Some Properties of the DCMU-Binding Site in Chloroplasts

Wolfgang Tischer and Heinrich Strotmann

Tierärztliche Hochschule, Botanisches Institut, Abteilung für Biochemie der Pflanzen, Bünteweg 17, D-3000 Hannover

Z. Naturforsch. **34 c**, 992–995 (1979); received June 2, 1979

Metribuzin, Inhibition of Photosynthetic Electron Transport, Trypsin Sensitivity of the DCMU Binding Site

Binding of the triazinone derivative metribuzin to thylakoid membranes was studied in dependence of temperature and trypsin pre-treatment of the chloroplasts. The temperature curve of the binding constant suggests that the lipid phase of the membrane contributes to inhibitor binding. Trypsin pre-treatment of the membranes removes the receptor sites for the inhibitor, while the trypsin effect on the affinity of the receptor to the inhibitor is marginal. Concomitantly trypsin treatment releases the inhibitory effect of metribuzin on the electron transport from DPC to DCPIP.

Introduction

Several herbicides of different chemical structure were shown to interact with the DCMU binding site of the thylakoid membrane, besides the phenylureas *e. g.* triazines, triazinones and pyridazinones [1]. Binding of these compounds to the membrane is quantitatively related to their inhibitory effect on photosynthetic electron transport. Maximum binding is 1 mol per 300 to 500 mol chlorophyll, suggesting that the inhibitor receptor is an electron carrier or a membrane component present in a stoichiometric amount to most of the redox systems of the photosynthetic electron transport chain [1].

In the present studies, the triazinone derivative metribuzin is employed as a DCMU-type inhibitor. The inhibitory effect of the 1,2,4-triazinones on electron transport was first described by Draber *et al.* [2]. Their interaction with the DCMU site was established by us in competition binding experiments [1]. The dissociation constant (K_d) corresponds to the inhibition constant (K_i). The numerical value for both is 0.07 μM.

In the present paper, some other physicochemical and biochemical characteristics of the inhibitor-receptor interaction are described. The results indicate that the receptor molecule is a membrane protein which is decomposed by treatment of the membranes with the peptidase trypsin. After digestion, electron transport becomes insensitive to the inhibitor. Moreover, the lipid phase of the membrane also appears to be involved in the binding of the inhibitor.

Reprint requests to Prof. Dr. H. Strotmann.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenol indophenol; DPC, diphenyl carbazide; metribuzin, 4-amino-6-isopropyl-3-methylthio-1,2,4-triazin-5-one.

0341-0382 / 79 / 1100-0992 $ 01.00/0

Experimental

Chloroplast preparation from spinach and binding of [^{14}C]ring-labeled metribuzin to the isolated thylakoid fractions were carried out as described [1]. The reaction medium contained 25 mM Tricine buffer, pH 8.0, 50 mM NaCl, 5 mM $MgCl_2$ and [^{14}C]metribuzin at varying concentrations added as a methanol solution. The final methanol concentration was constant (1%). Usually separation of the chloroplasts from the medium was attained by centrifugation as described [1]. In one experiment (Fig. 1) a filtration technique was employed (details in the legend).

Electron transport to DCPIP was measured by following directly the decrease in 580 nm absorbance in a spectrophotometer (PMQ II, Zeiss) with cross illumination equipment. The red actinic light (filter RG 630, Schott) was 8.7×10^5 ergs cm^{-2} sec^{-1}. The reaction medium contained 25 mM Tricine buffer, pH 8.0, 50 mM NaCl, 5 mM $MgCl_2$, 0.1 mM DCPIP and, where indicated, 0.8 mM DPC. The chlorophyll content was around 5 μg/ml, the total volume was 2 ml, the temperature 20 °C.

