Electron Transport from Q_A to Thymoquinone in a *Synechococcus* Oxygen-Evolving Photosystem II Preparation: Role of Q_B and Binding Affinity of Thymoquinone to the Q_B Site

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Q_B Site, Photosystem II, Thymoquinone, Plastoquinone, *Synechococcus*

We have recently shown that binding affinities of benzoquinones can be estimated by two methods in photosystem (PS) II particles (K. Satoh *et al.*, Biochim. Biophys. Acta **1102,** 45–52 (1992)). Using these methods we calculated the binding affinity of thymoquinone (2-methyl-5-isopropyl-*p*-benzoquinone) to the Q_B site and studied how the quinone accepts electrons in oxygen-evolving PS II particles isolated from the thermophilic cyanobacteria, *Synechococcus elongatus* and *S. vulcanus*. The results are as follows: (1) The binding constant of thymoquinone to the Q_B site determined by several methods was around 0.33 mM. (2) At low thymoquinone concentrations the quinone was supposed to accept electrons *via* Q_B-plastoquinone, whereas at high concentrations the quinone seemed to bind to the Q_B site and accept an electron directly from Q_A^-. Lower rates of photoreduction of the quinone at high concentrations were attributed to a slower turnover rate of the quinone at the Q_B site than that of endogenous plastoquinone. (3) A model for the function of plastoquinone at the Q_B site, which can explain all the results, was presented. According to this model, the plastoquinone molecule at the Q_B site is not replaced by another plastoquinone molecule. Instead, it transfers electrons to pool plastoquinone molecules by turning over its head group but remaining its long side chain bound to the PS II complexes.

Introduction

On the reducing side of PS II, the two bound plastoquinone acceptors, Q_A and Q_B, are involved in electron transport to the plastoquinone pool in higher plants, algae and cyanobacteria [1–3]. Q_A is a one-electron carrier, whereas Q_B functions as a two-electron gate: Q_B^- binds tightly to a specific site of the D-1 protein called 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 supposed to be replaced by a free plastoquinone molecule after protonation [2]. 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 non-heme 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, 10]. Recently, we have shown that the binding affinities of benzoquinones can be calculated by two methods [11]. The first method consists of measurements 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 an advantage that binding of DCMU to the Q_B domain can be directly estimated by measuring the magnitude of the fast decaying component at 413.5 nm after flash excitation which reflects electron transport from Q_A^- to Q_B [12]. The binding constants of benzoquinones were determined from their effectiveness to replace DCMU bound to the Q_B domain [11]. The results we obtained were that most benzoquinones bound to the Q_B site and that, with an increase in the number of methyl-substitution, the binding affini-

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

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ties of the quinones were decreased but their turnover rates at the Q_B site were increased. The only exception was duroquinone which has little or no affinity to the Q_B site and accepts electrons from plastoquinone functioning as Q_B [11].

In this paper we will show that thymoquinone accepts electrons either from plastoquinol or Q_A^- depending upon thymoquinone concentrations used. At concentrations lower than 0.25 mM, thymoquinone accepts electrons mainly from plastoquinone which is turning over at the Q_B site, but at higher concentrations, the quinone replaces the plastoquinone at the Q_B site and accepts an electron from Q_A^-. A model for plastoquinone function at the Q_B site is also presented.

Materials and Methods

The thermophilic cyanobacteria, *Synechococcus elongatus* and *S. vulcanus*, were grown at 55 °C and the thylakoid membranes were prepared as reported previously [13, 14]. Oxygen-evolving PS II particles were prepared from *S. elongatus* with β-octylglucoside as in [15] or from *S. vulcanus* with lauryldimethylamine-N-oxide as in [16].

Oxygen evolution was measured at 30 °C with a Clark-type oxygen electrode [17]. 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 [12]. 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 [12]. The reaction mixture contained 1.0 M sucrose, 5 mM $MgCl_2$, 10 mM NaCl, 50 mM 2-(N-morpholino)ethanesulfonic acid/NaOH (pH 6.0), indicated concentrations of electron acceptors and PS II particles equivalent to 3.5 μg chlorophyll/ml.

Thymoquinone and 2,5-DCBQ were purchased from Tokyo Kasei Co., Japan. Silicomolybdate was a kind gift from Dr. Oettmeier, Ruhr University, Bochum. Other chemicals were obtained from Wako Chemicals, Japan.

