Studies about the Mechanism of Herbicidal Interaction with Photosystem II in Isolated Chloroplasts

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Functional and Structural Organization of System-II-Electron Transport, Regulation of Electron Transport, Mechanism of Inhibition by DCMU-Type-Herbicides, Properties of Trypsinated Chloroplasts

Based on the functional organization scheme of system-II-electron transport and its modification by different procedures a proteinaceous component enwrapping the redox components (plastoquinone molecules) of the acceptor side (thereby acting as regulatory element) is inferred to be the unique target for herbicidal interaction with system II. This proteinaceous component, which is attacked by trypsin, provides the receptor sites for the herbicides. Studies of the release kinetics in trypsinated chloroplasts of the inhibition of oxygen evolution with $K_3[Fe(CN)_6]$ as electron acceptor indicates, that there exists a binding area with different specific subreceptor sites rather than a unique binding site for the various types of inhibitors. Furthermore, trypsination of the proteinaceous component enhances the efficiency of the plastoquinone pool to act as a non-photochemical quencher for excitation energy.

Based on the interpretation of fluorescence induction [1] a great variety of potent herbicides with DCMU (Diuron) as its prototype were inferred to interrupt the electron transport on the reducing side of system II between the "primary" acceptor X 320 and the plastoquinone pool (for rev. s. ref. [2]). This site appears to be a unique target for the interference of various chemicals with system-II-electron transport. Accordingly, the question arises about the properties which make the acceptor side of system II so highly susceptible to inhibition by a great number of herbicides. It is now generally accepted that the redox components involved in the electron transfer at the acceptor side are plastoquinone molecules, which are not known to react specifically with the above mentioned herbicides. Experiments with mildly trypsinated chloroplasts led to the postulation of a model for the structural and functional organization of the electron transfer between the plastoquinone molecules (X 320, B and the plastoquinone pool), which basically assumes that the redox components X 320 and B [3, 4] are embedded into a proteinaceous component [5]. This proteinaceous component is assumed to act as the indispensible regulator for the electron transport from X 320 into the plastoquinone pool and as a shield which concomitantly prevents the reaction between X 320 and exogeneous redox components. Furthermore, the proteinaceous component was claimed to contain also the binding site (s) for DCMU-type inhibitors and therefore an allosteric mode of action is postulated [5]. Recent binding experiments of Tischer and Strotmann [6] as well as studies by Arntzen *et al.* on chloroplasts isolated from atrazine resistent plants [7] support the existence of a DCMU-type susceptible protein attached to the acceptor side of system II. Moreover, the data of Arntzen *et al.* and a refined analysis of structure-activity correlations of various derivatives of different classes of herbicides interfering with system-II-electron transport led to the conclusion that there exists a binding area with different receptors and subreceptors for all DCMU-type inhibitors [8].

Mild trypsination of chloroplasts was found to remove the blockage of electron transport by DCMU-type inhibitors provided that an appropriate electron acceptor is present, such as $K_3[Fe(CN)_6]$ [5, 9]. Accordingly, trypsin appears to be a promising tool for the investigation of the molecular mechanism of the inhibition by DCMU-type herbicides.

In the present communication the effect of trypsin incubation on the system-II-electron transport and on the kinetics of the release of blockage is reported

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MES, morpholinethanesulfonate; Metribuzin, 4-amino-6-isopropyl-3-methylthio-1,2,4-triazine-5-one; Ph-p-BQ, phenyl-*p*-benzoquinone; SN 58132, 3-isopropoxycarbonylaminophenyl-N-ethyl carbanilate; Tricine, N-tris-(hydroxymethyl)-methylglycine.

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for different DCMU-type inhibitors. Furthermore, it is shown that trypsination decreases the photochemical quantum yield. The present results corroborate the model described in ref. [5].

Materials and Methods

The preparation of the class-II-chloroplasts according to the method of Winget *et al.* [10] and the procedure of mild trypsination are described in ref. [11].

The oxygen generated under repetitive flash ($\sim 20\ \mu s$) excitation conditions was detected with a Clark-type electrode by the method outlined in ref. [12].

The reaction mixture contained: chloroplasts (50 μM chlorophyll), 10 mM KCl, 2 mM $MgCl_2$ and 20 mM tricine-NaOH at pH = 7.0 or MES-NaOH at pH = 6.0 and 6.5. The addition of electron acceptors (1 mM $K_3[Fe(CN)_6]$ or 100 μM *p*-benzoquinone or 300 μM phenyl-*p*-benzoquinone) is indicated in the figures.

