Biochimica et Biophysica Acta, 1102 (1992) 45–52

BBABIO 43652

Binding affinities of benzoquinones to the Q_B site of Photosystem II in *Synechococcus* oxygen-evolving preparation

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(Received 31 March 1992)

Key words: Q_B site; Photosystem II; Benzoquinone; Plastoquinone; (*Synechococcus elongatus*)

We have investigated interactions of various *p*-benzoquinones with the Q_B-binding domain in oxygen-evolving PS II particles isolated from *Synechococcus elongatus* by two different methods. First, rates of oxygen evolution were determined in the presence of various concentrations of benzoquinones and the two kinetic parameters, K_m and V_{max}, were estimated from double reciprocal plots. The V_{max} value increased with increasing hydrophobicity of quinone molecules, suggesting that the hydrophobicity of acceptor molecules is an important factor affecting the terminal limiting step of quinone reduction. The K_m values agreed with the binding constants of the Q_B site for corresponding benzoquinones which were determined by the second spectrophotometric method. Thus, the first method offers a simple and convenient procedure to estimate the affinities of quinone acceptors to the Q_B site. In the second method, fractions of the Q_B domains occupied by 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) were determined by measuring the magnitude of the $Q_A^-Q_B$ to $Q_AQ_B^-$ transition. DCMU reduced the magnitude of Q_A^- oxidation by 50% at 60 nM. The inhibition was significantly reversed on addition of benzoquinones, indicating that quinone molecules bind to the Q_B domain in competition with DCMU and, once bound, serve as electron acceptors of Q_A. The binding constants of the Q_B site for benzoquinones were estimated from quinone-induced changes in the concentration of DCMU required to occupy 50% of the Q_B domains. No evidence was obtained to indicate the occurrence of oxidation of the iron (II) located near Q_A and Q_B under the assay conditions, which otherwise would affect the estimation of the binding constant. The relationship between the binding affinity and the molecular structure of benzoquinones is discussed.

Introduction

The two bound plastoquinone acceptors, Q_A and Q_B, are involved in electron transport to the plastoquinone pool on the reducing side of PS II in higher plants, algae and cyanobacteria (for review, see Refs. 1–3). On illumination, an electron is transferred from P680, the primary donor chlorophyll *a*, to Q_A via pheophytin *a* and Q_A^-, in turn, gives its electron to Q_B. Q_B functions as a two-electron carrier: Q_B^- binds tightly to a specific site of the D-1 protein called the 'Q_B site', whereas Q_B^{2-}, produced with the second electron from Q_A^-, has a low binding affinity to the site and is replaced by a free plastoquinone molecule after protonation. Herbicides such as DCMU bind to the Q_B-binding domain and block the binding of plastoquinone.

There are several lines of evidence indicating that various synthetic benzoquinones bind to the Q_B binding domain. Oettmeier et al. showed that various benzoquinones inhibit intersystem electron transport near PS II [4,5]. The DCMU-type herbicides bound to the Q_B binding domain were replaced by benzoquinones [6–8]. The iron located near Q_A and Q_B is oxidized after a single flash in the presence of several benzoquinones and the oxidation is ascribed to semiquinones bound to the Q_B site [9]. In particular, reconstitution of bound quinone acceptors with synthetic quinones has been reported with quinone-extracted reaction center preparations of photosynthetic bacteria [10–12], Photosystem I [13,14] and Photosystem II [15]. The quinone-binding sites of bacterial and Photosystem I reaction center complexes were shown to have low specificities to quinones, binding 1,4-benzoquinone, 1,4-naphthoquinone and 9,10-anthraquinone, as well as their various substituted forms [10–12,16].

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DBMIB, dibromothymoquinone (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone); duroquinone, tetramethyl-*p*-benzoquinone; Q_A, primary quinone acceptor; Q_B, secondary quinone acceptor.

Oxygen-evolving PS II particles isolated from the thermophilic cyanobacterium, *Synechococcus elongatus*, serve as excellent material for investigation on interactions of various *p*-benzoquinones with Q_A and Q_B because they lack the pool plastoquinone but are highly competent in oxygen evolution [17,18]. The PS II particles are also amenable to spectrophotometric investigation because they contain only about 50 chlorophyll *a* per PS II [17]. The previous study resolved the relaxation kinetics of flash-induced absorbance changes in the blue region into three exponential components labeled 'f', 'm' and 's' in the order of appearance [19]. The 'f' component (the fastest decay component, with a half-time of 1.3 to 2.0 ms at 25°C) was ascribed to electron flow from Q_A^- to Q_B, based upon its difference spectrum and sensitivity to DCMU. The m and s components are quinone-dependent oxidation of Q_A^- and Q_B^-, respectively [19].