Results and Discussion

The binding constant (K_b) of thymoquinone to the Q_B site was calculated from the competition of the quinone with DCMU for the Q_B site [11]. The concentration of DCMU needed to inhibit 50% of the electron flow from Q_A to Q_B (I_{50}) was shifted from 60 nM to 79 nM by the addition of 0.1 mM thymoquinone, and from this shift the K_b value was estimated (see appendix of ref. [11]) to be 0.33 mM (Table I). However, unlike other benzoquinones [11], dependence of the rates of oxygen evolution on the concentration of thymoquinone did not show a simple saturation curve (Fig. 1). The rate increased with an increase in the thymoquinone concentration at first, but it reached a maximum at around 0.25 mM and started to decrease at higher concentrations. The half inhibition (from V_{max} – see Fig. 2) concentration of thymoquinone was between 0.35 and 0.4 mM (Fig. 1), which was very close to the K_b value. Plots of (oxy-

Table I. The I_{50} values of DCMU for the electron flow from Q_A^- to Q_B and binding constant (K_b) of thymoquinone to the Q_B site calculated from the shift of the I_{50} value by thymoquinone (see ref. [11]). The electron flow from Q_A^- to Q_B was measured by plotting the fast decaying component at 413.5 nm (ref. [12]).

e^- Acceptor (concentration)	I_{50} of DCMU	K_b of thymoquinone
Ferricyanide (0.1 mM)	60 nM	–
Thymoquinone (0.1 mM)	79 nM	0.33 mM

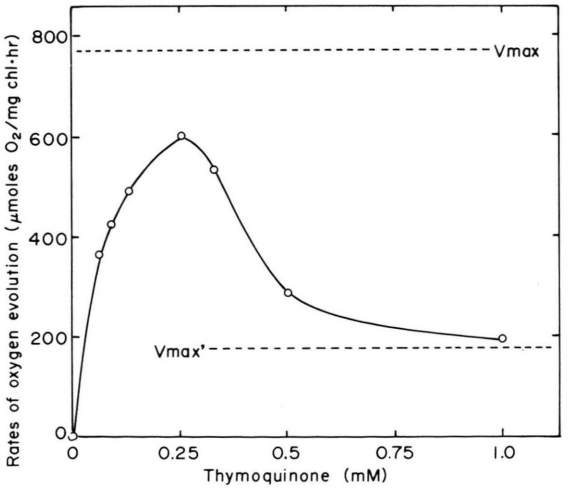

Fig. 1. Dependence of rates of oxygen evolution on thymoquinone concentrations. The reaction conditions, see Materials and Methods. V_{max} and $V_{max'}$ values correspond to intercepts of abscissa (see Fig. 2).

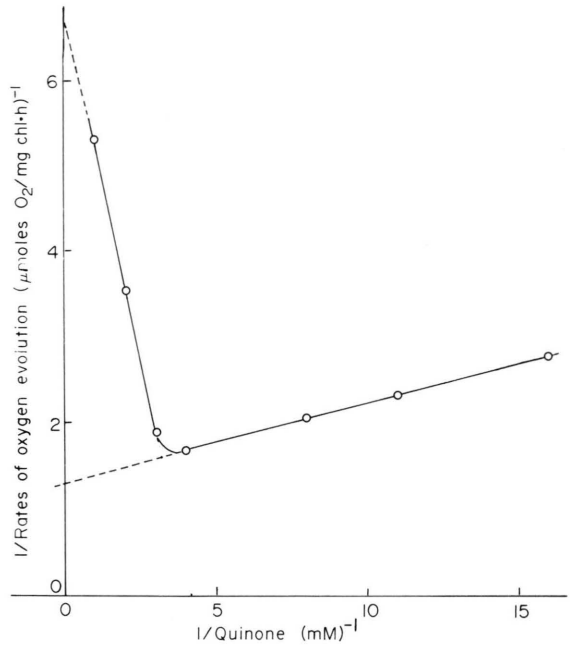

Fig. 2. Double reciprocal plots of rates of oxygen evolution and thymoquinone concentrations. Experimental conditions were the same as in Fig. 1.

gen-evolving rates)$^{-1}$ against (quinone concentrations)$^{-1}$ showed a set of two straight lines which cross at the quinone concentration of 0.25 mM (Fig. 2). This is the first example that the double reciprocal plots did not yield a single straight line in *Synechococcus* PS II particles (see Fig. 1 in ref. [11]). The V_{max} and K_m values estimated from the straight line corresponding to lower quinone concentrations were 770 μmol O_2/mg Chl·h and 0.07 mM, respectively. Note that the K_m value was about one fifth of the K_b value estimated above (see Table I) but very close to the half-binding concentration of duroquinone to plastoquinol (0.1 mM, see ref. [11]).

