Comparative Binding of Photosystem II – Herbicides to Isolated Thylakoid Membranes and Intact Green Algae

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The binding of the photosystem II herbicides diuron (DCMU), atrazine (s-triazine), ioxynil and dinoseb (substituted phenols) to isolated spinach thylakoids was saturated in less than 2 min in the dark. In intact cells of the green alga *Ankistrodesmus b.* it took 10 to 20 min to reach the binding equilibrium.

Binding affinity of diuron, atrazine, dinoseb, measured as equilibrium binding constants, was found to be comparable in isolated thylakoids and intact algal cells. For ioxynil, reduced binding affinity was observed in algae.

The concentration of binding sites in thylakoids and intact cells was determined to be 300–500 chl/inhibitor binding site, suggesting a 1:1 stoichiometry between bound herbicide and electron transport chains. In intact cells only the phenol herbicides ioxynil and dinoseb showed increased concentrations of binding sites.

Strong correlation of herbicide binding and inhibition of electron transport was found for diuron in isolated thylakoids and intact cells. In thylakoids this is valid also for atrazine and dinoseb. For ioxynil a difference between the amount of binding and inhibition was found. This correlation of herbicide binding and inhibition proves that binding specifically occurs at the inhibition site at photosystem II.

In addition to the specific binding, for all four herbicides studied, (except for ioxynil in thylakoids) unspecific binding was observed in thylakoids as well as in algae, which was not related to inhibition.

Introduction

Inhibition of photosynthetic electron transport by photosystem II (PS II) herbicides occurs after binding of these inhibitors to specific receptor sites at the photosystem II complex. There are several studies which indicate that the PS II herbicides act by inactivating the second functional acceptor quinone B after binding to the B-protein [1–4]. At concentrations up to 3–5 nmol herbicide/mg Chl, specific (high affinity) binding is predominantly observed [5–7]. At higher concentrations for several herbicides, the presence of a second, unspecific (low affinity) binding was described [5–8].

Abbreviations: Atrazine, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine; DCPIP, dichlorophenolindophenol; dinoseb, 2-sec-butyl-4,6-dinitrophenol; diuron, DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EDTA, ethylenediamintetraacetic acid; HEPES, n-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; I_{50}, concentration of a herbicide, giving 50% inhibition; K_b, equilibrium constant of herbicide binding; K_i, equilibrium constant of inhibition; MES, 2(N-morpholino)ethanesulfonic acid; PS II, photosystem II; PSU, photosynthetic unit; x_g, concentration of herbicide binding sites.

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The relationship between specific herbicide binding and inhibition of photosynthetic electron transport was demonstrated for several PS II herbicides by Tischer and Strotmann [5] by showing a correlation of the specific binding constant K_b and the inhibition constant K_i.

Recently, accumulation of pesticides in living systems has become of increasing interest. Until now, very limited information has been available about quantitative herbicide uptake and binding in algae [9, 10] or other intact cells.

The present study was initiated to compare herbicide binding in isolated chloroplast membranes and intact green algae, using representatives of several chemical groups of herbicides *i.e.* a phenylurea (diuron, DCMU), a s-triazine (atrazine) and phenol herbicides (ioxynil and dinoseb). It was of interest to know how intact cells with their various lipophilic structures influence herbicide binding and inhibitor efficiency. Studies were performed to clarify whether the correlation of binding (K_b) and inhibition (K_i) is valid also for the phenol herbicides, which show high unspecific binding [8].

In order to perform binding experiments under equilibrium conditions, the incubation time for saturation of herbicide binding was determined.

Materials and Methods

The green algae *Ankistrodesmus braunii* (Naegeli) were grown in a synchroneous culture in an inorganic medium [11] at a light/dark cycle of 14/10 h. The culture was illuminated with white fluorescent light at an intensity of 8 W/m² at pH 6.3 and was gassed with air and 1.5% CO_2. Cells were harvested 5 h after beginning of the light period.

Thylakoid membranes were isolated from freshly harvested, greenhouse grown spinach (*Spinacia oleracea* var. Monatol) in a medium containing 0.33 M sorbitol, 1 mM $MgCl_2$, 2 mM EDTA and 50 mM MES-KOH (pH 6.5). After grinding, filtration and centrifugation the pellet was resuspended in an isolation medium of the same composition but with 50 mM HEPES-KOH (pH 7.6). Chloroplasts were osmotically disrupted by suspending them in distilled water. Addition of the same volume of double strength isolation medium (pH 7.6) restored isomolarity. The preparation was stored in the dark on ice.