In the present study, the two approaches were made to determine the binding affinities of the Q_B site of the *Synechococcus* PS II preparation for a variety of substituted *p*-benzoquinones. The first method consists of measurement of oxygen evolution in the presence of various concentrations of quinones and estimation of the kinetic parameters by the double reciprocal plot analysis of the data. The second method takes advantage of the fact that binding of DCMU to the Q_B domain can be directly estimated by measuring the magnitude of the f component. The binding constants of benzoquinones were determined from their effectiveness to replace DCMU bound to the Q_B domain.

Materials and Methods

The thermophilic cyanobacterium, *Synechococcus elongatus* was grown at 55°C and the thylakoid membranes were prepared as reported previously [20,21]. Oxygen-evolving PS II particles were prepared with β-octyl glucoside as in Ref. 17. Where indicated, oxygen-evolving particles from another thermophilic cyanobacterium *S. vulcanus* were used [22,23].

Oxygen evolution was measured at 30°C with a Clark-type oxygen electrode [24]. Flash-induced absorbance changes of Q_A and Q_B were measured at 413.5 nm at 25°C with a Union-Giken single-beam spectrophotometer as described previously [19]. Flashes from a xenon lamp (5 μs duration at the half-maximum height) were fired 100 or 200 times at 1 Hz and averaged signals were analyzed with a microcomputer [19]. The reaction mixture contained 1.0 M sucrose, 5 mM $MgCl_2$, 10 mM NaCl, 50 mM 2-(*N*-morpholino)-ethansulfonic acid/NaOH (pH 6.0), indicated concentration of benzoquinone and PS II particles equivalent to 3.5 μg chl/ml.

DBMIB and 2,3-dimethyl-*p*-benzoquinone were generous gifts from Dr. Oettmeier, Rhur-University Bochum. Duroquinone, *p*-benzoquinone, methyl-*p*-benzoquinone, 2,5-dimethyl-*p*-benzoquinone, 2,6-dimethyl-*p*-benzoquinone, 2,6-dichloro-*p*-benzoquinone were purchased from Tokyo Kasei, Japan. Other chemicals were obtained from Wako Chemicals, Japan.

Results

Rates of oxygen evolution were determined in the presence of various concentrations of *p*-benzoquinones and the results were analyzed by plotting (oxygen-evolving rates)$^{-1}$ against (quinone concentrations)$^{-1}$. The double-reciprocal plots yielded straight lines for all the benzoquinones examined (Fig. 1 and data not shown). Table I shows the V_{max} and K_m values estimated for various benzoquinones including six methyl-substituted quinones. The V_{max} values correlate well with the hydrophobicity of quinone molecules; the value increased with increasing number of methyl groups (except for duroquinone) and phenyl-*p*-benzoquinone showed a larger K_m value than any of the methyl-substituted benzoquinones. Similar V_{max} values were obtained for 2,3-, 2,5- and 2,6-dimethyl-*p*-benzoquinone, indicating that V_{max} is independent of the position of methyl substitution.

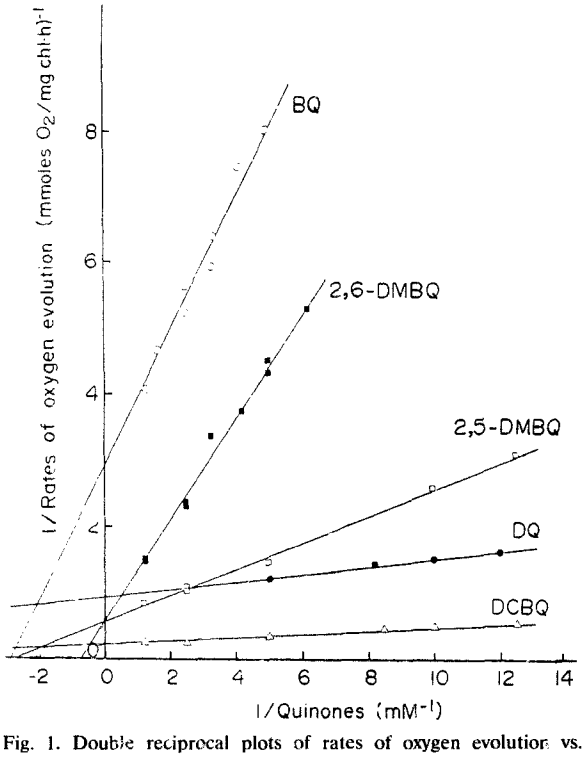

Fig. 1. Double reciprocal plots of rates of oxygen evolution vs. concentrations of benzoquinones. For experimental conditions, see Materials and Methods.

