Biochimica et Biophysica Acta, 408 (1975) 164-169

BBA 46982

ON THE INHIBITORY ACTION OF CADMIUM ON THE DONOR SIDE OF PHOTOSYSTEM II IN ISOLATED CHLOROPLASTS

M. A. VAN DUIJVENDIJK-MATTEOLI and G. M. DESMET

Association Euratom-Ital, P.O. Box 48, Wageningen (The Netherlands)

(Received April 1st, 1975)

SUMMARY

The inhibitory effect of the Cd^{2+} in the electron transport of the isolated chloroplasts has been observed by measuring the oxygen uptake from the solution and the fluorescence induction. Cd^{2+} is found to be an inhibitor on the donor side of Photosystem II and its action site, as determined by experiments using hydroxylamine and exogenous Mn, is supposed to be on the water-splitting enzyme itself. Moreover, physicochemical and physiological studies indicate that only the ionic form of Cd is acting at the level of the manganoprotein. It is not possible, from this work, to define precisely in which form Cd is taken up through the thylakoid membranes.

INTRODUCTION

Elements like cadmium, lead and nickel, although not known to be involved in any metabolic process, are taken up by plants [1] and affect the metabolism. In organelles like mitochondria, Cd was found to uncouple the oxidative phosphorylation at low concentrations and to produce a significant inhibition of the oxidative rate at higher concentrations [2, 3]. The present report describes the effects of Cd upon the light reactions of photosynthesis in isolated chloroplasts.

METHODS

Fresh spinach leaves (*Spinacea oleracea* L, cultivar verbeterd breedblad) were obtained from plants grown on a Steiner solution [4] in climate-controlled rooms. The deveined leaves were macerated in an omnimixer at 5 °C. The juice was filtered through 4 layers of nylon cloth and centrifuged at $4000 \times g$ for 4 min.

The resulting pellet was resuspended in a grinding medium containing at pH 7.6 : 0.05 M TES, 0.35 M sucrose, 0.01 M $MgCl_2$, 0.035 M KCl. The same medium was later used in the experiments. The chlorophyll concentration was diluted to 30 μg/ml as described by Bruinsma [5]. All the assays were performed at 20 °C, with an incubation time of 5 min in darkness.

Abbreviations: TES, *N*-tris (hydroxymethyl)-2-aminoethanesulphonic acid; DPIP, 2,6-dichlorophenol indophenol; Ioxynil, 3,5-diiodo-4-hydroxybenzonitrile.

Publication no. 1189 of the Biology Division of the Commission of the European Communities.

The electron transport was measured with a modified Mehler reaction [6], using 35 μM methyl viologen (paraquat; 1,1'-dimethyl-4,4'dipyridilium dichloride) as terminal electron acceptor and 0.01 M NaN_3 as inhibitor of the hydrogen peroxide catalase. The oxygen uptake from the solution was measured with an YSI Clark-type oxygen sensor.

The fluorescence induction was excited at 475 nm and observed at 680 nm. The chloroplasts were illuminated for 4 s.

A stock solution of cadmium nitrate (15 mM), diluted in the grinding medium and maintained at pH 7.6, was used. The extent of Cd^{2+} complex formation with the different components of this medium was measured with a Cd^{2+}-activity electrode Orion, Model 94-48.

RESULTS

Cd complex formation

Cd in aqueous solution is known to form a number of complex species (particularly with hydroxide and chloride anions and ammonia) [7]. Because of $CdCO_3$ precipitation at pH 7.6, it was impossible to use a concentration higher than 15 mM. The stock solution contained also 0.05 M TES and 0.055 M Cl^-, which complexed the Cd at 35 % ±5 and 60 % ±5, respectively (Fig. 1). The complete medium complexed approx. 75 % ±5 of Cd. The effect of this Cd complex formation has also been observed in some physiological measurements. The fluorescence intensity at the steady-state level [8] has been measured on isolated chloroplasts incubated in two reaction media, differing only in the Cl^- concentration (3 mM in a and 55 mM in b, Fig. 3). A shift of 70–80 % along the concentration axis was observed, due to the Cd · Cl complexes. A Cl deficiency is known to decrease the electron flow from Photosystem II into the quencher; the chlorine functions as a cofactor in the electron transport chain and its action site is near the water-splitting step [9]. In the present experiment, the Cl^- concentrations were chosen in a range which disturbed the Cl function, in the absence of Cd, to a very small extent (about 10 %). In order to

