Inhibition of Photosynthetic Electron Transport by the Quinone Antagonist UHDBT

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UHDBT (5-n-undecyl-6-hydroxy-4,7-dioxobenzothiazole) is an efficient inhibitor of photosynthetic electron transport in chloroplasts from spinach (pl50-value = 7.61) and the green alga Chlamydomonas reinhardii. At low concentrations of UHDBT its site of inhibition is located at the reducing side of plastoquinone, identical to that of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). This became evident from functional as well as binding studies. At higher concentrations of UHDBT a secondary inhibition site at the oxidizing side of plastoquinone, identical to that of 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone (DBMIB) becomes evident.

Introduction

UHDBT [1] has recently been introduced as a powerful inhibitor of ubiquinone function in respiration [2, 3] and in bacterial photosynthesis, where it inhibits the oxidation of ubiquinone [4, 5]. It was of special interest, therefore, to investigate the inhibitory activity of UHDBT on the plastoquinone function in the electron transport system of isolated chloroplasts.

As we wish to report here, UHDBT proved to be an efficient inhibitor of electron flow in chloroplasts from spinach and the green algae Chlamydomonas reinhardii as well. Its side of inhibition at low concentrations, however, is located at the reducing site of plastoquinone. UHDBT in its inhibition pattern thus resembles the type of herbicides commonly referred to as DCMU-type inhibitors [6]. At high concentrations of UHDBT, a secondary inhibitory effect at the oxidizing site of plastoquinone, i.e. identical to that of the well known plastoquinone antagonist DBMIB [7] can be observed.

Abbreviations: BSA, bovine serum albumin; Chi, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMDBOQ, 2,3-dimethyl-5,6-methylenedioxy-1,4-benzoquinone; DNP-INT, 2,4-dinitrophenylether of 2-iodo-4- nitrothymol; DQH2, durohydroquinone; metribuzin, 4-

Materials and Methods

Chloroplasts from spinach were isolated according to Nelson et al. [8] and stored in liquid nitrogen in the presence of 10% glycerol until use. Chloroplasts from the mutant Chlamydomonas reinhardii CW-15 [9] were prepared according to [10] with minor modifications. Harvested cells were washed in isolation buffer which in addition contained 0.6 M sucrose and 1 mg BSA/ml. After osmotic shock in 5 mM Hepes-buffer, pH 7.5, containing 1 mg BSA/ml, whole cells were centrifuged down at 300 x g and suspended in isolation buffer containing 0.3 M sucrose and 1 mg BSA/ml.

Photosynthetic NADP-reduction by spinach chloroplasts was measured at 340 nm in a Zeiss PMQ II spectrophotometer modified for illumination with white light at an intensity of 0.2 W m⁻².

MV-catalyzed photosynthetic oxygen consumption was determined in a Clark-type teflon covered oxygen electrode (Rank Brothers, Bottisham, England) illuminated with red light (filter OG 570, Schott, Mainz, Bundesrepublik Deutschland) at an intensity of 0.8 W m⁻².

Displacement experiments with [¹⁴C]metribuzin were performed as described recently [11]. UHDBT and [¹⁴C]metribuzin were generous gifts by Dr. A. R. Crofts, Urbana, Ill. (USA), and Dr. W. Draber, Forschungszentrum Wuppertal, Bayer AG (Bundesrepublik Deutschland), respectively.
Results

Like in mitochondria and in bacterial chromatophores, UHDBT is an efficient inhibitor of electron flow in isolated spinach chloroplasts. In Fig. 1 this is demonstrated for uncoupled photosynthetic electron transport from water to NADP. From the data of Fig. 1, a $p_{50}$-value (i.e. negative logarithm of the concentration which leads to 50% inhibition) of 7.61 could be calculated. This $p_{50}$-value is practically identical to that of DBMIB (7.52 [12]).