The apparent K_m value also tended to increase as the number of methyl-substitution increases. However, there were exceptions; the K_m value of 2,5-dimethyl-*p*-benzoquinone was smaller than that of methyl-*p*-benzoquinone, and duroquinone and phenyl-*p*-benzoquinone showed lower K_m values than did trimethyl-*p*-benzoquinone. It is also to be noted that there were notable differences in the K_m values of 2,3-, 2,5- and 2,6-dimethyl-*p*-benzoquinone. Thus, the position of methyl substitution is an important factor that affects K_m. Halogen-substitution seems to increase the affinity of *p*-benzoquinone to the binding site. The K_m values of 2,6-dichloro-*p*-benzoquinone were smaller than those of *p*-benzoquinone. DBMIB, which supports a low rate of oxygen evolution (see Table I and Fig. 2), showed the lowest K_m value, of less than 10 μM. However, the accurate K_m value could not be determined by this method.

DBMIB is a potent inhibitor of photosynthetic electron transport at the plastoquinol oxidation stage [25,26] but has a second inhibition site near the PS II reaction center [27,28]. Fig. 2 shows that oxygen evolution with 2,6-dichloro-*p*-benzoquinone as electron acceptor was strongly suppressed by DBMIB in the cyanobacterial PS II particles which lack the plastoquinone pool. The inhibition was incomplete and with increasing concentrations of the inhibitor the rate of oxygen evolution approached a low level that is supported by DBMIB alone. The result suggests that DBMIB inhibits oxygen evolution by competing with 2,6-dichloro-*p*-benzoquinone for a reducing site of PS II, but once bound, serves by itself as a relatively poor electron acceptor. The DBMIB concentration required for the half-maximum inhibition was about 4 μM in the presence of 0.4 mM 2,6-dichloro-*p*-benzoquinone. Thus, the binding constant (K_b, the concentration necessary to occupy 50% of the binding sites in the absence of any competing benzoquinone) of DBMIB is less than 4 μM.

TABLE I

V_{max} and K_m values of p-benzoquinones

	V_{max} (μmol O_2/mg Chl per h)	K_m (μM)
Benzoquinone	340	340
Methylbenzoquinone	1080	500
2,3-Dimethylbenzoquinone	1900	710
2,5-Dimethylbenzoquinone	1750	360
2,6-Dimethylbenzoquinone	1890	1350
Trimethylbenzoquinone	3260	1670
Duroquinone	1320	100
Phenylbenzoquinone	3920	140
2,6-Dichlorobenzoquinone	4760	130
DBMIB	480	< 10

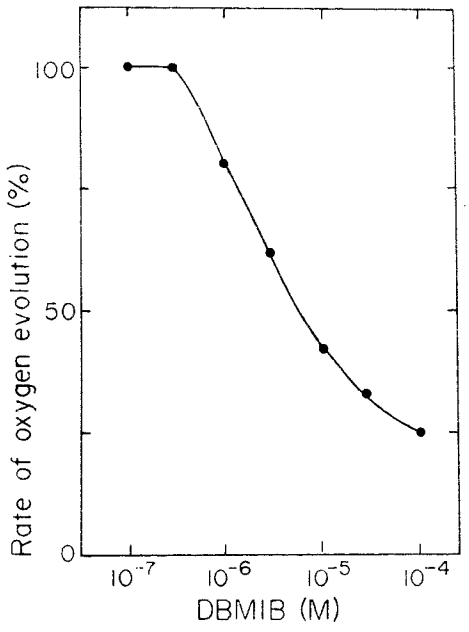

Fig. 2. Effects of DBMIB on the rate of oxygen evolution determined in the presence of 0.4 mM 2,6-dichloro-*p*-benzoquinone.

