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Investigation of the electron transfer site of *p*-benzoquinone in isolated photosystem II particles and thylakoid membranes using α- and β-cyclodextrins

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Abstract

The electron transfer sites of *p*-benzoquinone (*p*BQ) and 2,6-dichloro-*p*-benzoquinone (DCBQ) were investigated in thylakoid membranes and isolated photosystem II (PSII) particles from barley (*Hordeum vulgare*) using α- and β-cyclodextrins (CD) at concentrations up to 16 mM. In CD-treated thylakoid membranes incubated with DCBQ the electron transport through PSII, estimated as oxygen evolution (OE), is largely enhanced according to a S-shaped (sigmoidal) dose–response curve displaying a sharp inflection point, or transition. The maxima percent OE enhancement at cyclodextrin concentrations above 14 mM are about 100% (α-CD) and 190% (β-CD). On the contrary, in thylakoid membrane preparations incubated with *p*BQ as electron acceptor one observes an OE inhibition of about 30% which might result from the depletion of the thylakoid membrane of its plastoquinone content. It was also found that in isolated PSII particles incubated with either *p*BQ or DCBQ the cyclodextrins induce only a small OE enhancement. Moreover, the observation in CD-treated thylakoid membranes incubated with *p*BQ of a residual, non-inhibited oxygen-evolving activity of about 70% puts a twofold question. That is, either the plastoquinone depletion was not complete, or, *p*BQ binds to electron acceptor sites of different nature. From this and data published in the literature, it is concluded that in the thylakoid membrane (i) DCBQ binds to Q_B, as is generally accepted, and (ii) *p*BQ binds to the plastoquinol molecules in the PQ pool and most likely also to Q_B, thereby in accord with Satoh et al.'s model [K. Satoh, M. Ohhashi, Y. Kashino, H. Koike, Plant Cell Physiol. 36 (1995) 597–605]. An attractive alternative hypothesis is the direct interaction of *p*BQ with the non-haem Fe^{2+} between Q_A and Q_B.

Keywords: Benzoquinones; Cyclodextrins; Electron transport; Oxygen evolution; Photosystem II; PSII particles; Thylakoid membranes

1. Introduction

In oxygenic photosynthesis the photosystem II (PSII) complex catalyzes the photoinduced transfer of electrons from water to the primary and secondary quinone acceptors (Q_A and Q_B) in respectively the D2 and the D1 proteins in the PSII reaction center which is accompanied by the evolution of molecular oxygen and the formation of hydrogen bound to plastoquinone (PQ) in the PQ pool [1]. Photons are first transferred from an excited chlorophyll in the PSII antenna complex to the PSII reaction center Chl (i.e., P680) followed by the transfer of an electron from P680* to an oxidized peophytin (Phe) with concomitant formation of $P680^+$. Phe^- transfers an electron to the primary quinone Q_A [2], a PQ-9 molecule tightly bound non-covalently to the Q_A site in the PSII complex, which is reduced to the $Q_A^{-\cdot}$ form. Subsequently, $Q_A^{-\cdot}$ reduces the mobile plastoquinone Q_B, thereby forming the semiquinone $Q_B^{-\cdot}$ which has a high affinity to its binding site in the PSII complex, i.e., the Q_B site [3]. Upon a second

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reduction and a concomitant protonation, $Q_B^{-\cdot}$ becomes PQH_2 (plastoquinol) which, according to a generally accepted view, is rapidly exchanged for an oxidized plastoquinone from the PQ pool (see, e.g., [3–5]). Or, in conformity with a different conjecture, PQH_2 donates two electrons *in situ* to another plastoquinone molecule from the PQ pool through headgroup–headgroup interactions [6] that require a side-by-side arrangement of the molecules. The latter assumption, however, has recently been confronted with difficulties raised by new X-ray crystallographic determinations of the PSII structure at 3.2 Å resolution [7] and 3.5 Å resolution [8]. In short, the molecular models of the PSII dimer complex (see, e.g., 1W5C.pdb at http://www.rcsb.org/pdb/) show that the atomic displacement factor (or temperature factor, TF) of Q_B is about 25.5 $Å^2$ which is considerably larger than the TF of Q_A, i.e., 12.0 $Å^2$. What is more, comparison of these TF values with the average temperature factor for the polypeptide (21.7 $Å^2$) is an indication that the Q_B binding site is either only partly occupied or has a high flexibility degree [7]. Therefore, this might facilitate the exchange of plastoquinone molecules as is predicted in [3–5].

