Activity of Photosystem II Herbicides Is Related with Their Residence Times at the D 1 Protein

J. Dirk Naber* and Jack J. S. van Rensen

Laboratory of Plant Physiological Research, Agricultural University Wageningen, Gen. Foulkesweg 72, 6703 BW Wageningen, The Netherlands

Z. Naturforsch. **46c,** 575–578 (1991); received March 18, 1991

Chloroplasts, Photosystem II, D 1 Protein, Herbicides, Triazine Resistance

The reversible binding kinetics of atrazine, diuron and ioxynil were measured *via* their binding and release parameters during steady state inhibition of electron transport. The parameters were determined in isolated chloroplasts of peas and of triazine-resistant and -susceptible biotypes of *Chenopodium album* using a kinetic model. This model is based on the flash-induced oxygen evolution patterns of isolated broken chloroplasts.

It was found that the binding parameters were always significantly higher in the case of an oxidized acceptor quinone complex as compared with a semi-reduced complex. Triazine resistance seems to originate from a significant increase of the release kinetics. The release parameters could be used to calculate the residence times of the herbicides at the D 1 protein. The values of these residence times were always much higher for the herbicides than for Q_B; this explains the inhibition of electron transport. The only exception was the residence time of atrazine in the resistant biotype, where the value was close to that of Q_B.

It is concluded that the "on" kinetics of a compound to its binding environment at the D 1 protein are determined principally by the accessibility of the niche to the compound. The differences in activity between herbicides are mainly due to variations in the release kinetics.

Introduction

Photosystem II herbicides are potent inhibitors of electron transport near photosystem II. They include a large number of (chemically) different compounds. Two groups can be distinguished: the diuron-type herbicides, including the ureas and triazines, and the phenol-type herbicides [1–3]. The mode of action of these herbicides is studied in detail. They interrupt electron transport between Q_A and Q_B. Interaction of diuron-type herbicides with plastoquinone was first proposed by van Rensen [4]. It is now widely accepted that the mechanism of action is a displacement of Q_B from its binding site at the D 1 protein. This was independently and simultaneously proposed by Velthuys [5] for the PS II complex, and by Wraight [6] for the reaction center of purple photosynthetic bacteria. It is thought that the inhibitors reside at the D 1 protein for a relatively long time instead of Q_B; they cannot be reduced, and thus inhibit electron flow.

It is important to realize that there is a reversible exchange of Q_B and an added PS II herbicide at their common binding site at the D 1 protein. We have measured the kinetics of reversible binding of herbicides *via* their binding and release parameters. We compared the diuron-type herbicides DCMU and atrazine with the phenol-type herbicide ioxynil. In addition, we measured the kinetics of their binding in thylakoids of a triazine-resistant plant. We conclude that a strong inhibition is related to a long residence time at the D 1 protein.

Materials and Methods

Peas (*Pisum sativum* L. *cv.* Finale) and lambsquarters (*Chenopodium album* L.) were grown in growth chambers. The growth of the plants and the origin of the triazine-resistant and -susceptible lambsquarters were described earlier [7]. Broken chloroplast thylakoid membranes were isolated

Abbreviations: Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine; DCMU (diuron), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ioxynil, 3,5-diiodo-4-hydroxybenzonitrile; I_{50}, inhibitor concentration causing 50% inhibition of electron transport; pI_{50}, negative logarithm of I_{50}; PS II, photosystem II; Q_A, primary quinone electron acceptor of PS II; Q_B, secondary quinone electron acceptor of PS II; *R*, triazine-resistant biotype; *S*, triazine-susceptible biotype.

* *Present address:* Lehrstuhl Biochemie der Pflanzen, Ruhr-Universität, Postfach 10 21 48, D-4630 Bochum 1, Bundesrepublik Deutschland.

Reprint requests to Dr. J. J. S. van Rensen.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/91/0700–0575 $ 01.30/0

from the leaves according to a previously described procedure [8]. The thylakoids were suspended in a medium, which contained 0.3 M sorbitol, 50 mM tricine-KOH (pH 7.5), 100 mM KCl, 10 mM NaCl and 2 mM $MgCl_2$. Total chlorophyll concentration was determined spectrophotometrically according to [9]. Aliquots of 0.5 ml were stored at −80 °C, and slowly thawed on ice prior to use.

