Inhibition of the Photosynthetic Electron Transport by Pyrethroid Insecticides in Cell Cultures and Thylakoid Suspensions from Higher Plants

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Synthetic pyrethroid insecticides with different molecular structures have been investigated with respect to their effect on photosynthetic electron transport reactions in chloroplast suspensions and cell cultures from higher plants. The fluorescence induction curves of tobacco (Nicotiana tabacum) leaves and tomato cells were substantially affected by permethrin and cypermethrin resulting in a strong increase of the maximum fluorescence. Application of different concentrations (0.3–1.2 mM) of the respective chemical abolishes virtually any kinetics of the normal Kautsky effect. Oxygen evolution from cell cultures from tomato (Lycopersicon peruvianum) was completely inhibited by cypermethrin. Analysis of partial reactions of the photosynthetic electron transport showed that both a methylviologen-mediated Mehler reaction and a ferricyanide-driven Hill reaction were quantitatively inhibited by e.g. fenvalerate. On the other hand, neither a siliconolysimate-driven Hill reaction nor a methylviologen-driven Mehler reaction using dichlorophenol indophenol/ascorbate as electron donors could be inhibited by the pyrethroid. The analyses suggest that pyrethroid insecticides interfere with the photosynthetic electron transport at the same site as urca-type herbicides do. Depending on the molecular structure and on the halogen compound in the molecule, however, different pyrethroids are more or less phytotoxic to the investigated photosynthetic membranes – cypermethrin with two Cl-substituents requires much higher concentrations to be applied for significant inhibition of the electron transport reactions than the Br-derivative deltamethrin does. Moreover, qualitative differences have to be taken into account. In the case of fenvalerate the effect seems to exist in a type of all-or-nothing reaction when the reaction centres are inhibited by the pyrethroid. None of the S-states nor the transition probabilities are specifically influenced by increasing concentrations of fenvalerate. In the case of deltamethrin, however, it was found that the overreduced state S_i is significantly increased at the expense of both S_i and S_0. Moreover, the miss parameter a is increased in the case of deltamethrin addition. The results and the significance of different substituents for the investigated pyrethroids are discussed.

Introduction

Since years, herbicides are well-investigated with respect to their effect on photosynthetic electron transport reactions; dichlorophenyl dimethyurea (DCMU) as inhibiting herbicide has become the classical testing agent for a photosystem II reaction (for a review see Renger, 1986). Detailed analyses have been carried out investigating the inhibition of electron transport reactions in isolated cytochrome complexes, photosynthetic bacteria, cyanobacteria and higher plants (Oettmeier et al., 1991; Trebst et al., 1993; Oettmeier et al., 1994). Much work has also been done dealing with herbicides and the inhibition of carotenoid- and/or chlorophyll biosynthesis. Many of the so-called ‘bleaching herbicides’ have been shown to act on phytoene desaturase as one of the relevant enzymes introducing double bonds in the precursors of β-carotene (Böger and Sandmann, 1993; Sandmann et al., 1991). The correlation between the inhibitory effect of the carotenoid biosynthesis inhibitor norflurazon, illumination and D1 degradation has been described (Sandmann et al., 1993) as well as the mechanism of action of peroxidizing herbicides (Böger and Wakabayashi, 1995). How-
ever, in the general field of plant protecting agents, only herbicides have been thoroughly investigated concerning their interaction and interference with the photosynthetic electron transport chain. From such work it is known that herbicides like the above mentioned DCMU interact with Q_B on the acceptor side of PS II. (For a review see Oettmeier, 1992.) Moreover, substituted phenols have been shown to orientate differently in the Q_B-site in comparison to the urca-type herbicides. Mutation in the D1 protein lead to substantial tolerance of *Chlamydomonas* towards phenolic inhibitors (Trebst *et al.*, 1993). Details on the quinone binding sites on cytochrome complexes both in the photosynthetic and respiratory pathway including the use of site-specific inhibitors for mutagenesis of the respective binding site has been reviewed by Frank and Trebst (1995).

