylakoids. Using an artificially induced transmembrane electrical potential, Witt et al. [11] have been able to demonstrate the net synthesis of ATP in the absence of an appreciable transmembrane pH gradient. This technique, however, does not produce significant amounts of ATP synthesis.

Selman and Ort [12] recently developed a system that drives the synthesis of ATP in thylakoid vesicles and functions without the input of light. It derives its energy from an artificial transmembrane redox reaction. Chloroplasts are first incubated in a ferricyanide containing buffer, which causes the oxidant to be trapped internally. The chloroplast vesicles are then transferred to a medium containing an excess of the membrane-impermeable reductant, ascorbate [13]. When membrane-permeable mediators are included that in their reduced form can transfer both electrons and protons, the subsequent reduction of entrapped ferricyanide is accompanied by the liberation of protons. The proton gradient thus produced causes a short burst of ATP synthesis, which ends when the oxidant is completely reduced [12].

We have attempted to develop transmembrane electron transfer reactions which utilize the native electron transfer chain as a mediator, rather than the artificial mediators previously employed. Such reactions would be very useful in studying artificial liposomal systems into which chloroplast components have been incorporated. In this paper, several low potential mediators are tested for their ability to stimulate both phosphorylation in unilluminated thylakoid vesicles containing entrapped ferricyanide and cyclic photophosphorylation. The involvement of plastoquinone in shuttling both reducing equivalents and protons from hydrophilic external mediators to internally trapped ferricyanide is demonstrated. This function is similar to the function of plastoquinone in cyclic photophosphorylation.

Materials and Methods

Chloroplasts. Chloroplasts were isolated from spinach leaves and suspended in 0.3 M sucrose, 20 mM Tricine-KOH (pH 8.0), 10 mM KCl, and 5 mM $MgCl_2$ as previously described [14]. This preparation was used without further treatment when assaying cyclic phosphorylation. Alternatively, ferricyanide was entrapped within the thylakoids by resuspending chloroplast pellets (containing approx. 5 mg chlorophyll) in 5 ml of 0.1 M sucrose, 90 mM potassium ferricyanide, 10 mM potassium ferrocyanide, 10 mM Tricine-KOH (pH 8.0), 10 mM KCl, 5 mM $MgCl_2$, 2 mg/ml bovine serum albumin and 20 μm (DCMU). This was followed by diluting the suspension into 100 ml of the same buffer without added sucrose [11]. After 15 min incubation in the dark to allow the ferricyanide to gradually permeate into the vesicles, the chloroplasts were centrifuged at $4000 \times g$ for 5 min and the pellet resuspended in a small volume of the supernatant containing ferricyanide, thus preventing a ferricyanide diffusion potential across the vesicle membrane. Chlorophyll concentration was determined by the method of Arnon [15].

Cyclic phosphorylation. Reaction mixtures for cyclic phosphorylation contained 20 mM Tricine-KOH (pH 8.0), 10 mM KCl, 5 mM $MgCl_2$, 2 mM ADP, 2 mM [^{32}P]phosphate (containing approximately $1 \cdot 10^7$ cpm/ml), 10 mM glucose, 10 μM DCMU, 0.1 unit/ml hexokinase, 0.25 mg/ml bovine serum albumin, and 150 μM of the appropriate mediator. 1.5 ml of this solution was added to a 9 ml serum vial, stoppered, and depleted of oxygen by swirling during alternating cycles of gas evacuation followed by gassing with nitrogen. Chloroplasts were depleted of oxygen separately. A small volume of a dithionite solution was then added to provide the desired redox poise. Reaction mixtures containing 10 μg of chlorophyll per 1.5 ml were placed in a 20°C water bath and illuminated from 10 cm below by a 400 W high pressure sodium vapor lamp (output = 43 000 lumens). After 5.0 min illumination, reactions were stopped by the addition of 0.2 ml of 1 M $HClO_4$. [γ-^{32}P]ATP was measured as previously described [16].