Results and Discussion

Fig. 1 shows $\log 1/K_d$ for metribuzin binding as a function of the reciprocal of the absolute temperature. This plot exhibits a linear increase in the temperature range from 34 to 14 °C. From the

Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung") beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

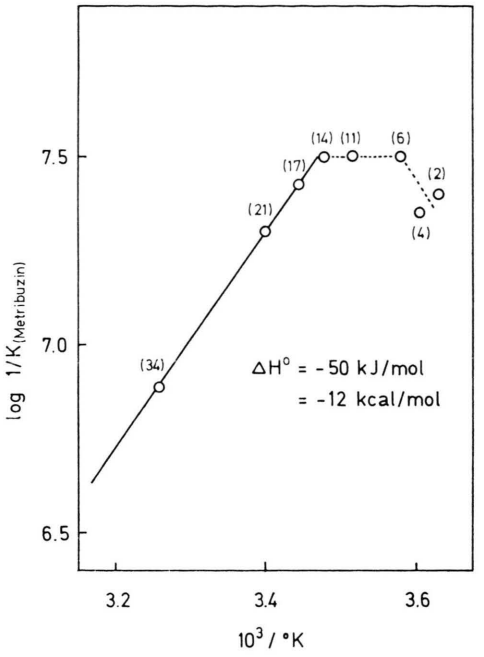

Fig. 1. Temperature dependency of the binding constant ($=1/K_d$) for metribuzin. Binding was measured by forced dialysis using plastic syringes which were connected with filter holders (Schleicher and Schüll, FP 025/1). Filters with a pore size of 0.2 μ (Schleicher and Schüll, RC 58) were employed. The complete reaction mixture including [^{14}C]metribuzin and chloroplasts, was pipetted into the syringes which were temperature controlled by a water jacket. After temperature equilibration, the plungers were inserted and depressed. In aliquots of the filtrate free [^{14}C]metribuzin was determined. The K_d values for each temperature were calculated from concentration curves. — The numbers in brackets indicate the temperatures in °C. ΔH was computed from the left linear branch of the curve.

slope, the standard free enthalpy change can be calculated. Under the experimental conditions employed, ΔH° is equal to -50 KJ/mol. At 14 °C the curve shows a characteristic break. Below this point, K_d is virtually temperature independent. Several other reactions related to the thylakoid membrane show similar changes in the slopes of activation energy [3 – 6] which occur in the same temperature range. They are probably due to a phase transition of the membrane lipids [7]. One could therefore speculate that the lipid phase of the membrane in some way contributes to the inhibitor binding process.

However, the intrinsic receptor molecule is probably a polypeptide constituent of the membrane. This is suggested by the sensitivity of inhibitor binding towards trypsin treatment.

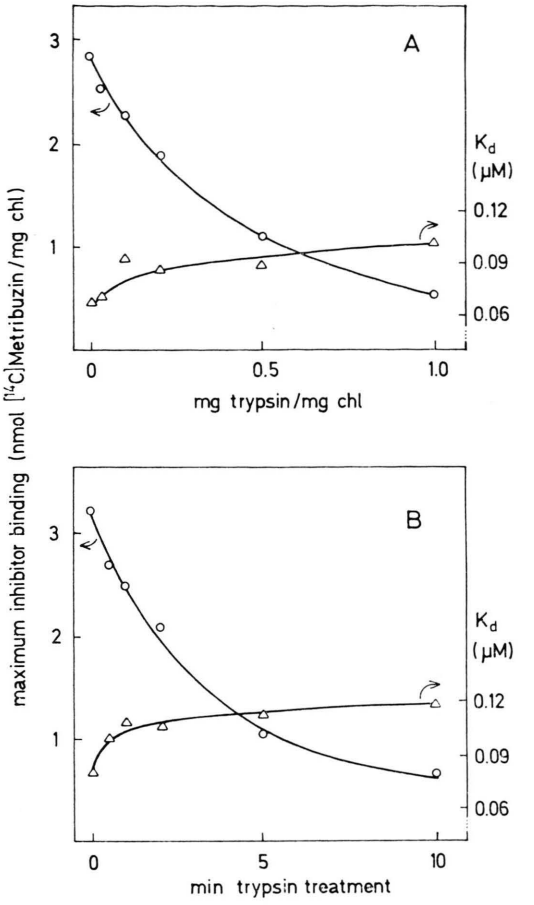

Fig. 2. Binding of [^{14}C]metribuzin by trypsin-treated broken chloroplasts as a function of trypsin concentration (A) and trypsin incubation time (B). For trypsin digestion the medium contained 70 mM Tricine buffer pH 7.8, and chloroplasts equivalent to 1 mg Chl/ml (A) and 0.9 mg Chl/ml (B), respectively. In A the trypsin concentration was varied, the incubation time was 6 min. In B the trypsin concentration was 1 mg/ml, while the incubation time was varied as indicated. Digestion was interrupted by the addition of trypsin inhibitor (1 mg/ml final concentration). After two washes with the trypsin-free medium, binding of labeled metribuzin was measured as a function of inhibitor concentration. From the double-reciprocal plots (1/bound versus 1/free inhibitor), maximum binding and K_d were evaluated. — Bovine pancreas trypsin and soy bean trypsin inhibitor were purchased from Boehringer.