Only scattered reports, however, deal with the effects of other plant-protecting substances like *e.g.* insecticides on plants (Bhunia *et al.*, 1993; Mishra *et al.*, 1992) what can easily be explained by the fact that insecticides (fungicides or others) are supposed to have a negative effect only on their specific target organism but should have rather good compatibility and tolerance with respect to the treated plants. From this point of view synthetic pyrethroid insecticides which can be derived from the naturally occurring (*Chrysanthemum*) pyrethrins might be of specific interest. Analysis of these substances in connection with photosynthesis might not only be relevant for physiological studies but should also allow new insights into structural necessities of modern and ecologically acceptable plant protection chemicals.

**Materials and Methods**

*Cell cultures* from tomato (*Lycopersicon peruvianum* LP 396) were kindly supplied by Prof. Dr. H.-P. Mühlbach, Institut für Allgemeine Botanik in Hamburg, Germany and cultivated in two-tire vessels containing the cells in culture medium and (separately) a 2m K_2CO_3/KHCO_3-buffer which provided 2% CO₂ in the gas atmosphere. Details of the procedure have been described earlier (Stöcker *et al.*, 1993; Hüseemann and Barz, 1977).

*Chloroplasts* from tobacco (*Nicotiana tabacum* var. John William’s Broadleaf) were isolated by mechanical homogenization of leaflets, centrifugation and suspension in buffer containing high concentrations of sucrose. Prior to the experiments, aliquots from the chloroplast suspension were diluted in reaction buffer (Tricine 0.03 m / KCl 0.06 m – pH 7.5) (Homann and Schmid, 1967). Some experiments have been carried out with intact leaves or leaflets also from this tobacco strain.

**Fluorescence measurements** were performed in a laboratory-built fluorometer. 1 ml reaction assay contained about 50–100 μg of chlorophyll in suspension buffer, reaction buffer (*vide supra*) and additions as indicated in a glass cuvette exposing a surface of approx 16 cm² to the light source. The light source illuminated the sample via an electrically triggered shutter, a blue plexiglas filter and a SCHOTT BG 28 filter. Fluorescence was detected with an EMI photomultiplier Type 9658R under a high tension of 1000–1100 V. Fluorescence induction curvess were recorded on a 5115 storage oscilloscope from Tektronix, equipped with a 5A22N differential amplifier and a 5B12N dual time base.

**Electron transport reactions** were carried out in a Clark-type electrode from Rank Bros., Bottisham, Great Britain. Oxygen evolution from a siliconolybate- or a ferricyanide-mediated Hill reaction, or oxygen uptake from a water → anthraquinone-2-sulfonate- or a dichlorphenolindophenol → anthraquinone-2-sulfonate Mehler reaction were measured and recorded on a SE 130 Recorder from BBC-Metrawatt. The assays were illuminated with a Leitz projector (Prado Universal) through a red plexiglas filter (580 nm < λ < 750 nm).

**Flash-induced oxygen evolution** was detected on the three-electrode-system developed by Schmid and Thibault (1979). The signals were processed by means of an Atari Mega ST 4 computer (Schulder *et al.*, 1992). Subsequently, the experimental data were fitted using the VOYONS general modelling from Thiery (1991) on an IBM compatible computer. Details have been described (Thibault and Thiery, 1981; Thibault, 1982).