Redox-induced phosphorylation. Reaction mixtures for measuring the phosphorylation arising from the reduction of internally entrapped ferricyanide contained 60 mM Tricine-KOH (pH 8.2), 10 mM KCl, 5 mM $MgCl_2$, 2 mM ADP, 2 mM [^{32}P]phosphate (containing $1 \cdot 10^7$ cpm/ml), 10 mM glucose, 0.1 units/ml hexokinase, 10 mM sodium ascorbate (to reduce external ferricyanide), and 150 μM of the appropriate mediator. 1.0 ml of this mixture was depleted of oxygen as described above and approx. 75 μl of chloroplasts containing 0.2 to 0.25 mg of chlorophyll were added in dim light and mixed well. After 15 s, 20 μl (0.75 mM) of sodium dithionite were added and the mixture was incubated for an additional 45 s. Reactions were stopped by the addition of 0.2 ml of 1 M $HClO_4$. ATP formation was measured as described above. All conditions to stoppered vials were made with gas-tight syringes.

Mediators. Ferredoxin was prepared as described by Petering and Palmer [17] as previously modified [18]. Sulfonated mediators were recrystallized from diluted alcohol or water, and phenylene diamines were recrystallized from concentrated HCl. 1,4- and 1,2-naphthoquinone were purified by sublimation, and all other mediators were recrystallized from alcochol. The isomeric purity

and concentration of 1,4- and 1,2-naphthoquinone and menadione were confimed by taking their ultraviolet spectra in an Aminco DW-2 spectrophotometer and comparing this data to published values [19,20].

Miscellaneous. Partition coefficients (*P*) for the dithionite-reduced mediators were determined essentially as described by Hauska [21] and are expressed as the ratio of the remaining absorbance at the ultraviolet absorption peak to the lost absorbance after extraction with chloroform.

Results

Cyclic phosphorylation catalyzed by various mediators

For a redox mediator to catalyze cyclic phosphorylation efficiently, there must be an optimal balance between the reduced and oxidized forms of the mediator, i.e., the mediator must be redox poised [5]. In these experiments with relatively low potential mediators (+100 to −400 mV), this poising was provided by varying the concentration of added dithionite. Under anaerobic conditions, which prevent oxidation of the mediators by oxygen, the redox poise of the mediators can be adjusted to the optimal potential for the given mediators.

Both Fig. 1 and Table I show that the optimum redox pose for cyclic phosphorylation varies with the mediator. Whereas the optimal rate for cyclic phosphorylation with anthraquinone-2-sulfonate is obtained when the mediator is fully reduced, the optimal rates with menadione and 1,2-naphthoquinone are obtained at 50% and 25% reduction, respectively. Most mediators become more efficient in catalyzing phosphorylation as they become fully reduced; however, after the titer of dithionite surpasses that of the mediator, the phosphorylation of ADP is strongly inhibited, probably due to the reduction of the Photosystem I acceptor [22]. The small amount of bisulfite produced by

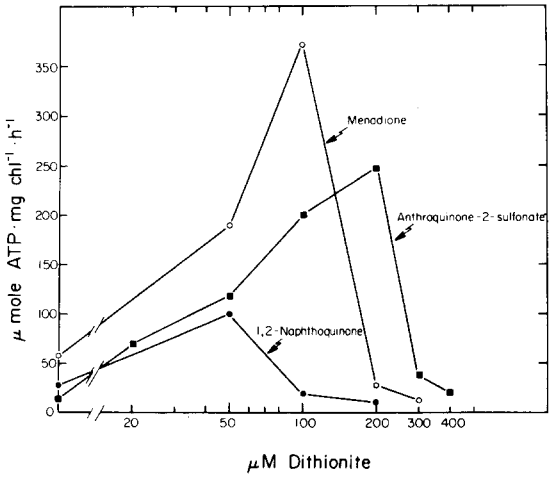

Fig. 1. Redox poising of quinone-catalyzed cyclic photophosphorylation by dithionite. The quinone mediators are present at a concentration of 200 μM. Anaerobic reaction mixtures were titrated with dithionite and assayed for phosphorylation as described in Methods.

TABLE I

RATES OF CYCLIC PHOSPHORYLATION AND YIELDS OF REDOX-INDUCED PHOSPHORYLATION CATALYZED BY VARIOUS MEDIATORS

Rates of cyclic phosphorylation were measured in each case under conditions of optimal redox poise. The fractional reduction of the mediator required for optimal poise was estimated from the ratio of dithionite to mediator for quinones, and two times this ratio for the monoequivalent ferredoxin. Conditions are as described in Methods. *P*, partition coefficient as described in Methods; N.D., not determined; DBMIB, dibromothymoquinone.