Oxygen evolution in continuous light was measured with a Gilson oxygraph [10] and for the measurement of flash-induced oxygen production a laboratory-designed Joliot-type apparatus [11] was used.

The exchange parameters were measured using a method, initiated by Vermaas *et al.* [12] and adapted by Naber [11]. It is based on the flash-induced oxygen evolution patterns of isolated broken chloroplasts, which are measured in the absence and in the presence of herbicides. The exchange parameters are obtained by fitting experimental data to those calculated with a kinetic model. This model is derived from the following equations:

$$\mathrm{Sn}\cdot Q_A\cdot Q_B + I \underset{E_3}{\overset{E_1}{\rightleftarrows}} \mathrm{Sn}\cdot Q_A\cdot I + Q_B$$

$$\mathrm{Sn}\cdot(Q_A\cdot Q_B)^- + I \underset{E_4}{\overset{E_2}{\rightleftarrows}} \mathrm{Sn}\cdot(Q_A\cdot I)^- + Q_B$$

In these equations, Sn (where $n = 0, 1, 2, 3$) represents the redox state of the oxygen-evolving complex. In the presence of slowly exchanging herbicides, having residence times on the D1 protein of the same order of magnitude as the duration of the flash train or longer, the oscillation is hardly altered compared to the control. In this case only the amplitude of the signal is diminished. However, when the herbicide exchange is occurring with the same or higher frequency than the firing of the flashes, the damping of the oscillation is considerably stronger. This is caused by the fact that then reaction centers are blocked for a certain time span, and start making turnovers at the moment the herbicide is displaced by a plastoquinone molecule. Thus, centers can get out of phase with each other, and produce oxygen at different flashes. By comparing flash patterns with different flash frequencies and herbicide concentrations, the exchange parameters E_1 to E_4 can be calculated. In Fig. 1 the results of a typical measurement are illustrated.

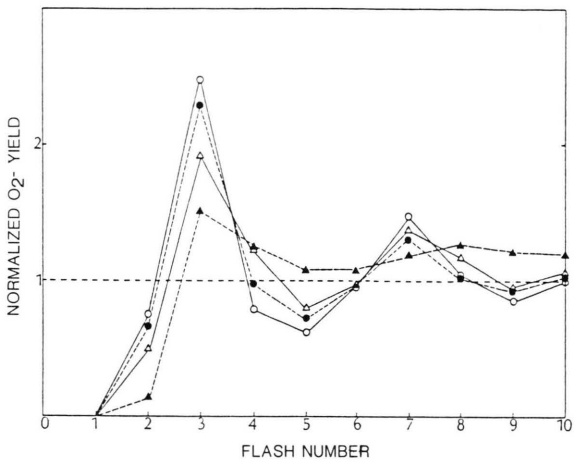

Fig. 1. Oxygen evolution patterns at different flash frequencies with and without atrazine. ○, 4 Hz flash frequency, no inhibitor; △, 0.5 Hz flash frequency, no inhibitor; ●, 4 Hz flash frequency, 0.5 μM atrazine; ▲, 0.5 Hz flash frequency, 0.5 μM atrazine.

Results and Discussion

The inhibitory activity of the herbicides atrazine, DCMU and ioxynil on oxygen evolution in continuous light as measured in chloroplasts from peas and lambsquarters is illustrated in Table I. The pI_{50}-values are between 6.5 and 7.5 for all three herbicides; in the triazine-resistant material there is very little activity of atrazine, a little decrease in activity of DCMU and a little increase in activity of ioxynil. This is in agreement with what is generally observed (*e.g.* [13]).

The results of the following tables are different from the action kinetics of PS II herbicides on thy-

Table I. Values of pI_{50} for the herbicides atrazine, DCMU and ioxynil measured in chloroplasts from peas and triazine-resistant (*R*) and -susceptible (*S*) *Chenopodium album* plants.