Binding of radioactively labelled herbicides was studied using [^{14}C]diuron (DCMU, 4.2 μCi/mg), [^{14}C]atrazine (27.2 μCi/mg), [^{14}C]ioxynil (33.5 μCi/mg) and [^{3}H]*i*-dinoseb (1.44 mCi/mg). [^{3}H]*i*-dinoseb is reported to have the same inhibitory activity as *n*-dinoseb [8].

Binding experiments were performed similar to the procedures given by Tischer and Strotmann [5]. Labelled herbicides were added in increasing concentrations to 1 ml of an algal or chloroplast suspension in a 1.5 ml vial. Algae were suspended in nutrient medium [11], chloroplasts in isolation medium at pH 7.6. Herbicides were generally incubated in the dark at 20 °C for 30 min (algae) or for 20 min (thylakoids). Sedimentation was performed in a Beckman Minifuge-B at 8000 × *g* for 1 min. An aliquot of 0.8 ml was taken from the clear supernatant, added to 3 ml of scintillation fluid (Zinnser, Miniria 20) and counted for radioactivity in a Berthold BF 8000 scintillation counter. Each sample was corrected for quenching. Calculation of the total added herbicide concentration was based on the specific activity of control samples without chloroplasts or algae. The amount of bound herbicide was calculated from the difference of total herbicide added and the concentration of free herbicide in the supernatant. Chlorophyll concentration was 150 μg/ml in dinoseb binding experiments and 50 μg/ml in all other experiments, giving 15–85% bound herbicide, depending on the total amount of herbicide. By plotting the concentration of bound herbicide vs. free herbicide, a hyperbola is obtained which approaches asymptotically the maximum concentration of herbicide binding sites. A double reciprocal plot of this saturation curve yields a straight line indicating at its ordinate intercept the concentration of binding sites (x_g) and at its absicssa intercept the binding constant K_b. All calculations and statistical analyses were performed on a Hewlett-Packard HP 85 desktop computer.

Time dependence of herbicide binding was followed in 10 ml of an algal or chloroplast suspension, containing a herbicide concentration which gave 2–4 nmol herbicide bound/mg Chl. At different times, an aliquot of 1 ml was withdrawn from the suspension and processed as described above. Inhibition of photosynthesis (I_{50}-determinations) in isolated chloroplasts was measured by photometrically following the rate of DCPIP reduction at 600 nm in a Shimadzu UV 210 modified for cross illumination. Exciting saturating red light (60 W/m²) was filtered through 2 mm Calflex C (Balzers, Liechtenstein) and 3 mm RG 645 (Schott, Germany). The photomultiplier of the photometer was protected by 3 mm BG 18 filters (Schott, Germany). Electron transport was measured in the isolation medium (pH 7.6) containing additionally DCPIP (10^{-4} M), NH_4Cl (5×10^{-3} M), gramicidin-D (10^{-7} M) and chloroplasts equivalent to 2 μg Chl/ml.

Two independent methods were used for the determination of the inhibition constant K_i [5]. By transforming the total herbicide concentration used in the inhibition experiments into the free herbicide concentration as described in detail in ref. [5], the inhibition constant K_i is obtained from the double reciprocal plots of inhibition *vs.* free herbicide concentration. The second method is based on the dependence of the I_{50}-concentration on the amount of chlorophyll present in the assays. By determining I_{50}-concentrations at different chlorophyll concentrations the binding constant is obtained after extrapolation to a chlorophyll concentration of zero [5].

Inhibition of photosynthesis for the green alga *Ankistrodesmus* was determined as inhibition of oxygen evolution in a Beckman oxygen electrode (2 ml volume, 30 °C, 1 mM $KHCO_3$, chlorophyll concentration 4 μg/ml, illumination with red light

630 nm, 100 W/m²). K_i determinations for *Ankistrodesmus* were performed by extrapolating I_{50} concentrations to zero chlorophyll concentration, as described above.

All herbicides used were dissovled in pure methanol. Final methanol concentration in the experiments never exceeded 1%. Chlorophyll was determined after Röbbelen [12].