A question arises as to what the K_m values determined by the double reciprocal plot mean. As stated in the Introduction, there are several lines of evidence suggesting that synthetic benzoquinones are reduced at the Q_B site [4–9]. Do the K_m values correspond to the binding affinities of the Q_B site for benzoquinones? We addressed the question by measuring binding constants of the Q_B site for benzoquinones as follows. The previous investigation showed that the fastest decay component (f component) of flash-induced absorption changes at 413.5 nm in the *S. elongatus* PS II particles represent electron transfer from Q_A^- to Q_B [19]. Fig. 3 shows that DCMU diminished the magnitude of the f component without affecting its rate. This indicates that DCMU blocks Q_A^- oxidation by binding to the Q_B domain and, furthermore, that binding and release of the inhibitor is much slower than oxidation of Q_A^-. Thus, a fraction of the Q_B sites that are occupied by DCMU can be estimated by measuring the magnitude of Q_A^- oxidation.

Fig. 4 shows effects of various concentrations of DCMU on the magnitude of the f component determined in the presence of ferricyanide, which has no affinity to the Q_B site [19]. Ferricyanide was added to reoxidize plastoquinol produced by repetitive flash excitation [19]. The result shows that the DCMU concentration required to block 50% of the Q_B domain (I_{50}) was 60 nM DCMU. The value agrees with the binding constants of DCMU determined with the radioactive herbicide [7]. When 10 μM DBMIB had been added,

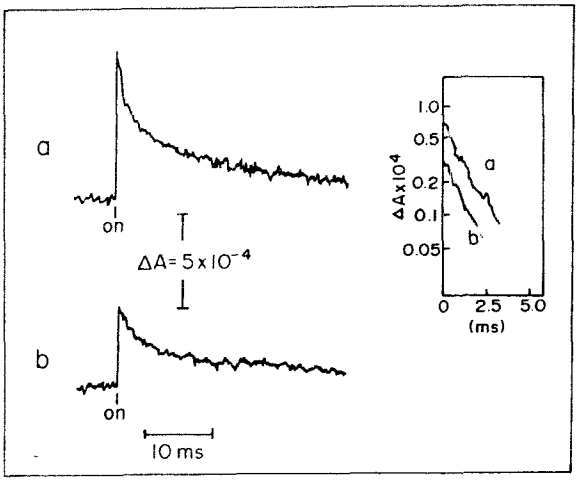

Fig. 3. Effects of DCMU on the decay kinetics of flash-induced absorbance changes at 413.5 nm. Absorbance changes were determined in the presence of 0.4 mM 2,6-dichloro-*p*-benzoquinone. (a), no addition; (b), 0.05 μM DCMU was added. Inset shows semilogarithmic plots of f components.

the inhibitory effect of DCMU was appreciably attenuated. This indicates that DBMIB binds to the Q_B site in competition with DCMU and accepts electrons from Q_A^-. The $I_{50'}$ value (I_{50} value determined in the presence of a competing benzoquinone) was 140 nM.

The $I_{50'}$ values estimated in the presence of other benzoquinones are summarized in Table II. All the benzoquinones examined were effective in attenuating the inhibition but in lesser extents than did DBMIB.

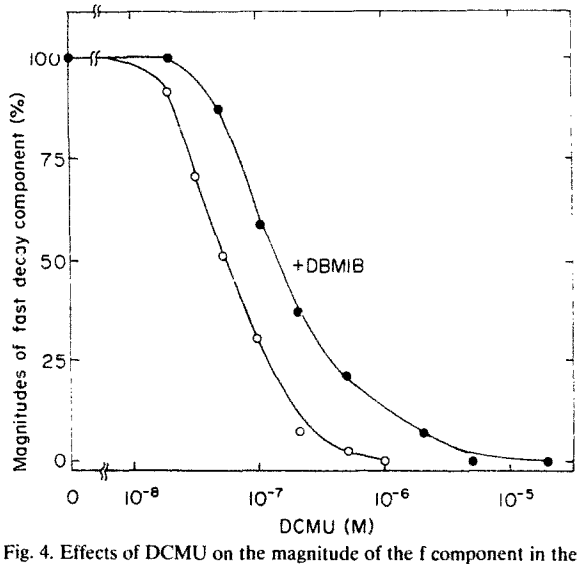

Fig. 4. Effects of DCMU on the magnitude of the f component in the presence and absence of DBMIB. (○), 0.4 mM ferricyanide; (●) 0.4 mM ferricyanide and 10 μM DBMIB.

