DCMU-Induced Fluorescence Changes and Photodestruction of Pigments Associated with an Inhibition of Photosystem I Cyclic Electron Flow

Stuart M. Ridley
ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, U.K.

and

Peter Horton
Department of Biochemistry and ARC Research Group on Photosynthesis, University of Sheffield, U.K.

Z. Naturforsch. **39 c,** 351–353 (1984); received October 10, 1983

Diuron (DCMU), Photodestruction, Fluorescence, Photosystem I, Cyclic Electron Flow

Diuron (DCMU) induces the photodestruction of pigments, which is the initial herbicidal symptom. As a working hypothesis, it is proposed that this symptom can only be produced when the herbicide dose is sufficiently high to inhibit not only photosystem II electron transport almost completely, but also inhibit (through over oxidation) the natural cyclic electron flow associated with photosystem I as well. Using freshly prepared chloroplasts, studies of DCMU-induced fluorescence changes, and dose responses for inhibition of electron transport, have been compared with a dose response for the photodestruction of pigments in chloroplasts during 24 h illumination. Photodestruction of pigments coincides with the inhibition of cyclic flow.

Introduction

The major initial symptom of phytotoxicity in plants treated with herbicides such as diuron (DCMU), whose mechanism of action is to block chloroplast electron transport, is the destruction of existing chlorophyll, and the extent of this depends on the intensity of the light under which the plants are grown. Much more is now known about the Q_B-protein in the lamellae which is involved in the block, as other papers in this Workshop report. What is now needed is an understanding of processes leading to this lethal symptom, beyond the time when DCMU binds to the Q_B-protein and up until the moment when the process of photodestruction actually starts. Few experiments have ever been done that follow the destruction of chloroplast components and processes in the time scale of hours, but attempts to gain a greater understanding of such events have been made using isolated chloroplasts [1–3], and individual leaves [4] illuminated and analyzed over periods of one or more days. This paper provides support for a working hypothesis proposing that natural cyclic electron flow associated with PSI must also be inhibited before DCMU can induce the photodestruction of pigments.

Abbreviations: BSA, bovine serum albumine; DCMU (diuron), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, photosystem.

Reprint requests to S. M. Ridley.

0341-0382/84/0500-0351 $ 01.30/0

Materials and Methods

Intact chloroplasts from pea seedlings were isolated by the method of Walker [5], and from maize mesophyll protoplasts [6] by the method of Day *et al.* [7]. Experiments on the photodestruction of pigments were carried out and measured as in [2] but in a medium additionally containing 0.1% BSA. Coupled noncyclic basal rate electron transport was recorded as O_2 evolution as described by Ridley [8]. The operation of PS I cyclic electron flow was recorded indirectly by measuring [^{14}C]leucine incorporation by a suspension of chloroplasts [9] following 30 min incubation (20 °C) and illumination (100 W/m^2) [2] in a medium (0.5 ml) containing 0.2 M KCl, 66 mM tricine (pH 8.3), 6.6 mM $MgCl_2$, 5.72 µM [^{14}C]leucine, 1 µM DCMU, and chloroplasts (180 µg/ml). Chlorophyll fluorescence was measured using an apparatus described by Horton [10].

Results and Discussion

The photodestruction of pigments induced by DCMU has been simulated *in vitro* by incubating isolated pea chloroplasts in the light for periods of about 24 h and monitoring pigment levels at intervals [2, 3]. Attempts have been made here to establish a relationship between the extent of pigment photodestruction induced by DCMU, and the degree to which DCMU can immediately inhibit electron flow. This has involved a study of the effects of varying doses of the herbicide on three different processes recorded as far as possible under similar conditions (100 W/m^2 illumination, 200 μM Chl) using intact or freshly ruptured chloroplasts (Fig. 1):

(a) chlorophyll and carotenoid levels in chloroplasts following 24 h illuminated incubation;

(b) coupled noncyclic basal rate electron transport, measured immediately in freshly prepared chloroplasts as O_2 evolution in the presence of ferricyanide;