Results

1. Binding of PS II herbicides to isolated thylakoid membranes and to intact green algae

In comparative experiments, binding of [^{14}C]-diuron (DCMU), [^{14}C]atrazine, [^{14}C]ioxynil and [^{3}H]dinoseb to isolated spinach thylakoids and to intact algal cells (*Ankistrodesmus*) was studied. Binding experiments have to be performed in the dark under equilibrium conditions, where no more time dependent penetration or binding is observed and when adsorption and desorption of the herbicide at its binding site is balanced. To evaluate the required incubations times, time dependent herbicide binding at a constant herbicide concentration was studied for all herbicides used in our experiments for thylakoids as well as for algae. Herbicide binding to isolated thylakoids was saturated for all four herbicides after about 2 min (data for diuron and ioxynil shown in Fig. 1). For diuron-type herbicides it is well known, that inhibition of electron transport reactions occurs almost instantaneously after addition of the herbicide. It has been reported for the phenol type herbicides ioxynil [13, 14] and bromonitrothymol [13] that there is a pronounced time lag of 2–4 min before maximum inhibition of light induced electron transport is reached. At concentrations of 2–4 nmol herbicide bound/mg Chl, however, the amount of herbicide bound did not change during 30 min. In contrast to isolated thylakoids, in green algae longer incubation times were required to reach binding equilibrium, as seen in Fig. 1. [^{14}C]diuron binding was saturated after about 10 min. Maximal [^{14}C]atrazine binding also required about 10 min (data not shown). Saturation of [^{14}C]ioxynil binding was observed after 7 min (Fig. 1), whereas [^{3}H]dinoseb required more than 20 min for equilibrium (data not shown). Based on these results all binding experiments using isolated thylakoids were performed after incubating chloroplast membranes for 10 min with herbicides. In experiments using algae, an incubation time of 30 min was used.

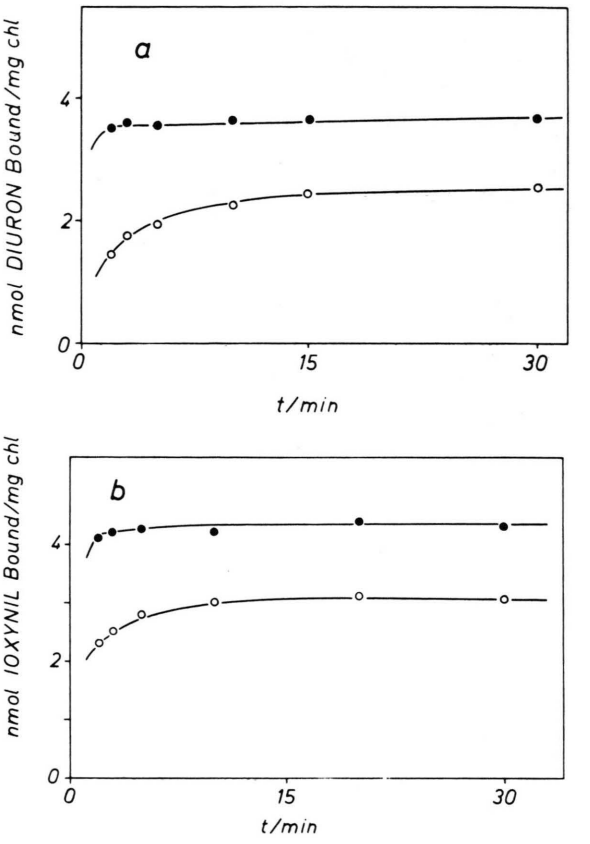
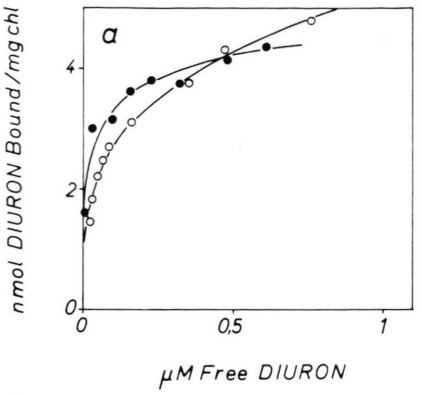


Fig. 1. Time dependence of the binding of [^{14}C]diuron (DCMU) (a) and [^{14}C]ioxynil (b) to isolated thylakoids of *Spinacia oleracea* (●—●) and intact cells of *Ankistrodesmus braunii* (○—○). The binding of a constant herbicide concentration (3×10^{-7} M diuron and 6×10^{-7} M ioxynil for isolated thylakoids and intact algal cells) was measured after different times of uptake. Binding was performed in the dark at 20 °C, pH 7.6 in the isolation medium and at pH 6.3 in algal nutrient solution.