Mediator (150 μM)	*P* (H_2O/$CHCl_3$)	Optimal fractional reduction	Cyclic photophosphorylation (μmol ATP/mg Chl/h)		Redox-induced phosphorylation (nmol ATP/mg Chl)	
			control rate	+6.7 μM DBMIB rate	control yield	+18 μM DBMIB yield
Phenanthrenequinone	0.029	1.0	250	29	85	62
Anthraquinone [a]	0.028	0.25	85	1.8	27	27
Anthraquinone-2-sulfonate	10.7	1.0	280	19	78	22
Anthraquinone-1,8-disulfonate	16.4	1.0	44	1.2	64	17
Anthraquinone-1,5-disulfonate	1.8	1.0	13	1.1	16	14
Anthraquinone-2,6-disulfonate	7.3	1.0	30	1.2	83	16
Anthraquinone-2,7-disulfonate	5.0	1.0	64	1.1	93	7.0
1,4-Naphthoquinone	0.20	0.40	190	5.1	42	26
1,4-Naphthoquinone-2-sulfonate	3.4	N.D.	13	2.9	11	10
1,2-Naphthoquinone	0.18	0.25	42	5.1	52	54
1,2-Naphthoquinone-4-sulfonate	1.5	N.D.	16	3.2	14	16
Menadione	0.062	0.50	490	11	88	70
Menadione, bisulphite salt	3.1	0.20	81	4.1	45	15
Phenylene diamine	N.D.	N.D.	10	N.D.	90	86
Phenylene diamine-2-sulfonate	N.D.	N.D.	8	N.D.	12	10
N,N,N',N'-Tetramethyl-phenylene diamine	N.D.	N.D.	4	N.D.	10	15
N-Methylphenazonium methosulfate	N.D.	N.D.	610	565	42	44
Ferredoxin	N.D.	1.0	295	5.4	8.8	7.6
Potassium ferricyanide	N.D.	N.D.	3.0	N.D.	N.D.	N.D.
No added mediator, no dithionite			16 ± 7	N.D.	12 ± 7	N.D.
No added mediator, 150 μM dithionite			13 ± 4	3.9 ± 2	11 ± 7	13 ± 6

[a] Anthraquinone concentration was limited to 30 μM due to its low solubility in aqueous solutions.

the oxidation of dithionite had no discernable effect on the rate of phosphorylation (data not shown).

Table I compares the ability of various non-polar mediators, and their polar sulfonated derivatives, to catalyze cyclic phosphorylation when the system is optimally redox poised. It is apparent that both polar and nonpolar mediators can catalyze cyclic phosphorylation, as previously noted [3], although the highest rates are obtained with nonpolar mediators. The plastoquinone antagonist dibromothymoquinone [2,23,24] inhibits cyclic phosphorylation catalyzed by all of the mediators tested, with the exception of phenazine methosulfate.

Of the nonpolar tricyclic mediators tested, phenanthrenequinone catalyzes

the highest rate of phosphorylation. The rate of phosphorylation with anthraquinone is probably limited by its marginal solubility in aqueous solution. Anthraquinone-2-sulfonate is as effective as phenanthrenequinone, but the disulfonated anthraquinone derivatives are much less efficient.

The unsulfonated, lipophilic naphthoquinones vary widely in their ability to catalyze cyclic phosphorylation, the highest rate being obtained with menadione. Their sulfonated derivatives, on the other hand, are very poor catalysts of cyclic phosphorylation. Experiments with sulfonated naphthoquinones require caution. An increase in the phosphorylation rate catalyzed by these compounds occurs when the reduced forms are allowed to preincubate in the reaction mixtures for one hour. This is accompanied by a concurrent decrease in the ultraviolet absorption peak of the sulfonate and probably indicates the decomposition of the compounds.

All phenylene diamines tested do not catalyze cyclic phosphorylation, regardless of whether they are reductively titrated with dithionite, or oxidatively titrated with ferricyanide. (It should be noted that ferricyanide stimulates negligible amounts of phosphorylation due to the presence of the electron transfer inhibitor DCMU.)