Because the PS II particles used in these experiments retain Q_A and Q_B but have almost no pool plastoquinone [18], there are at most two ways for thymoquinone to be reduced; reduction by plastoquinone functioning as Q_B or reduction by Q_A^- through binding of thymoquinone to the Q_B site. Similar K_m values of thymoquinone (Fig. 1) and duroquinone [11] suggest that thymoquinone, at low concentrations, accepts electrons from Q_B plastoquinone as duroquinone does [11]. On the other hand, at high concentrations, similarity of the half inhibition concentration of thymoquinone (see Fig. 1) with the K_b value (Table I) implies that thymoquinone binds to the Q_B site and accepts an electron directly from Q_A^-. According to this idea, the inhibition of electron flow at high thymoquinone concentrations (see Fig. 1) can be explained by a lower turnover rate of the quinone at the Q_B site than that of inherent plastoquinone. In order to verify this idea, we tried to find out the site inhibited by high concentrations of thymoquinone. Electron flow from Z to Q_A was not the site of inhibition because the extent of flash-induced Q_A reduction was not changed by the increase of thymoquinone concentration (data not shown). Fig. 3 shows effects of thymoquinone on the rates of Hill reaction with silicomolybdate or 2,5-DCBQ as an electron acceptor. In the presence of silicomolybdate and DCMU, the rate of oxygen evolution was very high, but the inhibition by thymoquinone was relatively small showing that the electron flow from the manganese complex to Q_A was not the main inhibition site. Larger inhibition of 2,5-

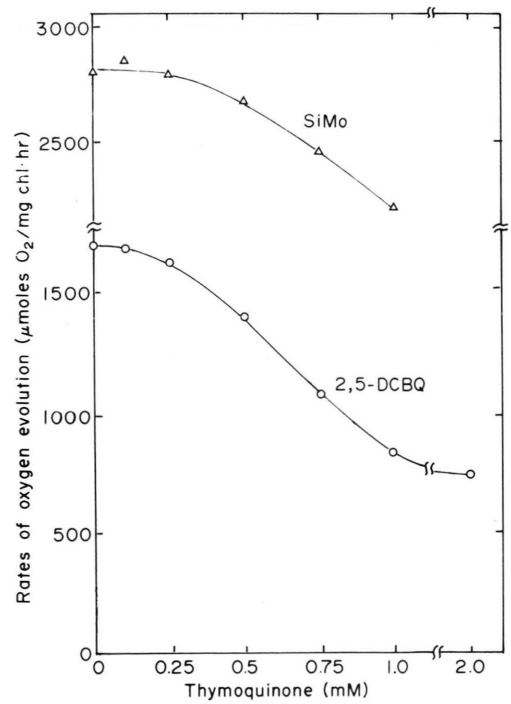

Fig. 3. Effects of thymoquinone on the photoreduction of silicomolybdate and 2,5-DCBQ. Where indicated, 10 μM DCMU, 1 mM ferricyanide and 0.5 μg/ml silicomolybdate (SiMo) or 0.4 mM 2,5-DCBQ were added. Other conditions were as in Fig. 1.

DCBQ-Hill reaction by thymoquinone indicates that the inhibition site is on the Q_B site because silicomolybdate accepts electrons from Q_A but 2,5-DCBQ accepts electrons *via* Q_B. The binding affinity of thymoquinone can be determined assuming competition of thymoquinone with 2,5-DCBQ for the Q_B site. Because the binding affinity (K_b value) of 2,5-DCBQ was estimated to be 0.20 mM, the K_b value of thymoquinone for the Q_B site was calculated to be 0.34 mM. This value was almost the same as that listed in Table I.