Binding of herbicides to isolated thylakoids and in algae was characterized by the equilibrium binding constant K_b (abscissa intercept in the double reciprocal plots) and by the maximum number of binding sites on a chlorophyll basis, x_g (ordinate intercept). Comparative binding of diuron is described in Fig. 2a, b. No saturation was obtained in the given concentration range. The presence of an unspecific diuron binding is clearly indicated in the double reciprocal presentation (Fig. 2b) by the deviation of the data from a straight line at high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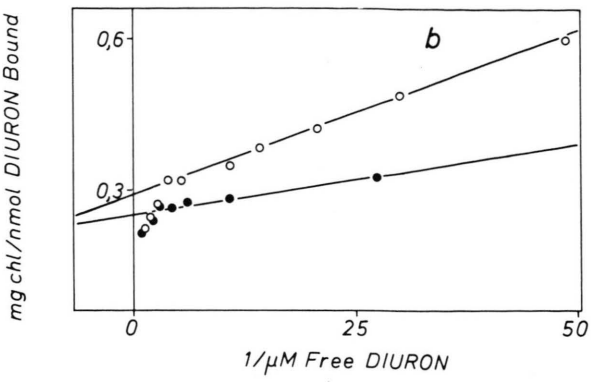
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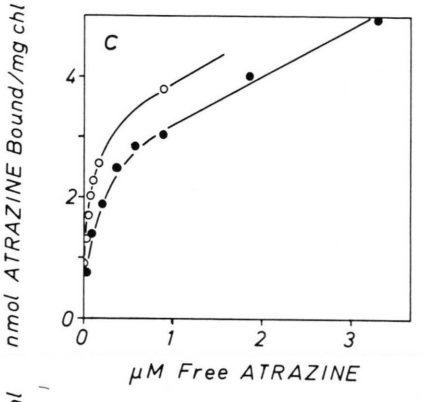
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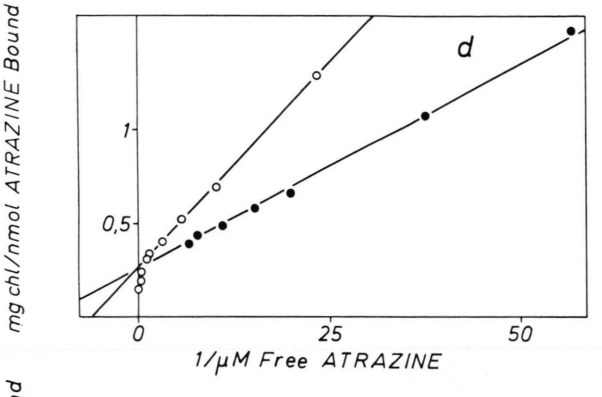
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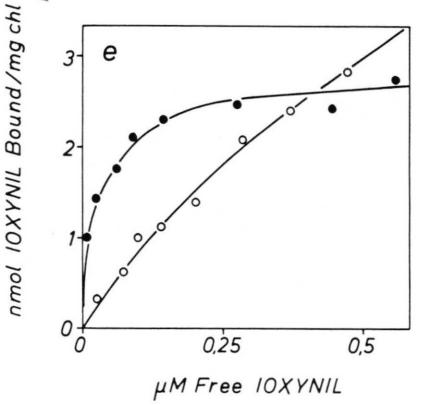
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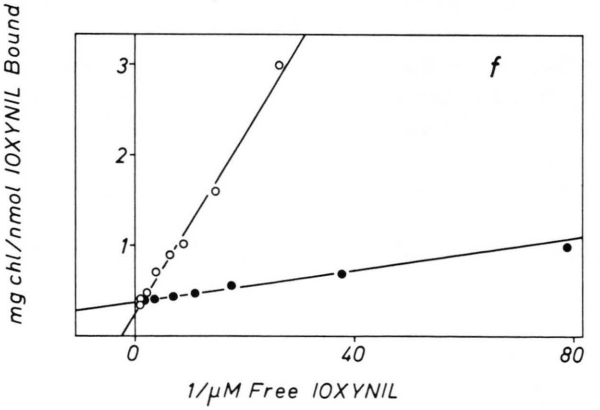
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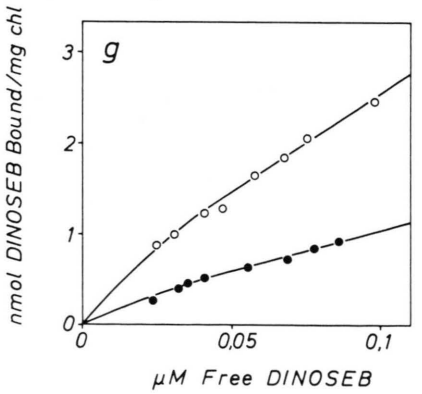
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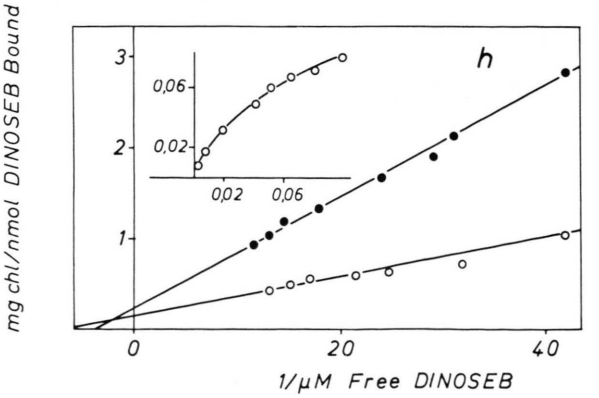
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mg chl/nmol DINOSEB Bound
1/µM Free DINOSEB