(c) cyclic electron transport, the operation of which was recorded indirectly by the light-dependent incorporation of [^{14}C]leucine into proteins by a suspension of freshly prepared intact chloroplasts containing 1 μM DCMU. When PS II is almost blocked by DCMU, ATP for protein synthesis comes from cyclic rather than noncyclic phosphorylation [11], and so we believe this to be a reasonable indicator of the operation of natural cyclic electron flow itself. The DCMU inhibitions (Fig. 1) are reversed by the addition of ATP, so the mechanism of protein synthesis is not being directly inhibited, only the production of ATP. It is apparent (Fig. 1) that pigment photodestruction after 24 h closely follows the progressive inhibition in fresh chloroplasts of cyclic electron transport rather than the progressive inhibition of noncyclic electron flow. I_{50} values of 0.36 μM and 4.3 μM for the inhibition of coupled noncyclic and cyclic electron flow, respectively, have been obtained. Concentrations of DCMU, that inhibit noncyclic electron transport up to about 98% in fresh chloroplasts, fail to induce any pigment photodestruction when chloroplasts are illuminated for 24 h. At this level of inhibition the Chl:DCMU ratio is about 200 : 1, which is similar to that needed to optimize fluorescence quenching through cyclic electron flow by providing the correct redox 'poising' of the electron carriers common to both cyclic and noncyclic electron transport [12]. Higher doses of DCMU induce a progressive destruction of pigments in chloroplasts during 24 h illumination, and also inhibit fluorescence quenching [12] and [^{14}C]leucine incorporation (cyclic flow) in fresh chloroplasts, presumably by causing the cyclic carriers to become over-oxidized as noncyclic flow becomes completely inhibited.

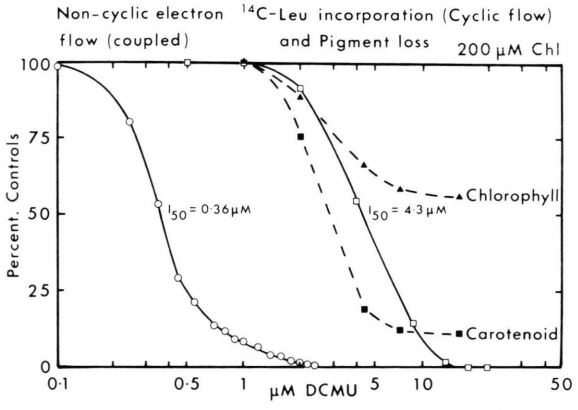

Fig. 1. Effects of DCMU doses on chlorophyll and carotenoid photodestruction in isolated chloroplasts (200 μM Chl) during 24 h illumination (as a % of illuminated controls), and on noncyclic and cyclic electron flow in freshly prepared chloroplasts. Controls rates (average of duplicates): coupled noncyclic flow, 48 μmol O_2 evolved/h/mg Chl; [^{14}C]leu incorporation in the presence of 1 μM DCMU (an indicator of the operation of natural cyclic flow), 7.0 nmol/h/mg Chl. The rate is the same without DCMU and comparable with rates in [9] and [11], but the ATP then comes from noncyclic phosphorylation.

The poising effect of DCMU [12] has been investigated further by fluorescence using maize mesophyll chloroplasts incubated with pyruvate (which enhances cyclic flow [13]). Since fluorescence yield is principally determined both by the redox state of the acceptor side of PS II (and hence the rate of noncyclic electron flow), and the transthylakoid pH gradient, it can be used to probe the metabolic state of photosynthetic material [10]. Figure 2A shows that fluorescence quenching (due to proton gradient formation) is stimulated by a low concentration of DCMU, but reversed by a high concentration. Antimycin A also reverses the DCMU-induced quenching, indicating the involvement of cyclic flow in the quenching (Fig. 2B). The responses to the yield of chlorophyll fluorescence titrated with DCMU in high light reveal an I_{50} for inhibition of cyclic flow of 3.0 μM [14], which is close to the I_{50} of 4.3 μM for the inhibition of cyclic flow recorded by