Ferredoxin catalyzes cyclic phosphorylation at an appreciable rate. In addition to being sensitive to dibromothymoquinone, this phosphorylation is inhibited by Antimycin A (Table II) as previously observed [2,7,8,25,26]. In contrast, anthraquinone-2-sulfonate-catalyzed cyclic phosphorylation is relatively insensitive to Antimycin A, but is inhibited by the uncoupler NH_4Cl. In addition, cyclic phosphorylation catalyzed by all of the other mediators is insensitive to Antimycin A. The dinitrophenyl ether of iodonitrothymol partially inhibits cyclic phosphorylation mediated by ferredoxin and anthraquinone-2-sulfonate to 53% and 67%, respectively, of the uninhibited rates.

TABLE II

RATES OF CYCLIC PHOSPHORYLATION MEDIATED BY ANTHRAQUINONE-2-SULFONATE AND FERREDOXIN

Mediators have been reduced to optimal levels, and are present at a concentration of 150 μM. Other conditions are as described in Methods. DBMIB, dibromothymoquinone; DNP-INT, dinitrophenyl ether of iodonitrothymol.

	Rate (μmol ATP/mg Chl/h)
Anthraquinone-2-sulfonate	294 ± 3
+6.5 μM DBMIB	2.2 ± 1
+5 mM NH_4Cl	19 ± 2
+1.9 μM Antimycin A	262 ± 32
+3.9 μM DNP-INT	198 ± 10
—ADP	2.5 ± 1
Ferredoxin	178 ± 1
+6.5 μM DBMIB	7.5 ± 1
+1.9 μM Antimycin A	58 ± 0
+3.9 μM DNP-INT	94 ± 7
—ADP	5.4 ± 1

Redox-induced phosphorylation

These same electron transfer mediators were tested for their ability to stimulate the transfer of both electrons and protons across the unilluminated thylakoid membrane to internally trapped ferricyanide [12]. Protons liberated in this redox reaction drive the synthesis of ATP. All of the anthraquinones tested stimulate redox phosphorylation in the dark, including three anthraquinone disulfonate derivatives which are poor catalysts for cyclic photophosphorylation (Table I). The lipophilic naphthoquinones and phenylene diamine also stimulate appreciable amounts of phosphorylation, whereas their sulfonated derivatives are ineffective. Phenazine methosulfate stimulates this reaction, as would be expected from its ability to shuttle protons [21]. In contrast, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine, which is able to transport electrons but not protons, yields no phosphorylation, as described [12]. Under these conditions, ferredoxin is also ineffective in stimulating redox induced phosphorylation. Dithionite by itself stimulates dark phosphorylation only negligibly above background.

Table I shows the sensitivity of redox induced phosphorylation to inhibition by dibromothymoquinone. The phosphorylation stimulated by all of the lipophilic, unsulfonated compounds is insensitive to dibromothymoquinone with the exception of 1,4-naphthoquinone, which is partially inhibited. In contrast, the phosphorylation catalyzed by all of the hydrophilic, sulfonated derivatives which have appreciable yields of phosphorylation is inhibited to a large extent by dibromothymoquinone. Fig. 2 shows dibromothymoquinone titration curves for four of the mediators. The two lipophilic mediators, menadione and phenanthrene-quinone, are completely insensitive to dibromothymoquinone up to a concentration of 40 μM. Phosphorylation mediated by anthraquinone-2,6-disulfonate and anthraquinone-2,7-disulfonate is inhibited 50% at about 1 μM dibromothymoquinone.

