

Studies on the inhibitory binding site of a new phenolic inhibitor of electron transfer in Photosystem II

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Introduction

PSII inhibitors, which have been classified as urea/triazine -type and the phenol-type on the basis of their structural features and their mode of inhibition (Oettmeier, 1992), had played an important role in photosynthesis research. Among these herbicides, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which inhibits the electron transport on the reducing side of the PSII at the level of Q_B side (Pfister 1983; Diner 1987), has widely been used in photosynthesis studies. Recently, a new group of highly efficient PSII inhibitor, derivatives of perfluoroisopropylidinitrobenzol, has been found (Klimov et al. 1993). Inhibitory effects of one of them, K-15 were studied in detail using PSII membrane fragments (Klimov et al. 1992; Allakhverdiev et al 1989; Kisselev et al. 1993; Zharmukhamedov et al. 1994). It is concluded that the inhibitory effect of K-15 involves a redox interaction with the components of reaction centers and on the formation of a short cyclic electron transfer that leads to the reversible separation of photoinduced charges in PS II. It is also suggested that K-15 occupies the Q_A-binding site and serves as a substitute for Q_A (Klimov et al. 1992; Zharmukhamedov et al. 1994). However, direct evidence has not been obtained and its precise binding site of K-15 remains unclear. In this report, we investigated the possibility of replacement of Q_A at its binding site by the inhibitor in core complexes by using the HPLC method.

2. Materials and methods

Thylakoid and photosystem II membrane fragments capable of O₂ evolution were prepared from spinach as described earlier (Berthold et al. 1981). Core complexes were isolated from BBY particles according to Enami et al (1989). Trypsin digestion of thylakoid was performed according to Yauella (1991). O₂ evolution activity was measured with a Clark-type electrode fitted with a circulating water jacket at 25°C with 1mM ferricyanide as electron acceptor.

The replacement of Q_A by inhibitor K-23 was performed by incubating the core complexes treated with the inhibitor in 0.4M Sucrose, 10mM NaCl, 5mM MgCl₂ and 40mM Mes-NaOH (pH 6.0) for 20 h at 20 °C in the dark. After centrifugation and lyophilization, the core complexes were extracted at 20 °C for 3h in hexane containing 0.1-0.2% methanol in the dark and collected by centrifugation. The content of plastoquinone was analyzed using Reverse Phase HPLC by the method of Zheleva et al (1997).

Absorption spectra were measured with UV-943 dual-beam spectrophotometer.

Results and discussion

Trypsinization of thylakoids:

Trypsin sensitivity of photosynthetic electron flow via Q_B in PSII is long known (Regitz and Ohad 1976). Trypsinization of thylakoids decreased O_2 -evolution using DCBQ as electron acceptor, while it remained practically constant in the presence of ferricyanide (Renger 1976; Yruela et al. 1991). Electron flow from water to ferricyanide after trypsin treatment of membranes became insensitive to inhibitors, like DCMU (Renger 1976; Regitz and Ohad 1976). This is shown again in Fig.1 to compare the inhibitory site of inhibitor K-23 with that of DCMU. Trypsin treatment decreased the inhibitory effectiveness of DCMU, which indicate that trypsin treatment significantly modified the Q_B niche and DCMU-bind site (Renger 1976; Trest et al. 1988). With the same trypsin treatment, however, it is found that K-23 becomes even more effective (Fig. 2). Stimulation of O_2 -evolution at low concentrations of K-23 observed in control disappeared after trypsin treatment. Therefore, trypsin treatment significantly modified the K-23-binding site and decreased the accessibility of K-23 to reaction center, which led to disappearance of the redox interaction between K-23 and the component of reaction center. From the comparison of K-23 with DCMU, we can conclude that the binding site of K-23 is different from that of DCMU.

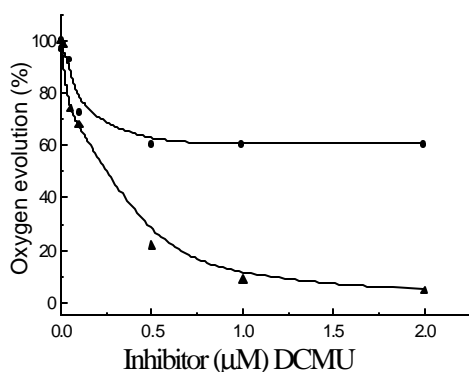


Fig. 1 Effect of DCMU on the oxygen evolution of control and trypsin-treated thylakoids. Control (closed triangle) and trypsin-treated (closed diamond) (100 μ g / ml trypsin for 3 minutes) are measured in the presence of 1mM ferricyanide.