Results and Discussion

The general effect of mild trypsination on the oxygen evolution in isolated chloroplasts with $K_3[Fe(CN)_6]$ as electron acceptor is schematically depicted in the insert of Fig. 1 (s. ref. [11]). In the absence of DCMU the average oxygen yield per flash gradually declines, probably reflecting the degradation of system Y. On the contrary, in the presence of DCMU at concentrations sufficient for a practically complete suppression of system-II-electron transport under repetitive excitation conditions, the average oxygen yield per flash firstly increases with the incubation time until it reaches nearly the same level and than decreases in the same manner as in the absence of DCMU. Accordingly, the difference between both curves, symbolized by $\Delta O_2(t_{inc})$, as a function of the incubation time can be interpreted as digestion kinetics of the native structure of the proteinaceous component which is required for an effective blockage of electron transport by DCMU-type inhibitors. As the modification of the proteinaceous component by trypsin simultaneously interrupts the electron flow from X 320 to the plastoquinone pool, one can anticipate that the kinetics of the latter effect closely resembles that of the release of DCMU-type blockage. In Fig. 1 the average oxygen yield as a function of the incubation in the presence of 100 μM *p*-benzoquinone (*p*-BQ) as electron acceptor is compared with

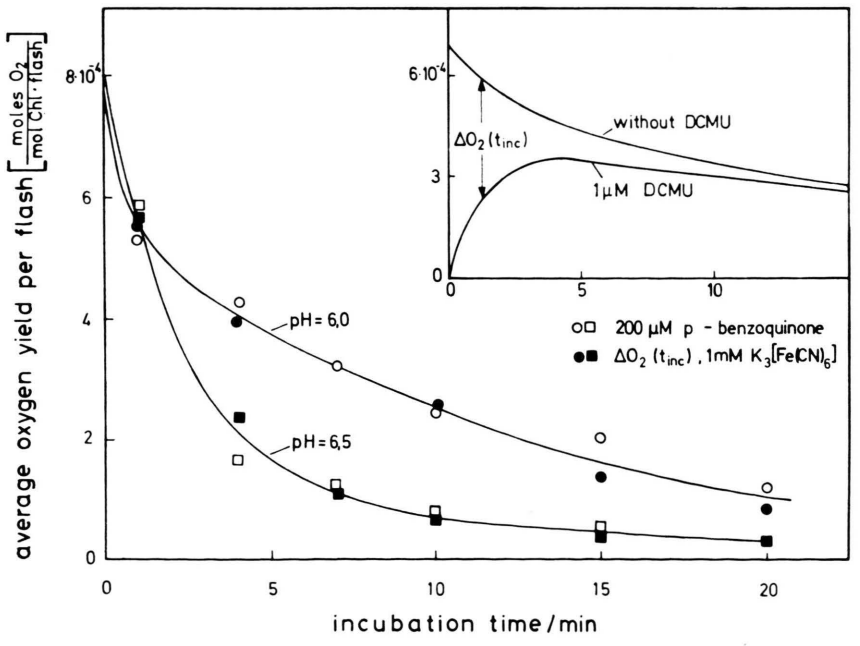

Fig. 1. Average oxygen yield per flash and difference ΔO_2 (t_{inc}) as a function of incubation time with trypsin in isolated chloroplasts at pH = 6.0 and 6.5, respectively. Electron acceptors (200 μM *p*-benzoquinone and 1 mM $K_3[Fe(CN)_6]$, respectively) as indicated in the figure, other conditions as in Materials and Methods.