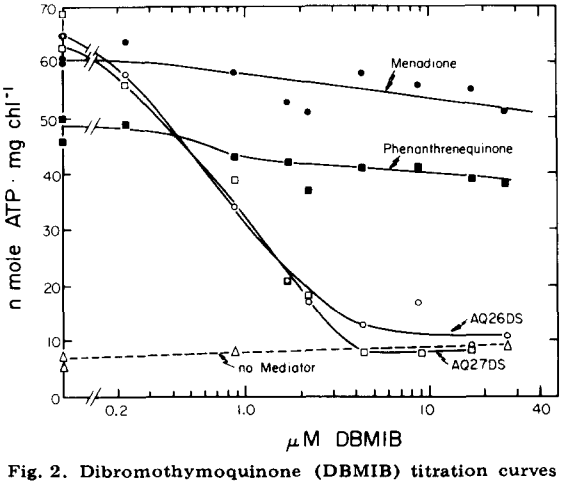

Fig. 2. Dibromothymoquinone (DBMIB) titration curves for redox-induced phosphorylation catalyzed by lipophilic or hydrophilic quinones. The quinone mediators were present at a concentration of 150 μM and the dithionite concentration was 750 μM. Ferricyanide was entrapped within thylakoids and redox-induced phosphorylation was assayed as described in Methods. AQ26DS, anthraquinone-2,6-disulfonate; AQ27DS, anthraquinone-2,7-disulfonate.

TABLE III

YIELDS OF PHOSPHORYLATION IN THYLAKOID VESICLES CONTAINING ENTRAPPED FERRICYANIDE MEDIATED BY ANTHRAQUINONE-2,7-DISULFONATE (150 μM)

Other conditions as described in Methods. DBMIB, dibromothymoquinone; DNP-INT, dinitrophenyl ether of iodonitrothymol.

	Yield nmol (ATP/mg Chl)
Anthraquinone-2,7-disulfonate	92 ± 2
+9.2 μM DBMIB	21 ± 3
+2.8 μM Antimycin A	101 ± 3
+5 mM NH_4Cl	18 ± 1
+4.6 μM DNP-INT	69 ± 1
no added dithionite	18 ± 1
no added ADP	14 ± 1

Redox-induced phosphorylation stimulated by a representative hydrophilic mediator, anthraquinone-2,7-disulfonate, was also tested for sensitivity to compounds that inhibit electron transfer or phosphorylation. Table III shows that this phosphorylation is uncoupled by NH_4Cl, insensitive to Antimycin A, and slightly sensitive to the dinitrophenyl ether of iodonitrothymol.

Discussion

Cyclic phosphorylation in chloroplasts often requires an optimal balance between the reduced and oxidized forms of the cyclic cofactor or mediator, i.e., proper redox pose [5]. In order to obtain this balance, previous workers have used a variety of methods to prereduce the mediators, including: (i) preillumination of the mediators in the presence of chloroplasts before the addition of DCMU and phosphorylation substrates [3], (ii) photoreduction of the mediator before the addition of chloroplasts [4], and (iii) the presence of a mild reductant, usually ascorbate, which can reduce the mediator without overreducing the Photosystem I acceptor [27]. Crowther et al. [28] have poised intact chloroplasts with 10 mM dithionite. Over-reduction of the system is prevented presumably by the hindered diffusion of dithionite across the chloroplast envelope. Cyclic phosphorylation mediated by anthraquinones and naphthoquinones ($E_{mp} < 0$ volts) requires redox poising with a reductant of lower potential than ascorbate and whose concentration can be adjusted so that an optimum oxidized/reduced ratio of the mediator can be achieved. Therefore, a different method was chosen to prereduce the mediators, involving the anaerobic titration of the mediators with dithionite. For most of the mediators, including all of the anthraquinone sulfonates, this optimal level is the fully reduced form, whereas the unsubstituted naphthoquinones, menadione and anthraquinone have optimal rates with only 25—50% of the compound in the reduced form. This partial reduction is difficult to achieve reproducibly with the prereduction methods used previously, and probably explains the discrepancy in the rates obtained by Hauska et al. [3] and those reported here.

When these low potential quinones are used as cofactors for cyclic phosphorylation, the rates of ATP synthesis vary widely. In general, however, the lipophilic quinones are better cofactors for cyclic phosphorylation. This trend

has also been observed by Hauska [21] for the phenazonium derivatives.

All cyclic phosphorylation mediated by these quinones or ferredoxin is sensitive to inhibition by dibromothymoquinone [2,23,24]. This indicates that either (i) the quinones are oxidized at the same dibromothymoquinone-sensitive site that plastoquinone is oxidized at, or (ii) plastoquinone is directly involved in the electron/proton transfer reactions. In the case of the hydrophilic sulfonated anthraquinones, the latter explanation is certainly the more tenable due to the difficulty these compounds would have entering or crossing the thylakoid membrane. However, only in the case of ferredoxin catalyzed cyclic phosphorylation, which is also inhibited by Antimycin A, can we conclusively invoke plastoquinone involvement, in that ferredoxin is too large and polar to enter the membrane.

