Increased Binding of [^{14}C]Ioxynil in Unicellular Green Algae under Anaerobiosis and by Addition of Other Phenolic Herbicides or Uncouplers

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Binding of the phenol-type herbicide ioxynil in intact algal cells is strongly increased under anaerobic conditions. This increase is most pronounced at low concentrations of bound ioxynil (< 3 nmol/mg chlorophyll). The affinity of binding sites is improved, rather than new sites are created.

Other phenol-type herbicides, as well as uncouplers like carbonylcyanidechlorophenylhydrazone, also significantly increase ioxynil binding in algae. Binding of DCMU-type herbicides, however, was not affected.

These results demonstrate high variability of ioxynil binding in algal cells and are discussed in respect of low affinity binding.

Introduction

In algal cells, the binding properties of phenol-type PS II herbicides turned out to be quite different from those of DCMU-type herbicides [1, 2]. Besides specific binding to the PS II acceptor side [2–4] phenolic herbicides display additional binding sites even at concentrations, where 'specific' binding is not yet saturated (< 3 nmol bound/mg Chl) [2].

In our experiments, algal cells turned out to be suitable objects for studies on the influence of physiological conditions on herbicide binding at PS II. In order to understand these additional effects of ioxynil in intact cells [5] binding experiments, *e.g.* under anaerobiosis or in presence of uncouplers were carried out.

Materials and Methods

Ankistrodesmus braunii was grown in synchroneous suspension culture with a light – dark cycle of 14:10 h in an inorganic medium at pH 6.3. The culture was gassed by air with addition of 1.5% CO_2.

[^{14}C]ioxynil, a benzonitrile (34.3 µCi/mg) and [^{14}C]-DCMU (34.4 µCi/mg) were used as radioactively labelled herbicides. Binding and competition experiments were performed as previously described [1, 2]. The experiments were carried out in the dark. Oxygen was removed by addition of 10 mM glucose and of 0.1 mg/ml glucose oxidase (250 units/mg). For presence of oxygen, samples were gassed with air during herbicide incubation. The incubation time of the herbicides was 20 min. In competition experiments [^{14}C]-labelled herbicides were bound to equilibrium [2] before incubation with competitor for 20 min. Incubation was stopped by centrifugation (Beckman, Minifuge B) at about 9000 × g for 30 sec. Aliquots of the clear supernatant were added to scintillation fluid (Zinsser, Miniria 20) and were counted for radioactivity (Contron Betamatic), corrected for quenching.

Results and Discussion

In Fig. 1 concentration dependent binding of [^{14}C]-ioxynil to *Ankistrodesmus b.* is demonstrated. Binding in the dark was significantly increased by anaerobiosis. This phenomenon was also found with other green algae, *e.g. Chlorella vulgaris* and *Eremosphaera viridis.* With increasing concentrations the effect becomes stronger (Fig. 1). At bound concen-

Abbreviations: CCCP, carbonylcyanidechlorophenylhydrazone; Chl, chlorophyll; Dinoseb, 2-*sec*-butyl-4,6-dinitrophenol; Diuron (DCMU), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Ioxynil, 4-hydroxy-3,5-diiodobenzonitrile; K_b, equilibrium constant of herbicide binding; PS II, photosystem II; X_g, concentration of herbicide binding sites.

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trations higher than 3–4 nmol/mg Chl, the binding isotherms in presence and in absence of oxygen converge again and reunite at about 8–10 nmol bound/mg Chl (data not shown). Obviously there is a rather limited supply of receptor sites which is influenced by anaerobiosis. For analysis of the binding constant (K_b) and the concentration of binding sites (X_g) a double reciprocal plot of the binding data is depicted (Fig. 2). The K_b-value is shifted from 4.7×10^{-7} M to 9.7×10^{-8} M by depletion of oxygen. In contrast, X_g is rather unaffected

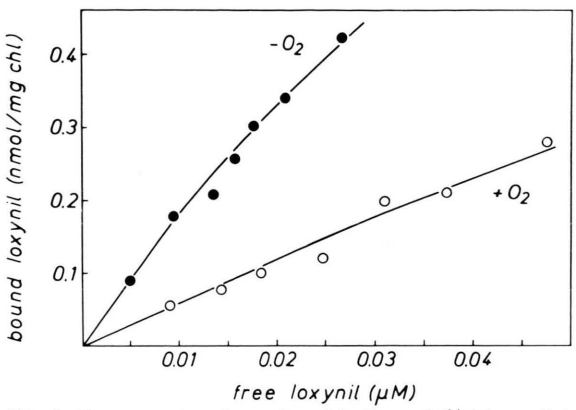

Fig. 1. Concentration-dependent binding of [^{14}C]ioxynil in the dark in presence (○) and absence (●) of oxygen. Oxygen was removed by glucose/glucose oxidase. Temperature 20 °C, pH 6.3, Chl, 100 μg/ml.