TABLE II

$I_{50'}$ values of DCMU determined in the presence of various p-benzoquinones and K_b values and $E_{1/2}$ values of quinones

	$I_{50'}$ (μM)	K_b (μM)	$E_{1/2}$ (mV)
Ferricyanide (0.4 mM)	0.060	–	–
Benzoquinone (0.4 mM)	0.12	400	286
Methylbenzoquinone (0.4 mM)	0.10	600	230
2,3-Dimethylbenzoquinone (0.4 mM)	0.089	830	174
2,5-Dimethylbenzoquinone (0.4 mM)	0.11	470	180
2,6-Dimethylbenzoquinone (0.4 mM)	0.080	1200	174
Trimethylbenzoquinone (0.4 mM)	0.074	1700	115
Duroquinone (1.0 mM)	0.060	–	52
2,6-Dichlorobenzoquinone (0.4 mM)	0.15	130	279
DBMIB (0.01 mM)	0.14	6.1	170

The $I_{50'}$ was, therefore, determined in the presence of 0.4 mM benzoquinones. An exception was duroquinone which failed to affect the I_{50} value of DCMU even at 1 mM.

The above observations indicate that benzoquinones compete with DCMU for the Q_B site and accept electrons directly from Q_A^-. Thus, the binding constants of the Q_B site for these benzoquinones can be estimated from the I_{50} and $I_{50'}$ values (see Appendix). The K_b values obtained are shown in Table II. Note that the K_b values agree with the K_m values determined from the double reciprocal plot of the oxygen-evolving rate vs. benzoquinone concentration. We conclude, therefore, that the K_m values represent the binding affinities of the Q_B site for benzoquinones.

Finally, it is to be mentioned that the binding affinity of DCMU to the Q_B site is considerably reduced when the non-heme iron(II) (R [29] or Q_{400} [30]), located near Q_A or Q_B, is oxidized [31]. The iron(II) is slowly oxidized with ferricyanide in the dark and the oxidized iron serves as a quencher of fluorescence from chlorophyll *a* [29]. More recently, the iron(II) was shown to be partially oxidized by illumination in the presence of several benzoquinones and the iron (III) in turn oxidized Q_A^- [9].

$$Q_A Fe(II)BQ \xrightarrow{h\nu} Q_A^- Fe(II)BQ \rightarrow Q_A Fe(II)BQ^- \rightarrow Q_A Fe(III)BQ^{2-}$$

$$Q_A Fe(III) \xrightarrow{h\nu} Q_A^- Fe(III) \rightarrow Q_A Fe(II)$$

Thus, if the iron(II) is oxidized during repetitive flash excitation, a resulting decline in the binding affinity of DCMU will lead to an overestimation of the K_b of benzoquinones. However, the K_b values were determined at pH 6.0, where the binding affinity of DCMU is relatively insensitive to the oxidation of the iron(II) [31]. EPR experiments showed that the extent of the iron(II) oxidation attained after illumination for 17 s at