In Fig. 2 the effect of increasing trypsin concentration on K_d as well as maximum inhibitor binding is shown. Whereas the K_d increases by a factor of less than 2 and this increase saturates at relatively low trypsin concentration, maximum binding continuously decreases with increasing trypsin concentration. Under the conditions employed, a trypsin/

chlorophyll (w/w) ratio of 1 leads to more than 80% inhibition of maximum binding. Similar results are obtained when the trypsin incubation time is varied at constant trypsin concentration (Fig. 2, *cf.* also Fig. 4). Since the maximum inhibitor binding reflects the number of inhibitor binding sites present, it has to be concluded that the receptor is removed or destroyed by the peptidase, while the remaining receptor molecules are only slightly modified in their binding properties.

Regitz and Ohad [8] and Renger [9] reported that, under certain conditions, the sensitivity of photosynthetic electron transport to DCMU is abolished by pre-treatment of chloroplasts with trypsin. If this is related to the above described decomposition of the inhibitor receptor site, the two effects should be parallel with respect to either the trypsin concentration or trypsin incubation time.

However, the effect of trypsin on electron transport is more complex. Trypsin is known to affect the oxygen evolving system [10, 11]. This type of inhibition can be overcome by the artificial photosystem II electron donor DPC [11]. In order to exclude this trypsin effect, the water oxidizing system is completely inactivated by Tris treatment [12] prior to trypsin digestion and the electron flow from DPC to the acceptor DCPIP is followed.

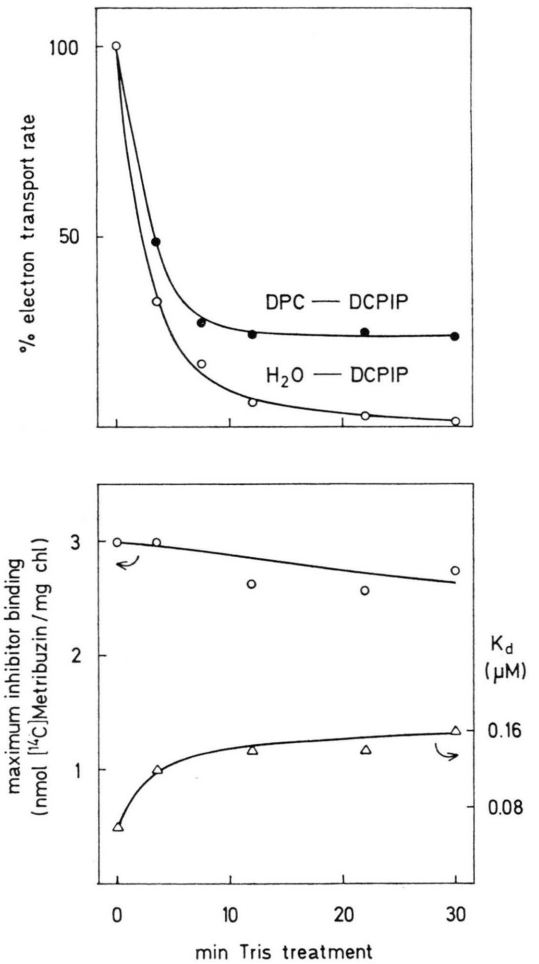

Fig. 3. Binding of [^{14}C]metribuzin and electron transport as affected by Tris treatment of the chloroplasts. Chloroplasts (0.1 mg Chl/ml) were suspended in 0.8 M Tris, pH 8.0. After the indicated times they were sedimented by centrifugation (2 min at 15 000×g) and washed twice with a medium containing 70 mM sucrose, 2 mM $MgCl_2$ and 2 mM Tricine buffer, pH 8.0. In parallel experiments electron transport with and without 0.8 mM DPC as well as [^{14}C]metribuzin binding as a function of inhibitor concentration was measured. The control rates for electron transport were 504 μmol DCPIP reduced/mg Chl per h (−DPC) and 420 μmol DCPIP reduced/mg Chl per h (+DPC), respectively.