However, as will be proved in the following experiments, its site of inhibition at low concentrations is different from that of DBMIB. In Fig. 2 (left side) is demonstrated that O$_2$-consumption in a MV-catalyzed Mehler-reaction is almost completely inhibited by UHDBT. The same is true for DBMIB (Fig. 2, right side). Inhibition of photosynthetic electron flow by UHDBT cannot be reversed by the photosystem II acceptor DMMDBQ [13] (Fig. 2, left side). The same experiment performed with DBMIB (Fig. 2, right side) shows that in this case photosynthetic electron flow is restored. The influence of UHDBT and DBMIB on electron flow from the artificial donor DQH$_2$ which feeds electrons into the plastoquinone pool [14, 15] was compared (Fig. 3, right side). The inhibition in MV-catalyzed oxygen consumption by UHDBT is overcome by addition of DQH$_2$. In contrast, electron flow in this system is inhibited by addition of DBMIB (Fig. 3, left side, see Fig. 2).

Fig. 1. Inhibition of uncoupled photosynthetic NADP-reduction by UHDBT. The reaction mixture contained in a volume of 2 ml: 20 mM Tricine/NaOH (pH 8.0), 5 mM MgCl$_2$, 17.5 $(x$ gramicidine, 1.5 mM NADP, 5 $(i$M ferredoxin from spinach and chloroplasts corresponding to a concentration of 14 $(ig Chl.

Fig. 2. Comparison of inhibitory effects of UHDBT and DBMIB on oxygen consumption in the systems H$_2$O to MV and H$_2$O to DMMDBQ. The reaction mixture contained in a volume of 3 ml: 26 mM Tricine/NaOH (pH 8.0), 3.3 mM MgCl$_2$, 0.5 mM MnCl$_2$, 0.33 mM NaN$_3$, 1 $(i$M gramicidine, 0.33 mM MV and chloroplasts corresponding to a concentration of 50 $(ig Chl.

Fig. 3. Comparison of effects of UHDBT and DBMIB on oxygen consumption in a MV catalyzed Mehler-reaction. DQH$_2$ was used as a photosystem I donor (left side). UHDBT-inhibition cannot be overcome by TMPD (right side). Same conditions like in Fig. 2.

Fig. 4. Displacement of [$^{14}$C]metribuzin (10$^{-7}$ M) from the thylakoid membrane by DBMIB (■ -■ ) and UHDBT (• - • ).
Inhibition of various electron transport systems in *Chlamydomonas* chloroplasts by UHDBT (closed symbols) and DNP-INT (open symbols). The assay medium contained in a volume of 3 ml: 60 mM Hepes/NaOH (pH 7.0), 3 mM MgCl₂, 0.3 mM NaN₃, 15 μg gramicidine, 0.1 mM MV and chloroplasts corresponding to a concentration of 50 μg Chl. Where indicated the medium contained in addition 4 mM NADH or 0.5 mM DQH₂.

Discussion

Substituted benzoquinones have drawn considerable interest as inhibitors of quinone function in respiratory and photosynthetic electron transport. In chloroplasts from higher plants DBMIB is the most widely used quinone-type inhibitor [7]. From studies with DBMIB-analogues we have concluded that the lipophilicity of the quinone governs its inhibitory activity of photosynthetic electron flux at plastoquinone function [12]. A quantitative structure activity relationship for inhibitory activity using the hydrophobic parameter π as the only variable could be established [12]. Recently, UHDBT has been introduced as a potent inhibitor of ubiquinone function in respiration, where it inhibits electron transfer in the cytochrome b-c₁ segment of the respiratory chain i.e. of ubiquinol oxidation [2, 3]. Similarly, in bacterial photosynthesis UHDBT interferes with the photoreduction of cytochrome b₅₆ and the rereduction of cytochrome c₂, possibly via the Rieske iron sulfur center [4, 5]. This indicates an interference with ubiquinol oxidation. UHDBT is also characterized by a high lipophilicity due to the long alky side chain in the 5-position.