$\Delta O_2(t_{inc})$ obtained with 1 mM $K_3[Fe(CN)_6]$ as electron acceptor in the absence and presence of 1 μM DCMU, respectively. The decline of oxygen evolution with incubation time in the presence of *p*-benzoquinone reflects the increasing degree of interruption of the electron transport [5], while $\Delta O_2(t_{inc})$ indicates the progressive release of DCMU-type inhibition. The experiments were performed at two different pH-values because the activity of tryptic digestion is known to be strongly pH-dependent (it must be emphasized that above pH = 7.0 the trypsination of chloroplasts concomitantly leads to a rapid damage of system Y). The data obtained indicate that the tryptic degradation kinetics of the electron transport nearly coincide with the release of the DCMU-type inhibition. Within the framework of the model discussed in ref. [5] this effect can be interpreted by two alternative explanations: a) Mild trypsination modifies the proteinaceous component in such a way that the electron transport is interrupted and simultaneously X 320 becomes accessible to exogeneous electron acceptor, *e.g.* $K_3[Fe(CN)_6]$, without changing the binding affinity for DCMU-type inhibitors. b) The tryptic attack concomitantly changes the properties of the binding area to DCMU-type inhibitors within the proteinaceous component. The data of Fig. 1 do not provide an answer to this problem. However, latest data of Tischer and Strotmann (s. contribution to this volume) indicating a correlation between release of DCMU-type (metribuzin) inhibition and the number of binding sites favour the hypothesis b). In order to get further information, the effect of trypsin on the release of the DCMU-type blockage has been investigated for different classes of inhibitors.

In Fig. 2 the average oxygen yield per flash as a function of the incubation time of trypsin is shown for 3 different DCMU-type inhibitors: DCMU, metribuzin and SN 58132. The data clearly indicate that there exist small, but distinguishable differences for the release of the inhibitory effect.

If one admits that the modification rate by trypsin of the proteinaceous component is independent of the chemical structure of bound DCMU-type inhibitors, then the results of Fig. 2 are only reconcilable with hypothesis b). Within the framework of this model the data further indicate that there does not exist a unique binding site for all DCMU-type inhibitors. They rather favour the

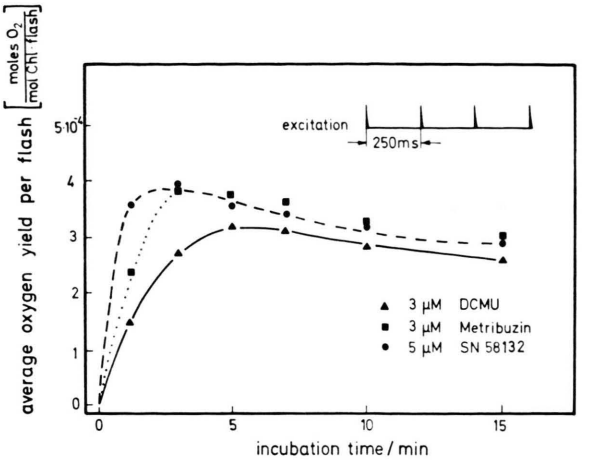

Fig. 2. Average oxygen yield per flash as a function of incubation time in the presence of different DCMU-type inhibitors. Addition of inhibitors as indicated in the figure, other conditions as in Materials and Methods.

existence of a binding area with different receptor and subreceptor sites, which are responsible for specific inhibition binding, despite of the common effect of blockage system II electron transport between X 320 and B. This model is consistent with the data of Arntzen *et al.* [7] obtained on chloroplasts isolated from atrazine resistant plants which still retain a rather high blockage efficiency by DCMU.

Now the question arises about the molecular mechanism of inhibition caused by the binding of various classes of chemicals to the proteinaceous component(s) embedding the plastoquinone molecules X 320, B and the outer segment of the pool. Generally, two different modes of action have to be considered: A) *Kinetic* effect, b) *Thermodynamic* effect. The structural modification of the proteinaceous component due to binding of DCMU-type inhibitors could change the mutual orientation and separation of the special plastoquinone molecules X 320 and B, so that the height and/or the width of the barrier for the electron transfer between X 320 and B becomes significantly increased, thus *kinetically* blocking this reaction. The electron transfer between X 320 and B can also *thermodynamically* be regulated via their redox potentials which are dependent on the microenvironment, including the protonation state. Accordingly, a modification of the proteinaceous component caused by inhibitor binding could shift the redox potential of the couple B^-/B markedly below that of $X320^-/X320$,

so that be electron transport would be prevented for thermodynamic reasons. A shift due to DCMU of the equilibrium of the reaction $X320^- + B \rightleftharpoons X320 + B^-$ towards $X320^- + B$ has been demonstrated by luminescence and fluorescence measurements [13, 14]. Furthermore, it was speculated that the bicarbonate effect on the electron transfer between $X320^-$ and B^- [15] might be caused by a redox potential shift accompanying the dissociation of a complex between B and HCO_3^- [16]. Recent studies of the proton transport pattern also led to the conclusion [17], that a modification of the proteinaceous component by mild trypsination changes the thermodynamic properties of X 320 (and/or B). Therefore, at the present stage of knowledge the interpretation of the DCMU-type induced blockage by a *thermodynamic* effect appears to be very attractive, but a kinetic interruption cannot be excluded.