	Pea	*C. album S*	*C. album R*
Atrazine	7.0	6.5	<4
DCMU	7.5	7.5	6.7
Ioxynil	6.5	6.6	7.0

lakoids that were reported by Ducruet *et al.* [14]. These authors added a herbicide to a thylakoid suspension and measured the kinetics of the progress of inhibition of electron transport by chlorophyll fluorescence; the time needed to reach 50% inhibition was defined as apparent half-time (t 1/2). In our experiments binding and release kinetics were determined while a steady state inhibition was obtained of about 50% inhibition of electron transport.

In Table II the E_1 to E_4 exchange parameters are presented for the herbicides atrazine, DCMU and ioxynil, measured in chloroplasts of pea, triazine-resistant and -susceptible *C. album*. The parameters E_1 and E_3 represent the exchange rates of herbicides to an oxidized binding environment. They are supposed to be much higher than the corresponding parameters for a reduced complex, E_2 and E_4. This is caused by the fact that the secondary acceptor Q_B binds very strongly to its binding niche when it is in the semiquinone form, whereas both the fully reduced and the oxidized forms are easily exchanged [5, 6]. In the semireduced state it is then difficult to replace the quinone by a herbicide molecule.

In all cases the binding parameters E_1 were significantly higher than E_2, which is in agreement with the expectation as described above. However, the release parameters E_4 were not always lower than E_3. In fact, in many experiments E_4 is found to be higher than E_3. This may be explained by the reasoning that a bound herbicide destabilizes a negative charge at the acceptor complex. Mutually, the presence of an electron on Q_A accelerates the release of the herbicide from its binding site, leading to higher E_4 values.

Table II. Values for the exchange parameters measured in isolated chloroplasts.

Pea	E_1	E_2	E_3	E_4
Atrazine	0.24	0.02	0.100	0.141
DCMU	0.01	0.001	0.135	0.141
Ioxynil	0.1	0.01	0.03	1.0
C. album S	E_1	E_2	E_3	E_4
Atrazine	0.1	0.03	0.11	0.04
DCMU	0.056	0.0015	0.008	0.0035
Ioxynil	0.4	0.02	2.0	2.0
C. album R	E_1	E_2	E_3	E_4
Atrazine	0.05	0.002	15	2.25
DCMU	0.064	0.002	0.001	0.001
Ioxynil	0.22	0.04	0.2	0.05

R = triazine-resistant; *S* = triazine-susceptible; E_1 and E_2: $\mu M^{-1} \cdot s^{-1}$; E_3 and E_4: s^{-1}.

Atrazine proved to bind faster than DCMU (E_1 parameter). In the resistant biotype of *Chenopodium*, the atrazine-binding constant E_1 is only slightly decreased as compared to the wild type. However, because the values of the release parameters E_3 and E_4 are much higher in the resistant chloroplasts compared to the wild type ones, triazine resistance seems to originate from a significant increase in the release kinetics. This can be explained on the molecular level. The "on" kinetics of a compound to the binding environment are determined principally by the accessibility of the niche to the compound. This is determined by the chemical structure of the herbicide, especially its molecular dimensions, charges and hydrophobicity. These properties are, of course, the same when atrazine is added to resistant or to susceptible chloroplasts. A very slight change of hydrophobicity of the binding pocket can be expected as a result of the serine to glycine substitution at position 264 in the triazine-resistant biotype. However, the atrazine molecule cannot be stabilized in its binding environment in the mutant protein, probably because the ser-OH group provides an important H-bonding possibility in the wild type. The result is a decrease in herbicidal activity of 2 to 3 orders of magnitude (Table I). In the case of ioxynil the situation is reversed, though the difference in activity in both biotypes is far less as compared with atrazine. For ioxynil only a slight difference in binding to the D1 protein is observed, but now the release from the resistant biotype is about 10-fold slower than from the wild type protein. The hydroxyl group of ser-264 apparently has a destabilizing effect on the binding of ioxynil.

The dissociation rates E_3 and E_4 can be used to calculate the time a herbicide stays at its binding site at the D1 protein. This residence time equals the inverse of the parameters $E_3 + E_4$. In Table III residence times are presented for atrazine, DCMU and ioxynil in chloroplasts from peas and from triazine-resistant and -susceptible *C. album* biotypes. Compared with the residence time of Q_B, which is about 20 ms [5], those of the herbicides

Table III. Residence times of herbicides (in seconds) at the D1 protein.