diuron concentrations (0.5 μM free diuron). This is observed with isolated thylakoids as well as with algae. The binding constant K_b for specific diuron binding was determined in 5 separate experiments to be very similar in thylakoids ($1.9 \pm 1.3 \times 10^{-8}$ M) and algae ($2.8 \pm 0.9 \times 10^{-8}$ M) respectively. The concentration of binding sites x_g was found to be 3.9 (± 0.9) and 3.3 (± 0.7) nmol inhibitor binding sites/mg Chl in thylakoids and algae.

Binding constants determined for atrazine are also similar in thylakoids ($6.7 \pm 2.6 \times 10^{-8}$ M) and algae ($1.6 \pm 0.3 \times 10^{-7}$ M) (Fig. 2c, d). The concentration of atrazine binding sites was comparable to the value found for diuron binding and was determined to be 3.3 ± 0.6 nmol sites/mg Chl (thylakoids) and 3.0 ± 0.6 nmol sites/mg Chl (algae). Also for atrazine the presence of unspecific binding becomes apparent in algae (Fig. 2d) and in isolated thylakoids (data not shown).

For the phenol herbicide ioxynil, however, a much smaller binding affinity was found in algae ($4.5 \pm 1.7 \times 10^{-7}$ M) as was observed in isolated thylakoids ($2.4 \pm 1.9 \times 10^{-8}$ M) (Fig. 2e, f). With algae no saturation of binding was obtained up to 3 nmol ioxynil/mg Chl, indicating a contribution of unspecific binding. In the double reciprocal plot, however, specific and unspecific binding could not be separated. The high concentration of ioxynil binding sites in algae (5.3 ± 0.8 nmol sites/mg Chl) also suggests the presence of unspecific binding. In thylakoids a concentration of 3.5 ± 0.8 nmol sites/mg Chl was detected which is comparable to the data obtained with diuron and atrazine.

Finally, in isolated thylakoid membranes as well as in algae no saturation of dinoseb binding was observed up to concentrations of 10^{-4} M free dinoseb (Fig. 2g, h). For the concentration range shown in Fig. 2g, h, comparable binding constants were calculated to be $3.6 \pm 2.6 \times 10^{-7}$ M (thylakoids) and $1.4 \pm 0.4 \times 10^{-7}$ M (algae). The corresponding concentration of dinoseb binding sites, however, was found to be quite different and was calculated to be 3.1 ± 0.7 nmol/mg Chl (thylakoids) and 6.5 ± 0.8 nmol/mg Chl (algae). The increased concentration of binding sites in algae indicates again a contribution of unspecific binding. It should be noted that K_b and x_g values for dinoseb are very much dependent on the dinoseb concentration used in the experiment. As seen from the inset in Fig. 2h, at high dinoseb concentrations (up to 100 nmol herbicide bound/mg Chl) a 100 fold higher K_b-value of approximately 10^{-5} M was estimated. It is apparent, that in case of dinoseb binding a multiphasic binding is observed instead of a biphasic binding. For this reason the calculated binding constants are valid only for the concentration range of dinoseb used on the particular experiment.

Fig. 2. Binding of [^{14}C]diuron (a, b), [^{14}C]atrazine (c, d), [^{14}C]ioxynil (e, f) and [^{3}H]dinoseb (g, h) to isolated thylakoids (●—●) and intact algal cells (○—○). K_b- and x_g-values were evaluated from the double reciprocal plots. Chlorophyll concentrations were 50 μg/ml in case of [^{14}C]-diuron-, [^{14}C]atrazine- and [^{14}C]ioxynil binding, respectively 150 μg/ml in case of [^{3}H]dinoseb binding.