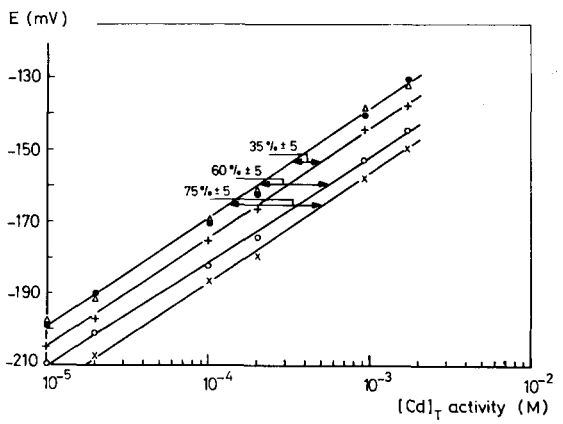


Fig. 1. Cd complex formation with the different components of the medium: ●, salts; △, salts+sucrose; +, salts+sucrose+TES; ○, salts+sucrose+$MgCl_2$+KCl; ×, sucrose+TES+$MgCl_2$+KCl.

eliminate the possibilities of a lower sensitivity of the chloroplasts due to an eventual Cl deficiency, the electron transport was measured again with a $CdCl_2$ stock solution instead of $Cd(NO_3)_2 \cdot 4H_2O$. Nearly identical results were obtained when using $CdCl_2$.

Electron transport

At pH 7.0, the Cd oxidoreduction potential is (−0.40 V) quite comparable to the methyl viologen potential (−0.47 V) [10]. Thus, Cd could be expected to function as an electron acceptor. To control this possibility, the modified Mehler reaction was measured, applying Cd instead of methyl viologen as an electron acceptor. No oxygen consumption or evolution was observed in the light.

When methyl viologen was used, Cd effects on the rate of O_2 consumption were observed (curve a, Fig. 2). Within the total range of Cd concentrations (1–10 mM), a double effect was obvious. Low Cd concentrations brought about an increase of oxygen consumption which could be explained by an uncoupling of the photophosphorylation resulting in an increase of the electron transport [11]. Maximum uncoupling was obtained at 4 mM total Cd. Higher Cd concentrations caused a decrease of oxygen consumption which should be due to a blockage of the electron transport chain since Cd does not act as an electron acceptor. In fact, the curve (Fig. 2a) was the result of opposite additive effects: uncoupling and inhibition by Cd.

In the following part of this paper, Cd inhibition has been emphasized. When preincubating isolated chloroplasts with a known uncoupler of the photophosphorylation (20 mM CH_3NH_3Cl), only the inhibitory effect of Cd was observed (Fig. 2b). Within the total Cd concentration range observed, (1–10 mM), the electron transport chain has never been completely inhibited.

In oxygen consumption measurements, with ascorbate-reduced DPIP (10 μM) as electron donor of Photosystem I and methyl viologen as electron acceptor [12], absolutely no Cd effect could be observed. Therefore, Cd inhibition must be localized on Photosystem II.

