Photosynthetic Electron Transport Inhibitors: Some Problems Related to an Accurate Determination of the Molecular Site of Action

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Z. Naturforsch. **39 c,** 338–341 (1984); received November 21, 1983

DCMU-Type Herbicides, Photosynthesis, Chlorophyll Fluorescence Transient, Area Growth

When DCMU and DCMU-type inhibitor concentrations vary over a large range, different effects may be observed on chlorophyll fluorescence parameters such as fluorescence transients, areas over fluorescence induction curves as well as kinetics of area accumulation. These results would indicate a heterogeneity of PS II electron acceptors, or an inhibitor partitioning behavior dependent on the concentration. Also, a threshold value can be reached at supra-optimal concentrations, beyond which the back reaction is almost completely blocked. Such an approach makes it possible to find out inconsistencies in the results, brought about by dual effects of some inhibitors such as phenol-type herbicides, as previously proposed.

Introduction

The biorational design of photosynthesis-inhibiting herbicides implies an accurate knowledge of their molecular chloroplastic target as well as of the interactions between inhibitors and the site of action, particularly when different chemical families have to be compared. In the past, several authors have accumulated evidence of a common site of action for various PS II inhibitors [1–4], and the term DCMU-type inhibitors has been proposed accordingly. Using a multi-methodological approach (partial photochemical reactions, fluorescence, luminescence, competitive binding, etc...), the various chemicals tested were shown to interact with a common site Q_B, the PS II secondary electron acceptor. However, by comparing the inhibitor concentrations with the corresponding effects upon these various phenomena, some peculiarities or discrepancies appeared. The purpose of this paper will be to discuss some of questions related to responses obtained when inhibitor concentrations vary over a wide range.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ioxynil, 4-hydroxy-3,5-diiodobenzonitrile; lenacil, 3-cyclohexyl-5,6-trimethyleneuracil; pyramin, 5-amino-4-chloro-2-phenyl-3-(2H)-pyridazinone; PS, Photosystem; Q_A, primary quinone electron acceptor of Photosystem II; Q_B, secondary quinone electron acceptor.

0341-0382/84/0500-0338 $ 01.30/0

Materials and Methods

The techniques used in this paper have been previously described [3, 4, 8].

Results and Discussion

Chlorophyll fluorescence

DCMU-type inhibitors cause various changes in chlorophyll fluorescence kinetics of treated chloroplasts or *Chlorella*.

Two parameters have been selected from the observed fluorescence induction curves:

- the area A_{max} over the fluorescence induction normalized to the steady-state fluorescence F_{max}, representing the size of the PS II electron acceptor pool [5] or the photochemical quenching capacity [6]. This value is expected to vary with DCMU-type inhibitor concentrations, because the re-oxidation of the primary PS II electron acceptor Q_A is inhibited.
- the fluorescence transient F_i, ascribed to the oxidation of an intermediary pool and Q_A by PS I [7] is also directly related to the DCMU-type herbicide concentrations [3, 4].

When values of either A_{max} or $F_{max} - F_i/F_{max}$ obtained with chloroplasts (in percent of the control) are plotted versus the log of inhibitor concentration, a typical sigmoidal pattern is observed, as shown in Fig. 1 a for the fluorescence transient. However, the

