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Effects of a new inhibitor K-23 on electron transport in photosystem II of higher plants

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Abstract

The effects of inhibitor K-23 on variable fluorescence, oxygen evolution and DCIP photoreduction were investigated. K-23 promotes the oxygen evolution and DCIP photoreduction at low concentration and inhibits them at relatively high concentrations, while an efficient inhibition at low concentration is found in variable fluorescence. These data further confirm that the inhibitor K-23 action is based on its redox interaction rather than quenching effect. Addition of DPC could not restore the DCIP photoreduction activity. It is suggested that the inhibitory site is at the acceptor side. Using ferricyanide as electron acceptor, the effect of K-23 and DCMU on the oxygen evolution of trypsin-treated thylakoids was investigated. It is found that oxygen evolution of trypsin-treated thylakoids was insensitive to DCMU, whereas became more sensitive to K-23 and also the promotion of K-23 at low concentration disappeared. This strongly indicates that trypsin treatment modified the binding site of K-23 and increased its accessibility to K-23 target site. From the comparison of K-23 with DCMU, we conclude that the binding site of K-23 is different from that of DCMU even though they both bind at the acceptor side.

Keywords: Inhibitor K-23; Variable fluorescence; Oxygen evolution; DCIP photoreduction; Binding site

1. Introduction

A wide range of chemicals is known to inhibit electron transport process of photosystem (PSII). Many of these chemicals have become important commercial herbicides. PSII inhibitors have

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usually been classified as urea/triazine-type and the phenol-type by reference to their structural features, as well as their mode of inhibition [1]. Among these herbicides, DCMU [3-(3,4-dichloro phenyl)-1,1-dimethylurea] has widely been used in photosynthesis studies. A precise location of the inhibitory binding site has been established for DCMU in plants [2,3]. This herbicide inhibits the electron transport on the Q_B site of reducing side of the PSII [4–6]. The electron transport activities of PSII can be inhibited completely by DCMU at very low concentration.

Recently, a new group of highly efficient PSII inhibitors, derivatives of perfluoroisopropyldinitrobenzol, has been found [7]. Inhibitory effects of one of them, K-15, were studied in detail using PSII membrane fragments [8–10] and reaction center [11]. It is concluded that the inhibitory effect of K-15 involves a redox interaction of this inhibitor with the components of reaction centers.

K-23(4-[benzoylhydroxy-bis-(trifluormethyl)methyl]-2,6-dinitrophenyl hydrazone), one of the derivatives of perfluoroisopropyldinitrobenzol, has also been found to inhibit the electron transfer in PSII and chloroplast preparation of higher plants [7]. However, its inhibitory effect was not investigated systematically and its inhibitory binding site is not clear. In this paper, the effects of K-23 on the electron transfer in PSII were investigated and its inhibitory binding site was also studied and compared with that of DCMU.

2. Materials and methods

Thylakoids and PSII membrane capable of O_2 evolution were prepared from spinach by the method of Berthold [12].

O_2 evolution activity was measured with a clark-type electrode fitted with a circulating water jacket at 25 °C. The assay medium for thylakoids comprised 330 mM sucrose, 10 mM NaCl, 10 mM Tricine–NaOH (pH 7.0) and with 0.5 mM DCBQ (2,6-dichlorobenzoquinone) or 1 mM ferricyanide as electron acceptor. The assay medium for PSII comprised 330 mM sucrose, 10 mM NaCl, 20 mM Mes–NaOH (pH 6.5) with 100 μM DCBQ plus 1 mM ferricyanide as electron acceptor.

The DCIP (2,6-dichlorophenolindophenol) photoreduction was monitored at 580 nm, in the presence of 30 μM DCIP using the UV190 dual-beam spectrophotometer. The extinction coefficient $\Delta\varepsilon$ at 580 nm is 12.9 $mM^{-1}\,cm^{-1}$. The Chl concentration of PSII and thylakoids in oxygen evolution and DCIP photoreduction measurements are 10 and 20 $\mu g\,ml^{-1}$, respectively.

The yield of Chl fluorescence was continuously monitored in a modulated fluorometer (PAM Chl fluorometer; Walz, Effelrich, Germany). PSII particle suspensions were placed in a stirred cuvette (25 °C). Maximum fluorescence level of light-adapted cells ($F_{m'}$) was measured using a 600 ms high-intensity white light pulse (1200 $\mu E\,m^{-2}\,s^{-1}$) which was produced by KL-1500 lamp (Schott, Germany). The chlorophyll concentration of PSII is 10 $\mu g\,ml^{-1}$.

Trypsin digestion of thylakoids was performed by incubating thylakoids with trypsin for 3 min and proteolysis was stopped by adding twice the amount of trypsin inhibitors [13]. Trypsin and trypsin inhibitors were dissolved in buffer consisted of 330 mM sucrose, 10 mM NaCl, 10 mM Tricine–NaOH (pH 7.0).

K-23 was provided by Professor V.V. Klimov and its synthesis was described in reference [14]. The DCBQ, DCMU, and inhibitor K-23 were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was less than 1%, which did not affect the electron transport in PSII.

3. Results

The inhibitory effect of K-23 on variable fluorescence is showed in Fig. 1. To gain deeper insight on the K-23-inhibitory effect, we also studied the inhibition curves of well-known diuron inhibitor DCMU for comparison. It is found that DCMU effectively inhibits the F_v at low concentration. 20% F_v was inhibited by 1 μM DCMU and then F_v remain invariable even with increasing concentration. However, K-23 performs different inhibitory behaviors from that of DCMU. K-23 strongly inhibits the F_v and 55% F_v was inhibited