2. Inhibition of photosynthesis in isolated thylakoid membranes and in intact algal cells

The correlation of binding and inhibition of electron transport was previously discussed by Tischer and Strotmann [5]. Our results of the determinations of the binding constant K_b, the inhibition constant K_i, of the I_{50}-value and of the number of specific binding sites on a chlorophyll basis are summarized in Table I. The K_i-value was determined by relating the degree of inhibition measured at saturating light intensities [5] to the free inhibitor concentration, as described in material and methods. For these calculations, the binding constant K_b from Table I was used. Determination of the K_i for diuron in isolated thylakoids is illustrated in Fig. 3a, b. The obtained K_i-value of 3.8×10^{-8} M is in good agreement with the K_i-value of 4×10^{-8} M as reported by Tischer and Strotmann [5]. Using a second method of K_i determination by extrapolating I_{50} concentration to a chlorophyll concentration of zero (see material and methods) a very similar K_i-value of 1.8×10^{-8} M was found for diuron (data not shown). Also in intact algal cells of *Ankistrodesmus* the K_i (3×10^{-8} M) is close to the K_b value (Table I). The good correlation of the calculated inhibition constants and the binding constants demonstrates for diuron that binding of the herbicide is strongly correlated with the inhibition of electron transport. This indicates that at these concentrations specific binding of diuron dominates. Similar results are obtained with atrazine in isolated thylakoids (Table I). In this case also the predominance of specific binding is indicated.

For the phenol herbicide dinoseb a reasonable agreement between the binding constant K_b, the

Table I. Comparison of the binding constants (K_b), inhibition constants (K_i), I_{50} concentrations for electron transport inhibition ($H_2O \rightarrow$ DCPIP, 2 µg Chl/ml) and concentrations of inhibitor binding sites (x_g) in isolated spinach thylakoid membranes and in the intact green alga Ankistrodesmus. The values given in the table are means from several independent experiments. K_b and x_g values are given for the specific binding only. K_b and x_g values for dinoseb are valid for the concentration range as shown in Fig. 2 g, h.

	Isolated spinach thylakoid membranes				Intact algal cells (*Ankistrodesmus b.*)			
	K_b [M]	K_i [M]	I_{50} [M]	x_g [nmol/mg Chl]	K_b [M]	K_i [M]	I_{50} [M]	x_g [nmol/mg Chl]
diuron	$1.9 \pm 1.3 \times 10^{-8}$	3.8×10^{-8}	3×10^{-8}	3.8 ± 0.9	$2.8 \pm 1.7 \times 10^{-8}$	3×10^{-8}	2.4×10^{-7}	3.2 ± 0.7
atrazine	$6.7 \pm 2.6 \times 10^{-8}$	4.5×10^{-8}	5×10^{-8}	3.3 ± 0.6	$1.6 \pm 0.3 \times 10^{-7}$	–	1.5×10^{-6}	3.0 ± 0.6
ioxynil	$2.4 \pm 1.9 \times 10^{-8}$	1.0×10^{-7}	3×10^{-7}	3.5 ± 0.8	$4.6 \pm 1.7 \times 10^{-7}$	–	6.2×10^{-6}	5.3 ± 0.8
dinoseb	$3.6 \pm 2.1 \times 10^{-7}$	7.8×10^{-7}	1×10^{-6}	3.1 ± 0.7	$1.4 \pm 0.4 \times 10^{-7}$	–	2.7×10^{-6}	6.5 ± 0.6