	Pea	*C. album S*	*C. album R*
Atrazine	4.1	6.7	0.058
DCMU	3.6	86.9	500
Ioxynil	0.97	0.25	4.0

are much higher; they vary from about 10-fold (ioxynil in *C. album S*) to about 25,000-fold (DCMU in *C. album R*). It thus appears that herbicides stay much longer at their binding site on the D1 protein than Q_B; since they cannot be reduced by Q_A they interrupt electron transport at the site of Q_B.

A special case is the residence time of atrazine in chloroplasts of the triazine-resistant *C. album* biotype. This time is 58 ms, which is in the same order as that of Q_B. The fact that the residence times of atrazine and Q_B are very close to each other may be the explanation for the resistance.

In Table IV the ratios of the resistant (*R*) over susceptible (*S*) values of the activity of the herbicides (in I_{50}) are compared with the ratios of their residence times. It appears that a high ratio of *R*/*S* in activity is correlated with a low *R*/*S* ratio in residence time (atrazine); a low *R*/*S* ratio in activity is correlated with a high *R*/*S* ratio in residence time (ioxynil). DCMU has an intermediate position for both *R*/*S* ratios. This means that the inhibition of a herbicide is stronger when the time it stays at the D1 protein is longer.

In conclusion, it appears that the "on" kinetics of a compound to a binding environment are determined principally by the accessibility of the niche to the compound. This is determined by the properties of the herbicide: its chemical structure, especially its molecular dimensions, charges and hydrophobicity. The differences in activity between herbicides are mainly due to variations in the release kinetics, which determine the residence times. A stationary binding, resulting in a significant electron transport inhibition, requires a strict molecular shape.

Table IV. Comparison of the activity of herbicides with their residence times at the D1 protein in triazine-resistant (*R*) and -susceptible (*S*) biotypes of *C. album*.

	R/*S* of I_{50} values	*R*/*S* of residence times
Atrazine	>322	0.0087
DCMU	6.5	5.8
Ioxynil	0.4	16.0

[1] A. Trebst, Z. Naturforsch. **42c,** 742–750 (1987).
[2] J. J. S. van Rensen, in: Herbicides and Plant Metabolism (A. D. Dodge, ed.), pp. 21–36, Cambridge University Press, Cambridge 1989.
[3] J. J. S. van Rensen, in: Photosynthesis and Plant Productivity (Y. P. Abrol, P. Mohanty, and Govindjee, eds.), Oxford University Press, Oxford 1991 (in press).
[4] J. J. S. van Rensen, Meded. Landbouwhogeschool Wageningen **71-9,** 1–80 (1971).
[5] B. R. Velthuys, FEBS Lett. **126,** 277–281 (1981).
[6] C. A. Wraight, Isr. J. Chem. **21,** 348–354 (1981).
[7] M. A. K. Jansen, J. H. Hobé, J. C. Wesselius, and J. J. S. van Rensen, Physiol. Veg. **24,** 275–284 (1986).
[8] J. J. S. van Rensen, D. Wong, and Govindjee, Z. Naturforsch. **33c,** 413–420 (1978).
[9] J. Bruinsma, Photochem. Photobiol. **2,** 214–249 (1963).
[10] J. J. S. van Rensen, W. van der Vet, and W. P. A. van Vliet, Photochem. Photobiol. **25,** 579–583 (1977).
[11] D. Naber, Ph.D. thesis, Agricultural University, Wageningen 1989.
[12] W. F. J. Vermaas, G. Dohnt, and G. Renger, Biochim. Biophys. Acta **765,** 74–83 (1984).
[13] W. Oettmeier, K. Masson, C. Fedtke, J. Konze, and R. R. Schmidt, Pest. Biochem. Physiol. **18,** 357–367 (1982).
[14] J.-M. Ducruet, S. Creuzet, and J. Viénot, Z. Naturforsch. **45c,** 348–352 (1990).