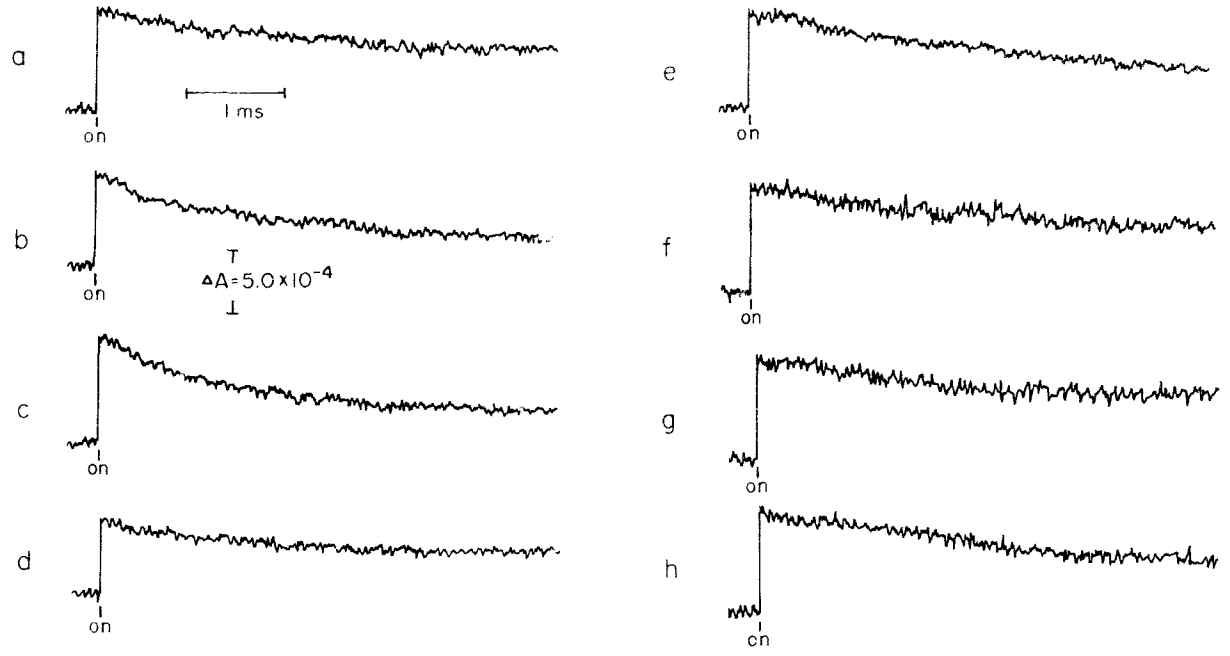

Fig. 5. Effects of benzoquinones on the decay kinetics of the f component in *S. elongatus* and *S. vulcanus* PS II particles. (a–d), *S. vulcanus* particles; (e–h), *S. elongatus* particles. (a) and (e), 0.1 mM ferricyanide; (b) and (f), 0.4 mM methyl-*p*-benzoquinone; (c) and (g), 1 mM benzoquinone; (d) and (h), 0.4 mM 2,5-dichloro-*p*-benzoquinone. 200 signals were averaged.

0°C decreases, depending upon substitutions of benzoquinones, in the following order [9]:

methyl → phenyl → 2,3-dimethyl → H-,2,6-dimethyl → 2,5-dichloro

→ trimethyl-, tetramethyl- (= ferricyanide)

As stated above, however, the K_b values agreed well with the K_m values for all the quinones examined (except for duroquinone). Because the K_m values were determined under experimental conditions, where no oxidation of the iron(II) is expected to occur, the agreement of the two binding affinities suggests that oxidation of the iron(II) is insignificant, or the binding affinity of DCMU is not affected by the oxidation of the iron(II).

The following experiment provided evidence which favors the absence of the iron(II) oxidation. When the iron(II) is oxidized with ferricyanide in the dark, a rapid oxidation of Q_A^- with a half-time of 25 μs [9] or 140 μs [32] occurs at the first of a series of short flashes, whereas Q_A^- is more slowly oxidized by Q_B at subsequent flashes because of slow oxidation of the iron(II) by ferricyanide. If the iron(II) is oxidized by semibenzoquinone at the Q_B site, a rapid oxidation of Q_A^- by the iron(III) occurs after every even number of flashes in the presence of benzoquinones. Then, averaging of the signals obtained with 100 flashes would result in time courses showing a faster kinetics of Q_A^- oxidation in the presence of benzoquinones of which the semiquinone forms are active in oxidation of the iron(II), than in the presence of ferricyanide, or benzoquinones, of which semiquinones are inactive. Fig. 5 compares time courses of Q_A^- oxidation determined at a fast time scale. For the comparison, the data obtained with oxygen-evolving PS II particles from another thermophilic cyanobacterium *S. vulcanus* are presented. The two preparations showed similar kinetics of Q_A^- oxidation with half-time of 1.3 ms for the f component in the presence of ferricyanide (traces a and e) On addition of *p*-benzoquinone and methyl-*p*-benzoquinone *S. vulcanus* particles showed a fast phase of Q_A^- oxidation with a half-time of 0.2–0.3 ms (traces b and c). However, 2,5-dichloro-*p*-benzoquinone (trace d) and duroquinone failed to induce a rapid oxidation of Q_A^- (not shown). Thus, the appearance of the fast phase of Q_A^- oxidation is well correlated with the ability of benzoquinone to induce oxidation of the iron(II). A similar pattern of benzoquinone effect was observed in spinach PS II membranes (data to be published elsewhere). It is, therefore, remarkable that PS II particles isolated from *S. elongatus* showed no fast phase of oxidation of Q_A^- even in the presence of *p*-benzoquinone and methyl-*p*-benzoquinone. The kinetics of Q_A^- oxidation determined in the presence of these quinones was essentially the same as that determined in the presence of ferricyanide. It is concluded,

therefore, that oxidation of the iron(II) is negligible under the conditions used for estimation of K_b in this cyanobacterial preparation.