Based on fluorescence measurements [18] the modificiation of the proteinaceous component by trypsin is expected to decrease additionally the photochemical quantum yield due to a significant increase of the nonphotochemical quenching by the plastoquinone pool [19]. As the electron transfer from photosystem II becomes interrupted, the plastoquinone pool remains in the oxidized quenching state and additionally the contact between plastoquinones and bulk chlorophylls might become closer by trypsin induced structural changes of the proteinaceous shield. In Fig. 3 there is depicted the dependence on actinic flash intensity of the relative average oxygen yield per flash in normal and in trypsinated chloroplasts with $K_3[Fe(CN)_6]$ as electron acceptor. If one assumes a simple Poisson-distribution of the light quanta and furthermore the quenching efficiency, Φ_Q, in normal chloroplasts is used as reference with $\Phi_Q = 0$, then trypsination increases the relative quenching efficiency by about 50%. If phenyl-*p*-benzoquinone (Ph-*p*-BQ) is used as electron acceptor instead of $K_3[Fe(CN)_6]$, then the quenching efficiency in normal chloroplasts is even higher, because external quinones are known to create nonphotochemical quenching traps within the bulk pigment system [20]. Accordingly, it is inferred that trypsination makes the endogeneous plastoquinones of the pool to act as efficient nonphotochemical quenchers.

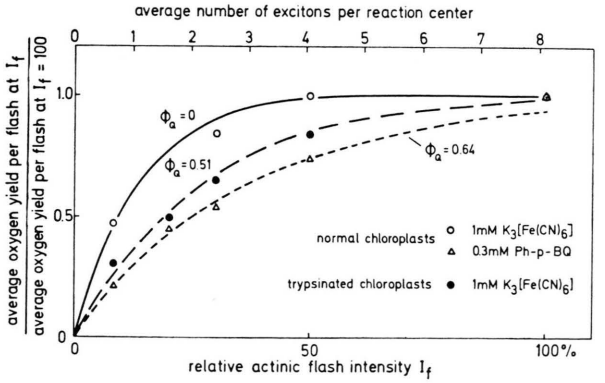

Fig. 3. Relative average oxygen yield per flash as a function of relative actinic flash intensity in normal and trypsinated chloroplasts. Addition of electron acceptor as indicated in the figure, other conditions as in Materials and Methods.

According to the proposed model for the functional and structural organization of system II electron transport [5], depicted in Fig. 4 the proteinaceous component enwrapping the plastoquinone molecules plays the central role as a regulatory element and as the target for DCMU-type herbicides. Inhibitor binding leads – probably via thermodynamic effects (redox potential shift) – to allosteric blockage of the electron transfer from X 320 to B. The nature of this proteinaceous trypsin sensitive component, especially a possible subunit structure, remains an unresolved problem.

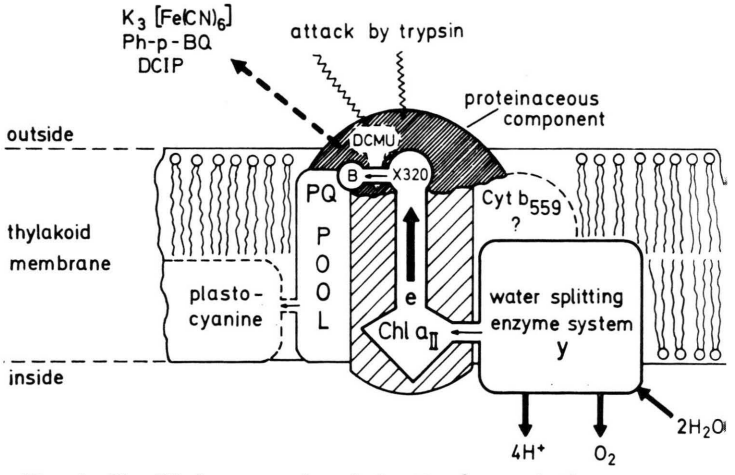

Fig. 4. Simplified structural and functional organization scheme of system-II electron transport in chloroplasts (for details s. text).

Note added in proof:

Latest data suggest, that the proteinaceous component enwrapping the acceptor side of system II

probably consists of two polypeptides with mol. weights of 27.000 and 32.000, respectively [21].

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