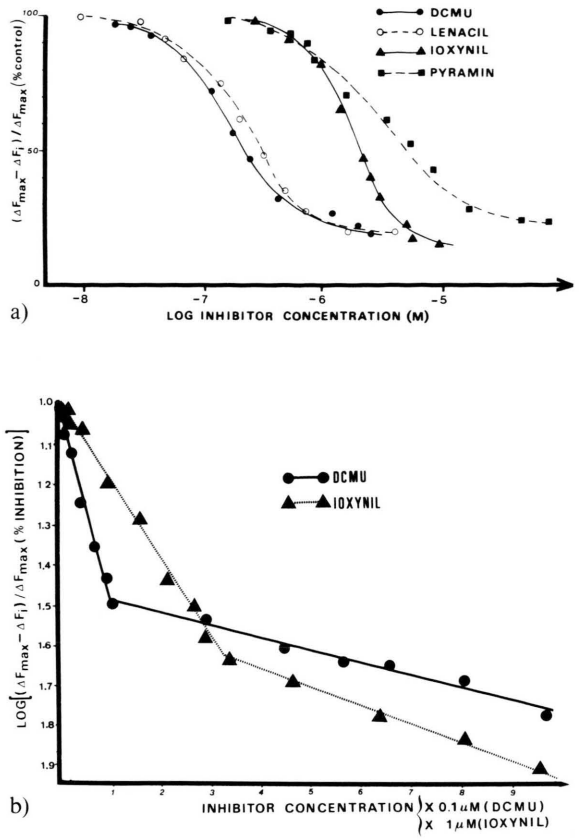

Fig. 1. Relation between the log of inhibitors concentration and fluorescence transient expressed in % of the control (Fig. 1 a), and between the inhibitor concentration and the log of fluorescence transient values, as percent of inhibition (Fig. 1 b, DCMU and ioxynil shown only), measured with tobacco chloroplasts. Chlorophyll concentration 30 γ/ml; photon flux density: 85 μE × m^{-2} × s^{-1}.

usual log-probit transformation for a sigmoid into a linear function does not apply in this case (low correlation coefficient). The best fitting is obtained by transforming values of $F_{max} - F_i/F_{max}$ or A_{max} as a percent of inhibition into their log versus the inhibitor concentration. A typical example is presented in Fig. 1 b (the concentration scales for DCMU and ioxynil have been displaced in order to have a better comparison between the two inhibitors) for DCMU and ioxynil, showing a biphasic pattern: a rapid change occurs in concentrations below the I_{50}, whereas a second linear relationship appears for higher concentrations, but with a different slope. Although this relationship is not shown here for all the DCMU-type inhibitors, similar responses were previously observed [4] with all the compounds, and half effect values corresponded well to the pI_{50} values, except in the case of ioxynil. This discrepancy has been further confirmed by chemically triggered luminescence and silicomolybdate studies, indicating that phenol-type herbicides (such as ioxynil and dinoseb) were acting on both side of PS II [4, 8].

The presence of two distinct portions obtained in plotting the log of effect versus inhibitor concentration could receive several tentative explanations. The rapid variation of both fluorescence transients (Fig. 1 b) and areas (not shown) over the induction curve in relation to the inhibitor concentration, as reflected by the first linear portion, occurs in a range of concentrations near the I_{50}, whereas above a threshold value, both parameters do not change drastically with the increasing inhibitor concentrations. This may indicate a fast saturation of the binding site (Q_B-protein) until a certain value is reached, beyond which inhibitor binding would proceed with a slower rate. However, this would not comply with usual partitioning or specific binding kinetics. Alternatively, the observed separation could arise from a threshold concentration above which all the back reactions are almost completely blocked leading to a limited effect on fluorescence when concentration increases. This hypothesis could be further tested by measuring the same parameters under conditions of a PS II single turn-over.

Kinetics of area accumulation over the fluorescence induction curves

The growth of the area over the fluorescence induction curve indicates the progress of photochemical events in PS II [9]. Kinetics of area accumulation in DCMU [9] or DCMU-type inhibitors poisoned chloroplasts [3] have been proposed to largely reflect the reoxidation of the primary PS II electron acceptor Q_A^-.

In *Chlorella*, various concentrations of DCMU and DCMU-type herbicides are tested for their effect on area growth kinetics. Two phases are observed for DCMU (Fig. 2 a) and lenacil (Fig. 2 b), a DCMU-type herbicide. However, by varying the inhibitor concentration, the two phases α and β are not equally affected. When concentrations of DCMU or DCMU-type herbicides are in the vicinity of the I_{50}, the two phases are not as distinct as with higher concentrations and the kinetics of