Discussion

In the present work, the two new approaches were made for determination of binding affinity of the reducing site of PS II for various benzoquinones. The first method is based on the finding that rates of oxygen evolution determined in the presence of various concentrations of benzoquinones follow a simple kinetics of the Michaelis-Menten type. Thus, dependence of the reaction rate upon benzoquinone concentration can be expressed by the two parameters, K_m and V_{max}. The K_m values estimated by the double reciprocal plot agree with the K_b values determined by the second spectrophotometric method. Thus, the method is a simple procedure to evaluate the binding affinity of the Q_B site for quinone acceptors. However, the method has limits; K_m cannot be accurately determined for a quinone with an extremely high affinity (like DBMIB) or an extremely low affinity (like duroquinone). Although duroquinone supports a substantial rate of oxygen evolution, showing a K_m value comparable to those of the good electron acceptors such as phenyl-*p*-benzoquinone or dichloro-*p*-benzoquinone, the second method revealed that the quinone has a very low affinity to the Q_B site. This indicates that duroquinone accepts electrons via plastoquinol and hence its K_m value is not related to the binding affinity to the Q_B site.

Plastoquinone is an extremely hydrophobic quinone. In this connection, of particular interest is the observation that V_{max} increased with increasing hydrophobicity of quinone molecules. The previous study showed that the overall electron transport in the *S. elongatus* preparations is limited by the final step of benzoquinone reduction [19]. Reduction of benzoquinone consists of a sequence of reactions, i.e., binding of benzoquinone to the Q_B site, reduction of benzoquinone or benzosemiquinone by Q_A^-, protonation of benzoquinol dianion and dissociation and diffusion of benzoquinol from the Q_B site. V_{max} is the rate of oxygen evolution with the Q_B sites fully occupied by benzoquinone and electron transfer from Q_A^- to benzoquinone (or benzosemiquinone) bound to the Q_B site is not rate-limiting [19]. Thus, the result suggests that the hydrophobic property of benzoquinone molecule is important for a terminal step of its reduction, such as protonation of benzoquinol dianion or dissociation and diffusion of benzoquinol from the Q_B site.

The spectrophotometric method provides an accurate estimation of the binding constants for quinones with a wide range of the binding affinities. The method depends upon competitive binding of benzoquinones and DCMU for the common Q_B domain. K_b or I_{50} values were estimated without taking competition of benzoquinone or DCMU with endogenous plastoquinone into account. However, this should not significantly affect our estimation because *Synechococcus* PS II particles contain only three plastoquinone molecules (including Q_A and Q_B) per PS II [18] and benzoquinone or DCMU was added in large excess over the endogenous plastoquinone molecules.

The present study showed that the iron(II), located on the reducing side of PS II reaction center, behaves differently under flash illumination in the presence of benzoquinones in the two cyanobacterial PS II particles. Addition of several benzoquinones to PS II particles from *S. vulcanus* resulted in a significant acceleration of oxidation of Q_A^-. This can be ascribed to the occurrence of rapid oxidation of Q_A^- by the iron(III) because the effectiveness of benzoquinones in this respect is well correlated with the abilities of their semiquinones to oxidize the iron(II). By contrast, no evidence was obtained for semibenzoquinone-dependent oxidation of the iron(II) in *S. elongatus* particles under the same experimental conditions. This is surprising because the two cyanobacteria are both highly active in oxygen evolution and share a common, thermophilic feature [20,22]. However, the absence of semibenzoquinone-dependent oxidation of the iron(II) is consistent with the observation that the K_b values which were determined from competition between benzoquinones and DCMU for the common binding domain agree well with the K_m values which were estimated by measuring steady-state rates of oxygen evolution. We conclude that the K_b values determined in the present study are not affected by oxidation of the iron(II).

Why are semibenzoquinones ineffective in oxidation of the iron(II) in *S. elongatus* particles? In this respect, of special interest is the previous observation that, by contrast to Q_B^-, which is produced by a single flash excitation in the presence of ferricyanide, the Q_B^- signal produced in the presence of phenyl-*p*-benzoquinone is unstable and disappeared with a half-time of about 300 ms [19]. The instability of the Q_B^- signal cannot be ascribed to reduction of the semibenzoquinone by the iron(II) because the signal disappeared at a comparable rate in the presence of dichloro-*p*-benzoquinone, of which the semiquinone form is a much poorer oxidant of the ion(II) than that of phenyl-*p*-benzoquinone [9]. It was suggested that the semiquinone forms of the synthetic benzoquinones have low affinities to the Q_B site and disappearance of the Q_B^- signal indicated dissociation of semibenzoquinones from the binding site [19]. The inability of semiquinone to oxidize the iron(II) may be related to its unstable binding to the Q_B site in *S. elongatus* particles.