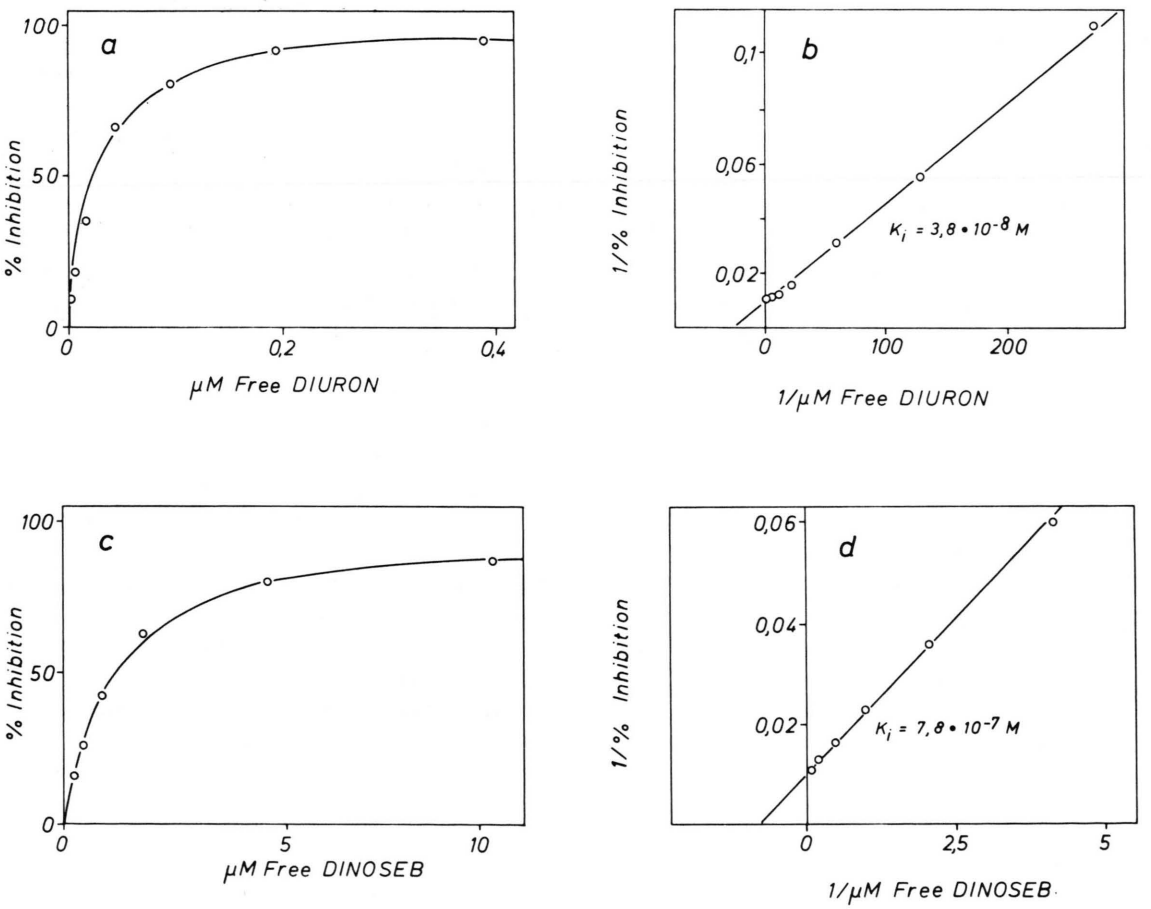

Fig. 3. Evaluation of K_i for diuron (a, b) and dinoseb (c, d) in isolated thylakoids. Inhibition of electron transport was measured photometrically as decreasing rates of DCPIP reduction. The free concentrations of herbicides were determined according to the equation of Tischer and Strotmann [5] using a K_b which was previously determined in a binding experiment. K_i is given by the abscissa intercept of the reciprocal plot (b, d).

inhibition constant K_i and I_{50} concentration was obtained (Table I). A K_i-value of 7.8×10^{-7} M was determined for dinoseb by relating the inhibition of the DCPIP reduction to the free dinoseb concentration (Fig. 3c, d). The extrapolation method yielded a K_i of 6×10^{-7} M (data not shown). The binding constant of dinoseb is approximately two times lower (3.6×10^{-7} M). It should be noted that the binding data obtained are strongly dependent on the dinoseb concentration used in the experiment. The reasonable agreement of binding and inhibition, found only at the given low dinoseb concentrations, is gradually lost with increasing dinoseb concentrations (inset Fig. 2h).

For the herbicide ioxynil an inhibition constant of 1×10^{-7} M (thylakoids) was calculated (Table I), which differs considerably from the binding constant (2.4×10^{-8} M). Also the I_{50} concentration (3×10^{-7} M) deviates much more from the K_b-value compared to all other herbicides studied.

Discussion

Binding of radioactively labelled herbicides was analysed to compare uptake of three representatives of different chemical classis of PS II herbicides by isolated thylakoid membranes and by intact cells of green algae. Binding affinity (K_b) of either diuron and atrazine was similar in intact algae as well as in thylakoid membranes (Table I). The values for K_b which are given in Table I are calculated for specific binding only. Unspecific binding of diuron, atrazine and dinoseb became more apparent at higher herbicide concentrations than those required for measuring specific binding. In the case of diuron and atrazine, unspecific binding is indicated in the double reciprocal binding plots by a deviation of the data at high concentrations from a straight line (Fig. 2b). Specific and unspecific binding can be easily separated if the two binding reactions occur at different concentration ranges. In such a case a second line can be fitted to the data of unspecific binding. Analysis of the data for unspecific diuron binding (Fig. 2a, b) yields an estimated K_b of $3-4 \times 10^{-7}$ M (thylakoids and intact cells respectively), which is about 10 times higher than the specific binding constants.