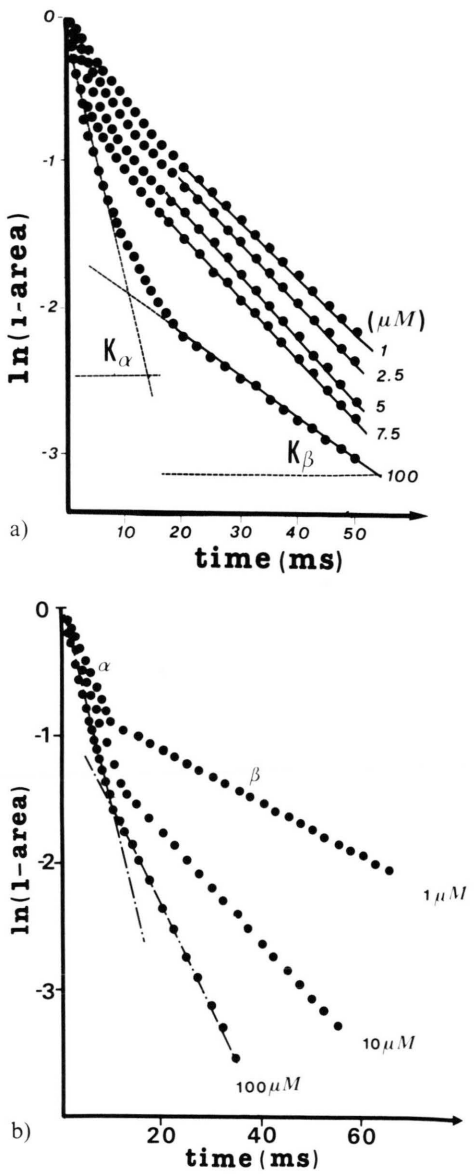

Fig. 2. First order reaction kinetic treatment of the area growth over the fluorescence induction curves measured with *Chlorella*, normalized to steady state fluorescence. AREA = fraction of the corresponding area size increases with time. Chlorophyll concentration: 30 γ/ml; photon flux density: 85 $\mu E \times m^{-2} \times s^{-1}$. 2a: DCMU; 2b: lenacil.

phase β do not change. By rising concentrations largely above I_{50}, both phases α and β are affected (Fig. 2b).

The presence of α and β components has been attributed, in higher plant chloroplasts, to the existence of two spatially separated photocenters [10]. Alternatively each PS II center would be connected to two primary electron acceptors Q_1 and Q_2 [11] both of which can be reduced in the presence of DCMU, but in a sequential manner. In turn, both primary electron acceptors may be linked to Q_B, or only one would transfer its electrons directly to the PQ pool, creating a relatively DCMU-insensitive pathway [12]. Also, Q_2 could be located in the membrane within an environment better protected from outside interferences than Q_1 [11].

From the results presented, it is difficult to explain that the variations of Q_A photoreduction arises from a heterogeneity in the organisation of the pigment units serving two types of PS II reaction centers. The differential effect of DCMU and DCMU-type inhibitors upon components α and β in relation with the herbicide concentration would rather indicate a heterogeneity of the PS II electron acceptors. Alternatively, the possible existence of a relatively less DCMU-sensitive electron transport pathway may also account for the observed differential effects on α and β components. On the other hand, reduced quinones diffuse rapidly in the membrane. Q_B can be exchanged with PQ (H_2) leaving vacant the binding site on the Q_B-protein, suggesting that DCMU could replace a PQ molecule [13]. Consequently, replacement kinetics could be controlled by inhibitor concentration, giving rise to variable fluorescence yield related to Q_A photochemical reduction.

Our results, obtained with *Chlorella*, are consistent with what has been found on higher plant envelope-free chloroplasts. It has been shown recently that DCMU-type and phenol-type herbicides show similar effects upon components α and β by increasing inhibitor concentrations, but the PS II β (or Q_B) component is suppressed at high herbicide concentration [14]. This result is in contradiction with our finding, since both phases α and β are observed even at high DCMU or DCMU-type inhibitor concentrations (Fig. 2b). The discrepancy may find its origin in a different plant material *i.e. Chlorella* and higher plant chloroplasts.

Concluding Remarks

As previously proposed [3, 4], the exact determination of PS II inhibitor mode of action implies a multimethodological approach, in order to accumu-

late evidence for a common site of action. Although very meaningful and easy to undertake, fluorescence induction studies do not provide a complete picture of possible interaction between inhibitors and one component of the photosynthetic electron transport chain. It has been widely demonstrated that fluorescence yield is not only controlled by the redox state of the acceptor side of PS II but also by the donor side. Phenol-type herbicides were shown to affect both sides of PS II [3, 4, 8]. Therefore, many results using only one herbicide concentration could have been masked by the dual effects. By varying largely the inhibitor concentration, it was possible to demonstrate some peculiar data particularly in comparing half-effect values obtained from variable fluorescence, initial fluorescence and chemical-triggered luminescence data, as well as with measurements of partial photochemical reactions. The results presented here put more emphasis on the necessity to work not only on qualitative aspects but also on quantitative determination, in order to display some differences between sub-lethal and lethal concentrations with respect to the photosynthetic electron transport.

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