Previously, radioactive herbicides were used to esti-

mate competition with various quinones [8]. An advantage of the spectrophotometric method is that the fraction of the Q_B sites that are occupied by DCMU can be determined by measuring the magnitude of the Q_A^- oxidation and hence independently of non-specific binding of the herbicide. Interaction of inhibitory quinones with the Q_B site has also been evaluated from their effects of inhibiting electron transport [4] or quenching chlorophyll fluorescence [33]. However, accurate values cannot be obtained if, like DBMIB, inhibitory quinones also serve as electron acceptors. It was also reported that binding of DCMU-type inhibitors to thylakoids is markedly lowered by depletion of plastoquinone with *n*-hexane [7]. In the present work, binding affinities of *p*-benzoquinones were determined without prior extraction of plastoquinone. Thus, our estimations are free from any artifacts which might be introduced by solvent-treatment employed for extraction of endogenous plastoquinones.

The binding affinity of benzoquinone varies depending upon the number, position and nature of substitution. *p*-Benzoquinone binds more strongly to the Q_B site than any methyl-substituted benzoquinone, suggesting that introduction of a methyl group(s) to the benzoquinone ring reduces the binding affinity of a quinone molecule to the Q_B site. However, the three dimethyl-*p*-benzoquinones with different substitution positions showed different K_b values. Thus, a steric parameter is an important factor that affects the binding affinity.

In general, our results agree with those of Soll and Oettmeier [7], who studied interactions of a wide variety of benzoquinones with the Q_B domain in spinach thylakoids. There are small differences in the K_b values determined by the two groups but they are ascribed to the differences in the materials and methods used. They showed that the structure-activity relationship can be quantitatively described by the $E_{1/2}$ for benzoquinone/benzoquinol transition (pH 7.0), a steric factor and an indicator parameter. The binding affinities determined here also showed a tendency to increase with the increasing $E_{1/2}$ of benzoquinones (see Table II). An implication is that the K_b values are apparent ones and involve not only the interaction of quinone with the Q_B site but also electron transfer between Q_A and the quinone occupying the Q_B site [16,34]. In this respect, however, more important are $E_{1/2}$ values for the Q/Q^- and Q^-/QH_2 couples. Experiments with quinones having a wider range of these $E_{1/2}$ values are in progress to examine this possibility.

Appendix

K_b of the Q_B site for benzoquinone is estimated from the I_{50} and $I_{50'}$ values of DCMU determined in the absence and presence of benzoquinone, respectively. In the absence of benzoquinone:

$$Q_A + DCMU \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Q_A \cdot DCMU \quad (1)$$

$$I_{50} = k_{-1}/k_1 \quad (2)$$

In the presence of benzoquinone, the following reaction takes place after flash excitation;

$$Q_A^- + \text{benzoquinone (BQ)} \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} Q_A^- \cdot BQ \overset{k_3}{\rightarrow} Q_A \cdot BQ^- \quad (3)$$

We neglect the two electron gate mechanisms involving $Q_A \cdot$ benzoquinol because the previous study showed that, in contrast to plastosemiquinone, benzosemiquinone dissociates from the Q_B site and dismutes during the flash interval of 1 s [19]. We also assume that reduction of Q_A by bound benzosemiquinone is negligible and that binding and release of DCMU are much slower than those of benzoquinone and Q_A oxidation by bound benzoquinone [35]. Then, the magnitude of the f component (Q_A^- oxidation by bound plastoquinone or benzoquinone) is linearly related to the fraction of the Q_B domain that is not occupied by DCMU.

$$[Q_A^-]_T - [Q_A^- \cdot DCMU] = [Q_A^-] + [Q_A^- \cdot \text{benzoquinone}]$$

where $[Q_A^-]_T$ stands for the total $[Q_A^-]$. We obtain:

$$[Q_A^-] = [Q_A^-]_T/(1 + [\text{benzoquinone}] \cdot k_2/k_{-2} + [DCMU] \cdot k_1/k_{-1}) \quad (4)$$

The concentration of DCMU which is required to block 50% of the Q_B sites in the presence of benzoquinone ($I_{50'}$) can be written as follows:

$$I_{50'} = k_{-1}/k_1(1 + [\text{benzoquinone}] \cdot k_2/k_{-2})$$

Thus, $K_b = k_{-2}/k_2 = [\text{benzoquinone}]/(I_{50'}/I_{50} - 1)$.

Acknowledgements

The present work was partly supported by a grant from the Ministry of Education, Japan. We also thank Mr. I. Ikegami for his skillful technical assistance.

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