**Pyrethroids** were diluted in water and applied in the concentrations indicated in the figures and in the figure legends. Cypermethrin (Ripcord 10) was obtained from C.F. Spiess & Sohn, Kleinkarlbach, fenvalerate (Sumicidin 10) from Amer. Cyanamid, Ingelheim, deltamethrin (Decis EC 2.5) from Hoechst, Frankfurt and permethrin (Ambush) from Deutsche ICI, Frankfurt.
Results

Leaves from higher plants show the typical fluorescence emission curves well-known from the literature and described as Kautsky effect during the initial phases of the illumination period. According to the current concept the fluorescence originates almost exclusively from photosystem II (at room temperature) and reflects the redox state on the acceptor side of PS II, as the first quinone acceptor, QA, in its oxidized state acts as a fluorescence quencher (Duysens and Sweers, 1963). The fluorescence can, however, be substantially influenced and affected by pretreatment of the leaf sections with the pyrethroids permethrin and cypermethrin (Fig. 1). (Such synthetic pyrethroids are often used in the field of plant protection as effective insecticides. Many of these chemicals have been derived from ‘Pyrethrum’ a mixture of the physiologically occurring pyrethrins from Chrysanthemum species.) Fig. 1a shows that the applied pyrethroids increase the fluorescence yield of leaf sections from tobacco (Nicotiana tabacum var. John William’s Broadleaf). This observation suggests that synthetic pyrethroid insecticides interact with the photosynthetic electron transport chain and influence the redox conditions of the primary electron acceptor of photosystem II in the in vivo conditions of intact leaves. Furthermore, it can be concluded that the interaction can be observed with various pyrethroids with different molecular structures. Permethrin increases fluorescence at a lower concentration to a higher extent than cypermethrin does. It should be noted that the only difference in the molecular structure between the two pyrethroids consists of the lack of the CN-group in the case of permethrin (Fig. 2). The effect of pyrethroids on fluorescence was analyzed in detail with cell cultures from tomato (Lycopersicon peruvianum). These cell suspensions show virtu-
Fig. 3. Effect of increasing concentrations of cypermethrin on the oxygen evolution capacity in cell cultures from *Lycopersicon peruvianum*. Photosynthetic oxygen evolution was detected without artificial electron acceptor.

All the same (principal) fluorescence induction curves and are, on the other hand, much more accessible for exogenously added chemicals than whole leaves are. Addition of the pyrethroid permethrin strongly increases the fluorescence yield of the cell cultures and nearly abolishes any kinetics of the normal Kautsky effect (Fig. 1b). This hints at an inhibition of the electron transport in the region between photosystems II and I. The same holds true for assays with chloroplast suspensions from tobacco (results not shown) although in most cases the kinetics of the fluorescence emission curves are less pronounced with these preparations due to a mechanical disruption of parts of the electron transport chain (membrane structure) during the isolation procedure.

Cell cultures from tomato are capable of performing photosynthetic electron transport using water as physiological electron donor at rates of about 40–50 μmol O₂ x mg Chl⁻¹ x h⁻¹. Consequently, an inhibition of the electron transport should result in an inhibition of the water oxidation capacity of the cells. Fig. 3 shows that this is indeed the case. Concentrations at about 600 μM of the pyrethroid cypermethrin reduces oxygen evolution of the cell cultures to zero. This means that the insecticide is capable of completely blocking the photosynthetic electron transport chain in higher plants. Following this observation, the question arises, whether the effect is based on a general and unspecific inhibition of the electron transport as a whole. Another possibility would be that the chemical exerts its effect in a specific region or even at a specific site which can be localized by analyzing specific and distinct parts of the electron transport chain. In order to clarify this question we measured the oxygen gas exchange of tobacco chloroplasts in water → methylviologen, water → ferricyanide, water → silicomolybdate and dichlorophenolindophenol/ascorbate → methylviologen partial reactions of the electron transport. For this and for the following experiments we used two other pyrethroids, namely fenvalerate and deltamethrin (Fig. 4). These substances have been chosen because of the (possibly) relevant structural differences of the molecules. In the case of deltamethrin the two chlorides on the dichlorovinyl-dimethylcyclopropyl side of the molecule are changed to two bromides. Fenvalerate shows a completely different structure in this part of the molecule. It contains an isobutyl component and a chlorophenyl ring instead. Fig. 5 clearly shows that these pyrethroids have the same inhibitory effect on the electron transport as permethrin and cypermethrin have as far as the overall effect on the whole chain is concerned. This effect must be