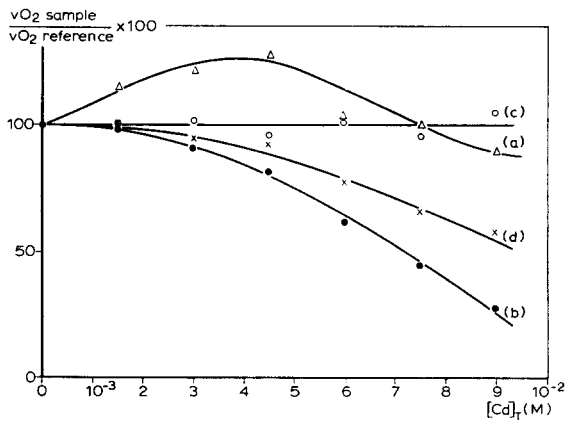


Fig. 2. Rate of O_2 uptake by the chloroplasts at different Cd concentrations: (a) effect of cadmium nitrate in the absence of any uncoupler; (b) Cd inhibition obtained in chloroplasts uncoupled with CH_3NH_3Cl; (c) Cd effect bypassed by hydroxylamine; (d) Cd effect partially reversed by exogenous Mn^{2+} in uncoupled chloroplasts.

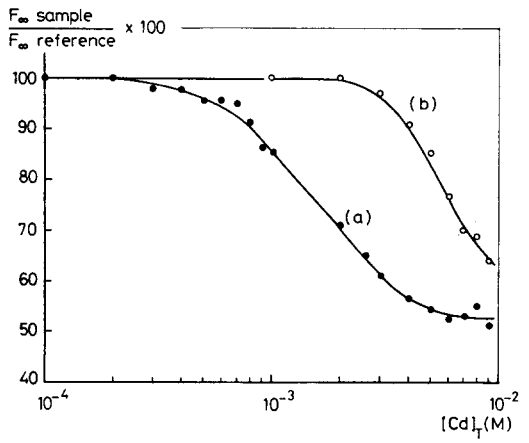


Fig. 3. Effect of different cadmium nitrate concentrations on the $F_\infty(Q/Q^-)$ ratio: (a) and (b) respectively at total Cl concentrations of 3 mM and 55 mM in the medium.

Fluorescence induction

When the light is turned on, the fluorescence is known to increase in a biphasic manner, from an initial level F_0 to a steady-state level F_∞, which reflects the reduction of the Q pool, primary electron acceptor of Photosystem II, into Q^- [8, 13]. The first phase did not change in the presence of Cd except that F_0 was slightly increased. The second phase was strongly affected by the Cd concentrations; the second rise phase, as well as F_∞, were decreased (Fig. 3). That meant that the first photochemical steps of O_2 evolution were still present, but Cd considerably affected the quantity of electrons that were able to reduce the Q pool. The same experiment was done in the presence of Ioxynil (5 μM) which is an inhibitor of Q reoxidation by Photosystem I [14]. When Ioxynil is present, the total Q pool is supposed to become reduced, at saturating light intensities, and the fluorescence induction rate is very fast.

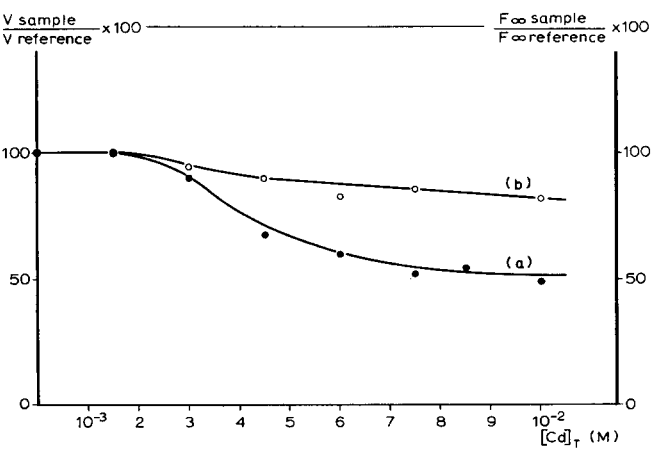


Fig. 4. Effect of different cadmium nitrate concentrations in the presence of Ioxynil on the fluorescence induction rate (a) and on the steady-state level F_∞ (b).