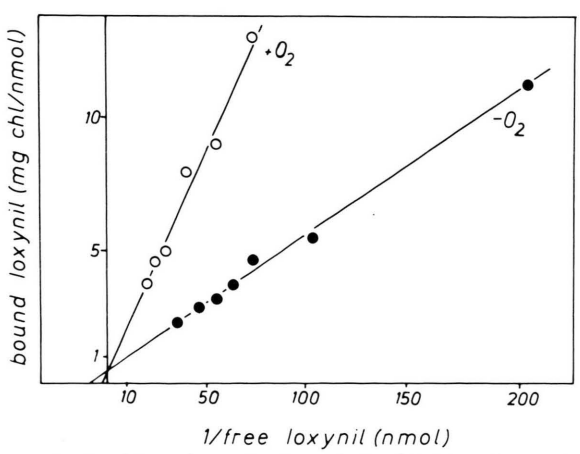

Fig. 2. Double-reciprocal plot of the binding data displayed in Fig. 1, in presence (○) and absence (●) of oxygen. K_b is taken from the abscissa intercepts, X_g from the ordinate intercepts of the straight lines.

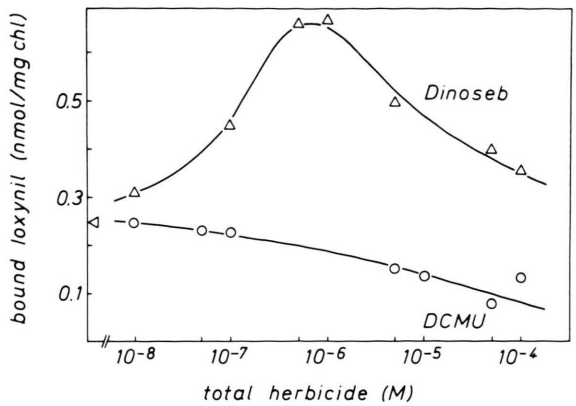

Fig. 3. Concentration-dependent influence of diuron (○) and of dinoseb (△) on [^{14}C]ioxynil binding. Temperature 20 °C, pH 6.5, Chl, 100 μg/ml.

(2.8 nmol/mg Chl). The result seems to indicate, that increased binding is caused by increased binding affinity rather than by generation of additional receptor sites.

Possibly a multitude of different ioxynil binding sites exists in algae which together reveal one total 'apparent' K_b- and X_g-value in graphical binding analysis (see [1] and Fig. 2). This variety of sites, even at low ioxynil concentrations is obviously the reason why the same phenol-type herbicide is much less effective as inhibitor of photosynthesis in algae as compared to isolated thylakoid membranes [1]. The actual reason for oxygen dependent ioxynil binding is under present investigation as there is multiple action of oxygen in the cell (*e.g.* shifting of redox potentials in photosynthesis and respiration [6]). It should be emphasized that there seems to be importance of this oxygen effect for studies on herbicide accumulation in microorganisms, as oxygen deficiency as well as the ioxynil concentrations used in the experiments may be realistic for aqueous systems polluted by herbicides.

Competition of previously bound [^{14}C]ioxynil with DCMU is depicted in Fig. 3. As displacement of ioxynil is incomplete, there is evidence that only a part of ioxynil binding takes place at the PS II acceptor complex [1]. [^{14}C]ioxynil binding in presence of dinoseb is in contrast to competition of herbicides reported before (Fig. 3). Up to about 10^{-6} M dinoseb, ioxynil binding was increasingly strength-

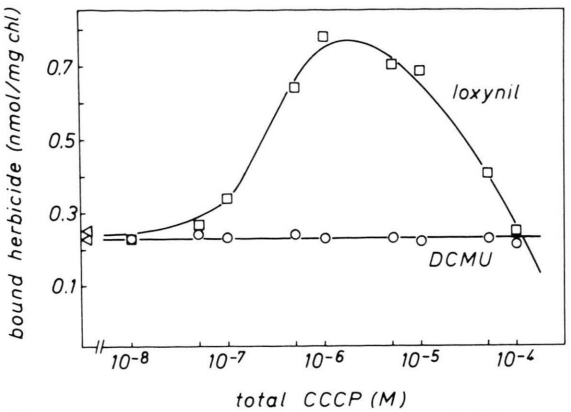

Fig. 4. Concentration-dependent influence of CCCP on binding of [^{14}C]diuron (○) and of [^{14}C]ioxynil (□). Temperature 20 °C, pH 6.5, Chl, 100 µg/ml.

ened, until at higher dinoseb concentrations displacement of bound ioxynil occurred again. A similar effect was found with the phenolic compound pentachlorophenol.

Extended ioxynil binding was also mediated by CCCP, a compound with uncoupler activity [7] (Fig. 4). Thus the 'stimulation' of ioxynil binding seems not to be correlated with herbicidal activity of the effectors. As demonstrated in Fig. 4, no influence of CCCP on [^{14}C]DCMU binding to algae was detected. There is no competition of DCMU-type herbicides and of CCCP for common binding sites. Under our conditions, we did not find this 'stimulation' effect in experiments with isolated thylakoid membranes. Therefore, the conductive effect of uncouplers and other phenolic herbicides seems not to take place directly at PS II. Further investigations should rather take into account possible existence of binding sites of phenolic herbicides in mitochondria (see uncoupling activity [5, 8]). Studies using isolated mitochondria will be helpful for elucidation of this effect.

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