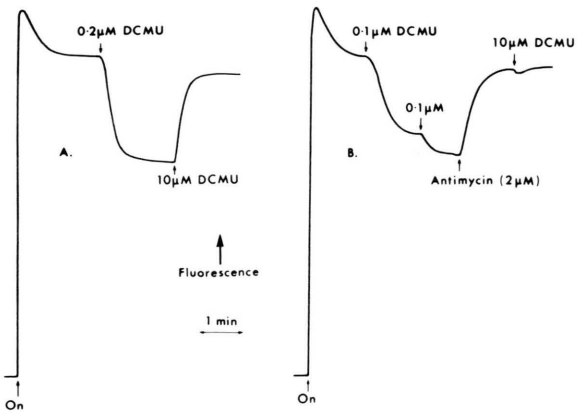

Fig. 2. DCMU-induced quenching of chlorophyll fluorescence in intact maize mesophyll chloroplasts, and inhibition of quenching by (A) 10 μM DCMU, and (B) 2 mM antimycin A. Pyruvate (10 mM) was present as a substrate [13], and 50 μg Chl/ml were used at a light intensity of 180 W/m^2.

measuring the incorporation of [^{14}C]leucine in high light (Fig. 1). In a preliminary experiment (data not shown), chloroplasts (200 μM Chl) were incubated and illuminated for 24 h with varying doses of antimycin A (up to 20 μM) in an attempt to inhibit cyclic electron transfer to a variable degree. This induced a photodestruction of pigments that became greater with increasing doses of antimycin, thus adding weight to the general argument of pigment photodestruction being related to inhibition of cyclic flow, although the possibility of additional actions of antimycin A [15] contributing to the chlorosis cannot yet be ruled out.

These experiments provide further evidence that the degree of DCMU-related inhibition of fluorescence quenching in freshly prepared intact chloroplasts can be equated to the extent to which DCMU-induced pigment destruction occurs following 24 h illumination of isolated chloroplasts; the hypothesis being that both of these are induced by the inhibition of cyclic electron flow by DCMU. Previous experiments have led to the suggestion that when P 700 activity is destroyed directly (which would cause a block in cyclic flow) the degree of DCMU-induced pigment photodestruction is greatly enhanced [3], and that the loss of pigments induced by DCMU can be prevented by restoring cyclic electron flow artificially [2, 3]. All of these findings are in general agreement with the working hypothesis that inhibition of PS I cyclic electron flow by DCMU (through over-oxidation of electron carriers when noncyclic flow is blocked) is necessary for inducing the primary herbicidal symptom of pigment photodestruction.

Acknowledgments

We wish to thank Ronald Swart at Jealott's Hill for the [^{14}C]leucine incorporation data in Fig. 1, and Paul Fernyhough in the ARC Unit at Sheffield for the data in Fig. 2.

[1] C. E. Stanger and A. P. Appleby, Weed Sci. **20,** 357–363 (1972).
[2] S. M. Ridley, Plant Physiol. **59,** 724–732 (1977).
[3] S. M. Ridley, Carotenoid Chemistry and Biochemistry (G. Britton and T. W. Goodwin, eds.), p. 353–369, Pergamon Press, Oxford 1982.
[4] K. E. Pallett and A. D. Dodge, J. Exp. Bot. **31,** 1051 to 1066 (1980).
[5] D. A. Walker, Methods Enzymol. **69,** 94–104 (1980).
[6] G. E. Edwards, R. McC. Lilley, S. Craig, and M. D. Hatch, Plant Physiol. **63,** 821–827 (1979).
[7] D. A. Day, C. L. O. Jenkins, and M. D. Hatch, Aust. J. Plant Physiol. **8,** 21–29 (1981).
[8] S. M. Ridley, Plant Physiol. **72,** 461–468 (1983).
[9] G. E. Blair and R. J. Ellis, Biochim. Biophys. Acta **319,** 223–234 (1973).
[10] P. Horton, Proc. R. Soc. Lond. B **217,** 405–416 (1983).
[11] J. M. Ramirez, F. F. del Campo, and D. I. Arnon, Proc. Nat. Acad. Sci. **59,** 606–612 (1968).
[12] J. D. Mills, R. F. Slovacek, and G. Hind, Biochim. Biophys. Acta **504,** 298–309 (1978).
[13] D. Crowther, R. C. Leegood, D. A. Walker, and G. Hind, Biochim. Biophys. Acta **722,** 127–136 (1983).
[14] T. Brearly and P. Horton, Proc. 6th Int. Congr. Photosynthesis, 1983, in press.
[15] D. S. Bendall, Biochim. Biophys. Acta **683,** 119–151 (1982).