![Fig. 4. Molecular structure of the synthetic pyrethroids fenvalerate (A) and deltamethrin (B).](image-url)
Electron transport running through both photosystems (water → MV) is completely inhibited what is also the case for a ferricyanide-mediated Hill reaction. A silicomolybdate Hill reaction, however, remains completely unaffected even by the highest concentration of the applied pyrethroids. A DCPIP/asc → MV Mehler reaction was also not inhibited; in this case even a slight stimulation was observed which appeared at relatively low concentrations and remained constant over the concentration range which we analyzed. (This stimulatory effect i.e. an interaction of the pyrethroids with photosystem I shall only be mentioned here and will be analyzed in a forthcoming paper.) From our results it can be concluded that the site of inhibition of pyrethroids can be localized on the acceptor side of photosystem II between the sites where silicomolybdate and ferricyanide accept electrons from the transport chain. Thus, it appears that these insecticides interact with the photosynthetic electron transport chain exactly at the same site as herbicides (e.g. diuron) do.

In further experiments we analyzed the effect of pyrethroids on the flash induced oxygen evolution in the frame of the coherent Kok model. All oxygen amplitudes under a series of short (5 μs) saturating flashes are inhibited by different concentrations of deltamethrin, cypermethrin (Fig. 6) and other pyrethroids. The highest sensitivity of photosynthetic membranes to the pyrethroids was observed with deltamethrin; a concentration of 5 μM completely inhibited the flash induced oxygen evolution, whereas in the case of fenvalerate about 5–10-fold higher concentrations were required (Fig. 6a,b). Furthermore, the kinetics of the concentration dependence appear to be different in the two cases and a specific effect of deltamethrin on individual S-states has to be regarded. In the case of fenvalerate the ratio \( Y_{\text{max}}/Y_{\text{min}} \) remains constant over a wider concentration range of the pyrethroid than it was observed with deltamethrin. This might be explained by the interpretation that the chemical inhibits all S states to about the same extent. For further interpretation of \( Y_{\text{max}}/Y_{\text{min}} \) see
also Bader and Höper, 1994. In the case of deltamethrin, however, $Y_{\text{max}}/Y_{\text{min}}$ is decreased already in the early phases of inhibition. As $Y_{\text{max}}$ in this case means $Y_3$, one might assume that $S_1$ is preferentially hit by the pyrethroid. In order to substantiate this idea we fitted the oxygen evolution sequences by means of the VOYONS mathematical program developed by Thiery (1991). Calculation of the 5-state distribution yielded values of 10–15% $S_0$, 83–88% $S_1$, approx. 1% $S_2$, and approx. 1% $S_3$ according to the 4-state Kok model for the controls. Fit of the oxygen amplitudes using the 5-state Kok model resulted in a value of approx. 10% for $S_1$ at the expense of $S_1$. Fig. 7 shows the dependence of the S-state distribution in the presence of increasing concentrations of deltamethrin and fenvalerate in the 5-state Kok model. From this fit it can be concluded that in fact $S_1$ but even more $S_0$ appear to be decreased by deltamethrin. The effect seems to consist of a (chemical) reduc-

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**Fig. 7.** Effect of deltamethrin (A) and fenvalerate (B) on the S-state distribution in tobacco chloroplasts calculated on the basis of a 5-state Kok model.

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**Fig. 8.** Effect of deltamethrin (A) and fenvalerate (B) on the transition probabilities misses ($\alpha$), successes ($\beta$) and double hits ($\gamma$) and on the quadratic deviations ($\Delta$ quad) of the fit.
tion of the reaction centres; this can also be derived from Fig. 7 showing that the ‘overreduced’ state $S_3$ increased by a factor of about 2.5. Moreover, Fig. 8 shows that the transition probabilities are attached. The miss parameter $\alpha$ was substantially increased upon deltamethrin addition with the concomitant decrease of successful transitions $\beta$. The double hit parameter $\gamma$ seems not to be influenced by the pyrethroid. The results show that insecticides like pyrethroids can interact very specifically not only with photosynthetic membranes as a whole but with the $S$-states of the OEC in particular. Redox states are converted from $S_1$ to $S_3$ and the functioning of the complex as evidenced by the transition probabilities is substantially influenced.