The dinitrophenyl ether of iodonitrothymol partially inhibits cyclic phosphorylation mediated by anthraquinone-2-sulfonate or ferredoxin (Table II). Trebst et al. [29] suggest that this compound inhibits the transfer of electrons from a bound plastoquinone ('B') to the plastoquinone pool. The concentrations of the dinitrophenyl ether of iodonitrothymol used here are very high, and lesser concentrations give little inhibition of cyclic phosphorylation. If their interpretation of the inhibition site is correct, we can conclude that anthraquinone-2-sulfonate or ferredoxin mediated cyclic phosphorylation does not require the function of a bound plastoquinone.

Three of the anthraquinone disulfonates which catalyze low rates of cyclic phosphorylation catalyze high yields of ATP synthesis in the unilluminated, entrapped ferricyanide system. This might be explained by the nature of the assays. Unlike the cyclic phosphorylation assay which measures a kinetic rate, the entrapped ferricyanide system measures the total yield of ATP synthesized in the reaction. For the lipophilic mediator, diaminodurene, the half-time for complete reduction of entrapped ferricyanide is approx. 4 s [12]. In these experiments, reaction mixtures were incubated for 45 seconds. This would allow even kinetically slow compounds to complete the catalysis of ferricyanide reduction. On the other hand, the rate of cyclic phosphorylation would be limited by the rate of diffusion of the sulfonated mediators through the thylakoid membrane.

The major point of interest here is that the phosphorylation stimulated by the sulfonated mediators in the entrapped ferricyanide system is inhibited by dibromothymoquinone (Fig. 2). This inhibition requires high concentrations of dibromothymoquinone, which is probably due to the high chlorophyll concentrations (0.23 mg/ml) used [30]. That dibromothymoquinone itself is not acting as a proton/electron mediator is shown in the no mediator control in Fig. 2. However, it has also been suggested [23] that high concentrations of dibromothymoquinone (>5 μM) might inhibit the reduction of the plastoquinone pool as well as its reoxidation. It is possible that this additional effect on plastoquinone reduction is the important effect here. Regardless of the precise location of the inhibition site, one would not expect the relatively hydrophilic mediators to enter or cross the thylakoid membrane at an appreciable rate. It is therefore presumed that the electron/proton transport stimulated by these compounds leading to ATP synthesis is also mediated by the native electron/proton shuttle, plastoquinone.

It was hoped that the electron transferring protein ferredoxin, which is the native indirect reductant of plastoquinone, would also stimulate ATP synthesis in this artificial system. Its failure to stimulate phosphorylation under these conditions might be explained by the fact that ferredoxin utilizes a slightly different and more complex pathway of electron transfer. This pathway includes an Antimycin A sensitive site, perhaps cytochrome *b*-563 [31], in cyclic phosphorylation. The exposure to high ferricyanide concentrations used in this assay might damage the components involved in electron transfer from ferredoxin [32,33].

Antimycin A does not inhibit redox induced phosphorylation catalyzed by quinones, as shown by data for anthraquinone-2,7-disulfonate in Table III. Apparently, the quinones used in both redox-induced and cyclic phosphorylation can transfer electrons directly to plastoquinone, without utilizing the Antimycin A sensitive site (Tables II and III). Because plastoquinone is accessible to reduction by the hydrophilic quinones, this suggests that the plastoquinone pool extends close to the thylakoid membrane surface. The dinitrophenyl ether of iodonitrothymol inhibits redox induced phosphorylation 25% when anthraquinone-2,7-disulfonate is the mediator (Table III), whereas the inhibition is even less when menadione is the mediator (data not shown). This indicates that a bound plastoquinone is probably not required for this reaction.

The quinones studied in these experiments can be useful in stimulating electron transferring functions in native or artificial membranes. One such liposomal system has been developed by Hauska [34] in which both electrons and protons are transported across lecithin vesicles by incorporated plastoquinone. Other components of thylakoid membranes could conceivably be studied in a similar manner, provided they can be incorporated into vesicles and then involved in appropriate redox reactions.

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