As demonstrated in this paper, UHDBT proved to be an efficient inhibitor of the photosynthetic electron transport chain in isolated chloroplasts from the green alga *Chlamydomonas reinhardii* (Fig. 5). Oxygen consumption in the system H₂O to MV is inhibited to 50% at a concentration of 2 × 10⁻⁷ M UHDBT. Evidence for the inhibition site comes again from studies with electron donor systems. As we have recently reported, NADH can function as electron donor for photosystem I in *Chlamydomonas* particles [10]. When NADH or DQH₂ are used as donors, 50% inhibition can only be achieved at the much higher concentration of 5 × 10⁻⁶ M UHDBT (Fig. 5). The same is true for the donor system DQH₂. The inhibition of the latter systems has to be compared again with the inhibition pattern as observed with the DBMIB-analogue DNP-INT [17]. DNP-INT impairs electron flow from NADH or from DQH₂ to MV to 50% at a concentration of about 10⁻⁷ M (Fig. 5).
spinach and the green alga Chlamydomonas reinhardti. However, despite its quinone character, UHDBT has an inhibition pattern which is different from DBMIB. This conclusion has been reached from the following observations: (i) photoreductions by photosystem II as mediated by the acceptor DMMDBQ [13] are inhibited by UHDBT but not by DBMIB. (ii) The opposite is true for the photosystem I donor DQH₂. This system is inhibited by DBMIB but not UHDBT. (iii) Inhibition by UHDBT cannot be bypassed by TMPD as compared against DBMIB [16]. In all these experiments DBMIB behaves in the opposite way to UHDBT. These results indicate that UHDBT at low concentrations inhibits at a site closer to photosystem II than that of DBMIB. This has also been assumed for four other benzoquinones (2,3-dimethyl-1,4-benzoquinones with the following additional substituent(s): 5-bromo-6-n-octadecyl-mercaptop; 5-n-hexadecylamino; 5-oleyl-amino; 5-n-propylamino), which in their substitution pattern are similar to UHDBT but much less effective (pL₅₀-values of 4.27, 3.90, 3.90 and 3.52, respectively) [18]. In our recent investigations on quantitative structure activity relationship of DBMIB analogues we have also found that some 1,4-benzoquinones differed from the others. From 29 1,4-benzoquinones tested, 9 (for instance trimethyl-, tetramethyl-, tetrafluoro-, tetrachloro-, tetrabromo-, tetrabromo-1,4-benzoquinone) did not exhibit a TMPD bypass [12]. For these an inhibition site identical to that of UHDBT has to be assumed.

Direct evidence for the inhibition site of UHDBT comes from displacement experiments with a radioactively labelled inhibitor. As Tischer and Strotmann [19] have shown, inhibitors which share identical binding and consequently inhibition sites can displace each other competitively from the thylakoid membrane. UHDBT efficiently displaces [¹⁴C]mitrajubin which has been shown to be a DCMU-type inhibitor [6]. Inhibitors of this type interrupt the photosynthetic electron transport chain by binding to the so-called B-protein, and in term by preventing the binding of plastoquinone. UHDBT at low concentrations, therefore, is a DCMU-type inhibitor. For that a possible explanation might be that the thiazole moiety in UHDBT in some respect has resemblance to the structural element N-C=X (where X=N or O) which is considered essential for inhibitory activity of DCMU-type inhibitors [6].

However, as concluded especially from the experiments with chloroplasts from Chlamydomonas at higher concentrations of UHDBT a secondary inhibition site of UHDBT becomes evident. Because at higher concentrations UHDBT prevents electron flow to plastocyanin, from artificial electron donors, this mode of inhibition is identical to that of DBMIB or DNP-INT. The concentration of UHDBT required for this type of inhibition corresponds to that reported previously for inhibition of electron flow in mitochondria and photosynthetic bacteria [2–5].

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