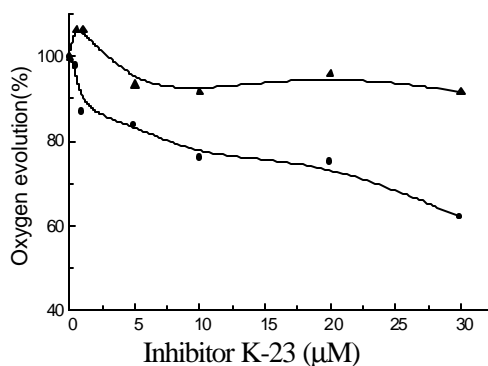


Fig. 2 Effect of K-23 on the oxygen evolution of control and trypsin-treated thylakoids. Activity of control and trypsin-treated (symbols as for Fig 1 (100 μ g / ml trypsin for 3 minutes) are measured in the presence of 1mM ferricyanide.

In order to identify the precise binding-site of K-23, the effects of K-23 on the content of plastoquinone in core complexes of PSII were investigated by using HPLC method to identify possible replacement of the primary electron acceptor of PS II, plastoquinone Q_A , at its binding site by K-23.

3.2 HPLC analysis of the content of plastoquinone

We investigated the exchange of Q_A by the inhibitor K-23 by HPLC method, which provide an effective method to investigate the biochemistry of exchange. The elution time of plastoquinone extracted from core complex of PSII in the absence and presence of K-23 (10 molecules per RC and 100 molecules per RC) was detected at 4.06, 4.14, 4.12 minutes, respectively. It is found (Fig.3) that inhibitor K-23 did not affect obviously elution times of plastoquinone, but it affected the absorption profiles. That could means that Q_A is disturbed by the inhibitor K-23 at its binding site, but it is not replaced by K-23. Presence of K-23 also results in decrease of peak area of plastoquinone. From the above results, it is suggested that K-23 may bind at the Q_A site by reacting with it. To further verify the results, absorption spectra of plastoquinone extracted from core complex of PSII were measured. The absorption spectra of plastoquinone in Fig. 4 showed the peaks at 359nm were strongly affected in the presence of K-23.

In short, we proposed from the above results that inhibitor K-23 binds at the Q_A site by reacting with it other than by replacement of plastoquinone. Further research is

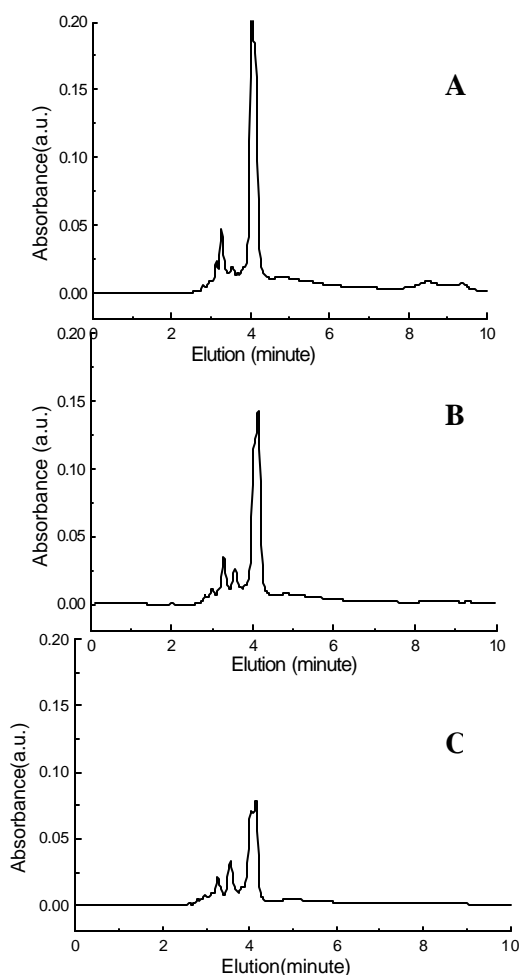


Fig. 3 Reverse phase HPLC elution profiles of plastoquinone detected at 255nm. in the absence (A) and presence (B, C) of K-23: B, 10 molecules per RC; C, 100 molecules per RC

required to verify it.

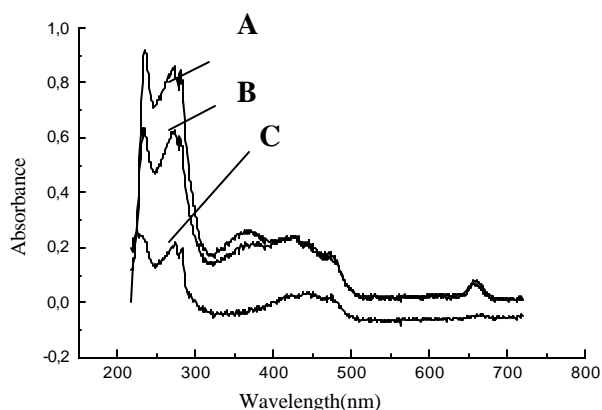


Fig. 4 The absorption spectra of plastoquinone extracted from core complex Control (A) and presence 10 (B) and 100 (C) molecules of K-23 per PSII RC

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