A different behaviour was observed with the phenol herbicide dinoseb. Binding of these herbicide does not saturate even at levels of several hundred nmol dinoseb bound/mg Chl (inset in Fig. 2h) [8, 13], indicating the presence of strong unspecific binding. In the double reciprocal binding plot at high concentrations a clear negative deviation from the straight line plot is observed. This deviation does not consist of a single additional binding reaction but instead is formed by a multitude of reactions each having a different binding affinity. The binding constant calculated from the linear branch of the reciprocal plot (Fig. 2h) was determined to be 3.6×10^{-7} M. This is higher than the value of 6.9×10^{-8} M given by Oettmeier and Masson [8, 13], which was calculated by substracting unspecific binding from the total dinoseb binding.

The binding affinity of ioxynil to *Ankistrodesmus* cells is approximately 20 times lower as compared to isolated thylakoids. No other herbicide studied showed such a difference between the binding constants of isolated thylakoids and of cells of green algae. The rate of ioxynil penetration into the algal cells is clearly not limiting under our conditions (Fig. 1). The lack of saturation indicates the presence of high unspecific binding in algal cells, although this is not becoming apparent in the concentration range described in the double reciprocal representation of the data (Fig. 2f).

Further evidence for the presence of unspecific binding sites can be obtained by performing competition experiments between phenol herbicides and the predominantly specific binding diuron-type herbicides. Results of such experiments will be reported in a forthcoming paper.

The relationship between herbicide binding and inhibition of photosynthetic electron transport was compared for isolated thylakoids and for intact algal cells. For the herbicide diuron, binding and inhibition constants are found to agree remarkable well for thylakoids and intact cells (Fig. 2a, b, Table I). Also for atrazine and dinoseb agreement of K_b and K_i was found. It should be noted that the K_i and I_{50} concentrations for ioxynil differ much more from the binding constant than was observed for the other herbicides studied. A K_b of 2.9×10^{-7} M was reported by Pfister *et al.* [16] for ioxynil. This constant was calculated from a competition experiment measuring the release of [^{14}C]atrazine from isolated *Amaranthus* thylakoids after addition of [^{12}C]ioxynil. The K_b-value reported by these authors is clearly higher than the K_b measured in a direct binding experiment (Fig. 2d, Table I). We propose from

these results that at least a part of the ioxynil bound at low concentrations does not contribute to an inhibition of PS II photoreactions. Inhibitory effects of ioxynil at the oxidizing site of PS II have recently been described [17]. These effects require ioxynil concentrations of more than 6×10^{-5} M for half saturation [15] and therefore should not have affected our binding or inhibition experiments.

The concentration of herbicide binding sites for all four compounds studied was calculated to be 3.1 to 3.8 nmol/mg Chl in isolated thylakoids and 3 to 3.2 nmol/mg Chl in intact algal cells. For the phenol type herbicides dinoseb and ioxynil in algal cells 6.5 and 5.3 nmol/mg Chl were determined, which may indicate contribution of unspecific binding sites to the total number of sites. Oettmeier and Masson [8] have calculated the concentration of dinoseb binding sites in spinach thylakoid membranes to be 1.45 nmol/mg Chl (830 Chl/inhibitor) by substraction of unspecific binding from total observed binding as compared to the value of 3.6×10^{-7} M as given in Table I. With the exception of the phenol herbicides binding data in intact cells, a concentration of 3 to 3.8 nmol herbicide bound per mg chlorophyll indicates the involvement of 300 to 240 chlorophyll molecules in creating a herbicide binding site. Other authors have reported 300–500 Chl/inhibitor (spinach, ref. [5]) and 420–450 Chl/inhibitor (Senecio, ref. [6]).

This stoichiometry agrees with the size of the photosynthetic unit (PSU) reported by several authors. It is generally assumed that in higher plants 300 to 500 chlorophylls and in green algae 250–300 chlorophylls comprise one photosynthetic unit [18–20]. The ratio of chlorophyll/PSU and of chlorophyll/herbicide binding site suggests a simple 1:1 stoichiometry between the number of electron transport chains and the number of inhibitor binding sites.

The results reported in this publication prove that unicellular microalgae are suitable for quantitative studies of herbicide uptake and accumulation. It should be noted that for all herbicides studied, herbicide binding primarily occurs to the specific herbicides receptors (B-protein) at the PS II complex [16]. Depending on the chemical structure of the herbicide an additional binding can occur which is not related to inhibition of PS II reactions and is therefore termed "unspecific". The nature of this secondary binding reaction and whether this influences any native biochemical reaction, is not yet known. The unspecific binding of herbicides may be important for accumulation and distribution of these compounds in plant cells. The results of the binding studies with phenol herbicides should be helpful for further investigation and the understanding of the reversible herbicide resistance in algal cells, which has been decribed recently [21].

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