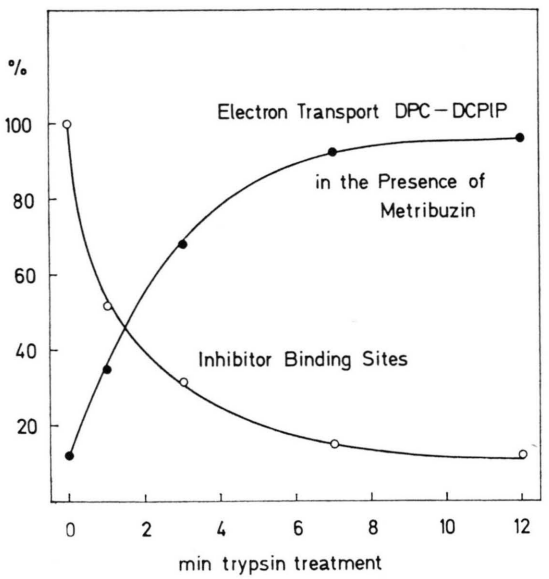

Fig. 4. Removal of inhibitor binding sites by trypsin treatment and release of the inhibitor sensitivity of electron transport. Chloroplasts were first treated with Tris as described in Fig. 3 for 12 min. Subsequently they were subjected to trypsin digestion (0.7 mg trypsin/mg Chl) for the indicated times as described in Fig. 2. With those preparations electron transport from DPC to DCPIP in the presence of 100 μM metribuzin as well as [^{14}C]metribuzin binding as a function of inhibitor concentration was measured. From the concentration curves the numbers of active binding sites (= maximum binding of inhibitor) were computed. Per cent electron transport rates are related to the control rate obtained with the Tris-treated, non-digested chloroplasts in the absence of metribuzin. The control rate was 74 μmol DCPIP reduced/mg Chl per h.

Fig. 3 demonstrates that about 20% of the control activity can be recovered by DPC in Tris treated chloroplasts. The effect of Tris on the maximum inhibitor binding is marginal; however, the dissociation constant K_d increases. When Tris treated chloroplasts are subjected to trypsin digestion, the number of inhibitor binding sites decreases with increasing incubation time in accordance with the results shown in Fig. 2. In parallel, the inhibition of electron transport through the indicated system by metribuzin is released (Fig. 4).

Renger [9] has suggested that the DCMU binding component may be a protein shield which covers the primary acceptor of photosystem II, X-320. Trypsin treatment is believed to remove this protein so that X-320 becomes accessible to an artificial electron acceptor. The results presented here, are in agreement with this hypothesis.

[1] W. Tischer and H. Strotmann, Biochim. Biophys. Acta **460,** 113–125 (1977).
[2] W. Draber, K. H. Büchel, K. Dickoré, A. Trebst, and E. Pistorius, Progress in Photosynthesis Research (H. Metzner, ed.) **Vol. III,** pp. 1789–1795, Laupp, Tübingen 1969.
[3] R. Kraayenhof, M. B. Katan, and T. Grunwald, FEBS Lett. **19,** 5–10 [1971].
[4] F. A. McEvoy and W. S. Lynn, Arch Biochem. Biophys. **150,** 632–635 (1972).
[5] P. Jursinic and Govindjee, Photochem. Photobiol. **15,** 331–348 [1972].
[6] P. Gräber and H. T. Witt, Proceedings of the 3rd International Congress on Photosynthesis (M. Avron, ed.), **Vol. II,** pp. 951–956, Elsevier, Amsterdam 1974.
[7] J. K. Raison, J. Bioenerg. **4,** 285–309 (1973).
[8] G. Regitz and I. Ohad, Proceedings of the 3rd International Congress on Photosynthesis (M. Avron, ed.) **Vol. III,** pp. 1615–1625, Elsevier Amsterdam 1974.
[9] G. Renger, Biochim. Biophys. Acta **440,** 287–300 [1976].
[10] K. E. Mantai, Plant Physiol. **45,** 563–566 (1970).
[11] B. R. Selman and T. T. Bannister, Biochim. Biophys. Acta **253,** 428–436 (1971).
[12] T. Yamashita and W. L. Butler, Plant Physiol. **43,** 1978–1986 [1968].