The described effects are largely specific for deltamethrin (or related molecular structures) as similar observations cannot be made (or to a much smaller extent) following the addition of fenvalerate. In Fig. 6 it was shown that the ratio $Y_{\text{max}}/Y_{\text{min}}$ remained constant over a relatively wide range of the pyrethroid concentration dependency. In this case, the inhibitory effect of the insecticides seems to consist of a more general and unspecific impairment of the OEC. Figs. 7 and 8 show part of the results of the mathematical fit for fenvalerate. It is clearly seen that neither the $S$-state distribution nor the transition probabilities are substantially influenced by this pyrethroid (Figs. 7b/8b). (Only a slight increase of the miss parameter $\alpha$ can be discussed.) This means that fenvalerate acts on the reaction centres in a type of all-or-nothing reaction whereas the OECs in the case of deltamethrin are attached in their specific redox and transition conditions before/when the function is blocked.

**Discussion**

Synthetic pyrethroids have been derived from the physiologically occurring pyrethrins and are often used as insecticides in the frame of modern plant protection. However, the present paper shows that first a strong impact of certain pyrethroids on photosynthetic electron transport reactions has to be considered and that second large differences exist between the effects of different pyrethroids depending on details of the chemical structure of the respective molecule. In this context it should be mentioned that structure-activity correlations have been described for fluridone derivatives in the case of phytoene desaturase. The significance of the methyl group as $N$-substituent and the lower indispensability of the keto group, the aromatic nature of the cyclic structure and other parameters have been investigated (Sandmann et al., 1992). From the detailed studies of partial reactions of the electron transport in chloroplasts we conclude that the investigated insecticides have a binding site and show interaction with the photosynthetic electron transport chain at the same site where several (the urea-type) herbicides interfere, namely $Q_b$. Like the herbicide DCMU, the investigated pyrethroids strongly inhibit PS II reactions like the ferricyanide-driven Hill reaction and the Mehler reaction $H_2O \rightarrow MV$. Neither a silicomolybdate-driven Hill reaction nor a PS I reaction are inhibited. Comparison of the effects of different pyrethroids yield further insights into the relevant structural components of the molecules which are responsible for a specific interaction with photosystem II.

As the sensitivity of the examined photosynthetic membranes to the pyrethroids permethrin, cypermethrin, fenvalerate and deltamethrin differed by one to two orders of magnitude, the two-carbocycles-side of the molecules can not be essential for the interactions. This side of the molecules is identical for all of the tested pyrethroids (apart from the ether bridge between the carbocycles in the case of deltamethrin). Thus, the halogen side of the molecules must be responsible for the observed phytotoxicity i.e. the specific interaction of the insecticides with the D1 protein. Consequently, the inhibitory effect of herbicides like DCMU must also be based on the dichlorophenyl-side of the molecule and can, possibly, be increased by exchanging the chloride to other halogens. This might be derived from the experiments showing that deltamethrin with two bromides inhibits photosynthesis to a higher extent than fenvalerate with a chlorophenyl or permethrin with its dichlorovinyl group does. Like the other structural components of the investigated pyrethroids also the cyclopropyl part of the molecule can not be responsible for the interaction with $Q_b$, as fenvalerate with a relatively good effect lacks this structure. Thus, it
can be concluded from the results that the inhibitory effect of the measured pyrethroids increases in the sequence cypermethrin < permethrin < fenvalerate < deltamethrin and that the two bromides are the structural components responsible for the strong effect in the case of deltamethrin.


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