In *in vitro* conditions several exogenous benzoquinones have been used to study the photosynthetic activity of isolated thylakoid membranes and PSII particles. For example, a widely used electron acceptor is 2,6-dichloro-*p*-benzoquinone (DCBQ) which is thought to bind at the Q_B site (see discussions in [2,6]), therefore receiving electrons from Q_A through the non-haem Fe^{2+} located between the Q_A and Q_B sites [7,8]. On the contrary, the electron acceptor site of *p*-benzoquinone (*p*BQ), i.e., Q_B or the plastoquinone molecules in the PQ pool, is still a matter of controversy (see discussions in [6,9,10]). It is important to note, in addition, that the experimental data reported in the literature were often obtained with isolated PSII particles where the *p*BQ binding site (most likely Q_B) could differ from the *p*BQ active site in the thylakoid membrane (e.g., the PQ pool). In an attempt to clarify further this matter, we undertake hereunder a comparative study of the electron transfer from water to *p*BQ and DCBQ in isolated thylakoid membranes and PSII particles from barley (*Hordeum vulgare*) treated with α- and β-cyclodextrins.

The cyclodextrins (CD) are cyclic oligosaccharides containing, e.g., six (α-CD) or seven (β-CD) α-D-glucose residues linked by α-1,4 glycosidic bonds [11], which were shown to be efficient tools in structure–function studies of photosynthesis [12–17]. In short, experiments performed with isolated thylakoid membranes revealed that the α- and β-CDs enhance the activity of the oxygen-evolving complex [14,15] on the one hand, and inhibit the ATP synthesis [16] and the whole chain electron transport from water to photosystem I [17] on the other hand. Of particular interest is the observation that in the thylakoid membrane, but not in isolated PSII particles, the electron transport through photosystem II, measured as oxygen evolution, varies with the α- and β-CDs concentration according to a S-shaped curve displaying a sharp inflection point, or transition [17].

We found in the present work that in contrast with the large enhancement of oxygen evolution observed in CD-treated thylakoid membranes incubated DCBQ [17], the electron transfer from water to *p*BQ is strongly inhibited by α- and β-CDs. These data shall be discussed in relation to the most probable electron acceptor site of *p*BQ in the thylakoid membrane.

2. Materials and methods

2.1. Chemicals

Cyclodextrins were purchased from Fluka-Chemie (Buchs, Switzerland) and 2,6-dichloro-*p*-benzoquinone was obtained from Pfaltz and Bauer (Waterbury, CT). All other chemicals were from Fisher Scientific Company (Fair Lawn, NJ).

2.2. Plant material and growth conditions

Two hundred grams of barley seeds were put in water overnight, then placed on a 3 cm thick vermiculite bed in a tray and covered with 1 cm thick layer of vermiculite. The vermiculite bed was soaked with one liter of water and the tray was kept in dark for two days. The germinating seedlings were then grown in a growth chamber at 293 ± 2 K for 6–8 days. The light intensity on the surface of the leaves was 200 μmol photons m^{-2} s^{-1}.

2.3. Isolation of thylakoid membranes and PSII particles

Primary leaves from 6- to 8-day-old barley seedlings were used to isolate thylakoids from chloroplasts according to the procedures described in [18,19]. Briefly, the leaves were homogenized in a buffer containing 50 mM Tricine–NaOH (N-tris[hydroxymethyl]-methylglycine–NaOH) (pH 7.8), 400 mM sorbitol, 10 mM NaCl and 5 mM $MgCl_2$ (buffer A) at 273 K. The resultant slurry was filtered through eight layers of cheesecloth. The filtrate was centrifuged at 1000*g* for 5 min at 277 K to precipitate the chloroplasts which were centrifuged again upon suspension in buffer A. This chloroplast preparation was collected in a buffer containing 50 mM Tricine–NaOH (pH 7.8), 10 mM NaCl and 5 mM $MgCl_2$ (buffer B), and centrifuged immediately at 1000*g* for 5 min at 277 K. The pellet contained the thylakoid membranes which were dispersed in a buffer containing 20 mM MES-NaOH (2-[N-morpholino]ethanesulfonic acid–NaOH) (pH 6.5), 400 mM sucrose, 15 mM NaCl and 5 mM $MgCl_2$ (buffer C), and centrifuged at 1000*g* for 5 min at 277 K. The final pellet was diluted in buffer C to give a final chlorophyll (Chl) concentration of 2 mg/mL, and stored at 143 K. The chlorophyll concentration in the thylakoid preparations was measured in 80% acetone [20].

The PSII particles were prepared from isolated thylakoids according to the procedures described in [18,19]. The thylakoids were diluted in a reaction medium containing