The concentration of thymoquinone needed to occupy a half of the Q_B site (binding constant, K_b) can also be estimated by another method. In *S. elongatus* PS II particles, the decay of absorbance at 413.5 nm after flash excitation had three components and the slowest component corresponded to the decay of semiquinone at the Q_B site [12]. If we assume that this decay is due to the release of semiquinone formed at the Q_B site, the extent of the slowest component can be regarded as the amount of bound exogenous quinone because endogenous plastosemiquinone is known to be tightly bound to the Q_B site [12] and, therefore, does not contribute to the slowest component. Fig. 4 shows that the ratio of the slowest component increased with an increase in thymoquinone concentration. The K_b value for the quinone estimated from this experiment was 0.30 mM.

In the preceding paper, we determined the K_b value for 2,5-dimethyl-*p*-benzoquinone as 0.36–0.47 mM [11]. Therefore, it seems quite reasonable to suppose that thymoquinone has a binding affinity to the Q_B site and a half of this site will be occupied by thymoquinone at around 0.33 mM.

All the data mentioned above support the idea that the oxygen evolution at low thymoquinone concentrations is maintained by the rapid electron flow from plastoquinol to thymoquinone and, at higher concentrations, the quinone binds to the Q_B site with a binding constant of about 0.3 mM and accept an electron from Q_A^-. The slower rates of oxygen evolution at higher concentrations of this quinone can be explained by a slower turnover rate of thymoquinone at the Q_B site than that of plastoquinone.

Low K_m values for duroquinone and thymoquinone and relatively high V_{max} values for both quinones (1320 and 770 μmol O_2/mg Chl·h) suggest that both affinities and rates of electron flow between these quinones and plastoquinone molecules are relatively high. In order to support high rates of oxygen evolution, the plastoquinone molecule at the Q_B site must turn over very quickly. However, the PS II particles used in these experiments have almost no plastoquinone pool [18]. Furthermore, although the molecular size of thymoquinone is much smaller than that of plastoquinone, thymoquinone was supposed to turn over more slowly than plastoquinone at the Q_B site. This suggests that only the quinone ring and its neighboring atoms (not the whole part) of the plastoquinone molecule are turning over at the Q_B site while its long side chain stayed bound to the PS II complexes (see Fig. 5). This hypothesis agrees with the results that the binding affinity of plastoquinone was mainly determined by the long side chain [19] and that various PS II particles

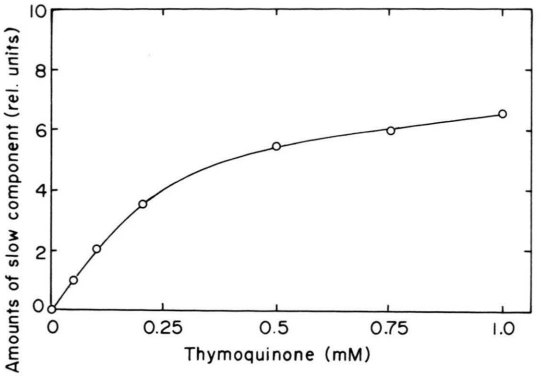

Fig. 4. Dependence of amounts of slow component (easily removable Q_B^-) on the concentration of thymoquinone. Flash-induced absorbance changes at 413.5 nm were measured and extents of the slow component were determined as in ref. [12].

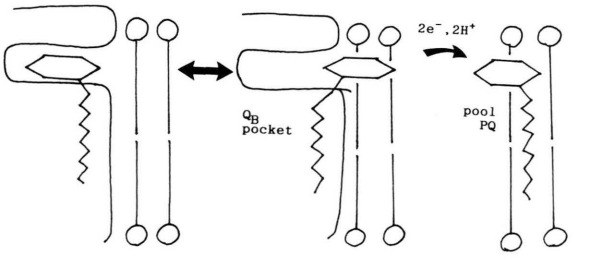

Fig. 5. A schematic model for plastoquinone function at the Q_B site. Electron flow through Q_B is supported by turnover of the head group (not the whole part) of the plastoquinone molecule at the Q_B site.

were obtained by detergent treatments with a plastoquinone molecule bound to the Q_B site but with no pool plastoquinone [15, 16, 20]. This hypothesis, however, does not agree with the data reported using bacterial reaction centers [21]. Further studies to resolve these problems are under progress.

Acknowledgements

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