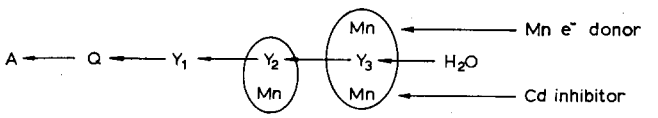
When Cd was added, this induction rate was decreased as well as the F_∞ level (Q/Q^-) (Fig. 4). These fluorescence experiments suggested that Cd inhibits the electron transport chain at the donor side of Photosystem II.

In the presence of Ioxynil or DCMU, the reduced Q pool can be reoxidized during dark incubation of the preilluminated chloroplasts in a reaction between Q^- and Y_1^+ [15, 16]. In the presence of Cd, this back-reaction was still possible. Therefore, Cd could not have its inhibition site on the first electron donor. Hydroxylamine (50 mM) is known to give electrons to Photosystem II, preventing the splitting of water [17, 18]. With oxygen measurements, it has been shown that hydroxylamine bypassed the Cd effect (Fig. 2c). Since this compound gives electrons to Y_2 [15], the second electron donor of Photosystem II, it was concluded that Cd was acting before Y_2.

Mn^{2+} is most often proposed to be an electron donor at the level of the manganoprotein required for the oxidation of water [15, 19]. It was of particular interest to see how Cd could act in the presence of uncoupled chloroplasts incubated with Mn nitrate (10 mM) (Fig. 2d). Mn could not suppress the Cd effect. But the inhibition percentage, at all Cd concentrations, was significantly lower in presence of Mn (see Figs 2b and d).

DISCUSSION

Our results have shown Cd to be an inhibitor of the electron transport chain at the water side of Photosystem II, which can be described by the following model based on several works [15, 20, 21]:



In the experiments with Ioxynil and Cd, a decrease in the steady-state level F_∞ was observed, less electrons reaching the Q pool from the water-splitting site. Hydroxylamine (50 mM), electron donor to Y_2, completely bypassed the Cd effect. Other tests done with added Mn instead of Cd confirmed that hydroxylamine also bypassed the Mn effect in the electron transport. Cd and exogenous Mn, respectively, as inhibitor and electron donor, were assumed to act on the same electron donor Y_3 required for the oxidation of water. This could be supported by the fact that exogenous Mn could not bypass the Cd effect, but only partially reversed the Cd inhibition.

Hg, Cu and Pb have been described to have at least one inhibition site on the donor side of Photosystem II [22–24]. Because the electron donors of Photosystem II are localized either inside or on the inner surface of the thylakoid membranes of the grana [25, 26] these divalent cations have to penetrate the membranes to act. The Cd inhibition or the transport through the membranes or both were dependent on the physicochemical form of Cd. Considering the physiological experiment (Fig. 3), the shift of the curve, when Cd was not in ionic form, suggested that the electron transport chain was more sensitive to Cd^{2+} than to some Cd complex. The uncoupling effect of Cd will be discussed in more detail later on.

ACKNOWLEDGEMENTS

One of the authors (M.v.D.-M.) wishes to thank the commission of the European Communities for a fellowship granted to her. We wish to thank Dr C. Myttenaere and Dr A. Ringoet for their interest and criticism.

REFERENCES

1 Turner, M. A. (1973) J. Environ. Quality 2, 118–119
2 Jacobs, E. E., Jacob, M., Sanadi, D. R. and Bradley, L. B. (1956) J. Biol. Chem. 223, 147–156
3 Miller, R. J., Bittel, J. E. and Koeppe, D. E. (1973) Physiol Plant. 28, 166–171
4 Steiner, A. A. (1968) in Proceedings of the 6th Colloquium of the International Potash Institute, p. 324, International Potash Institute, Berne
5 Bruinsma, J. (1963) Photochem. Photobiol 2, 241–249
6 Mehler, A. H. (1951) Arch. Biochem. Biophys. 33, 65–77
7 Posselt, H. S. (1971) Thesis, Univ. of Michigan, Mich., U.S.A.
8 Duysens, L. N. M. and Sweers, H. R. (1963) Microalgae and Photosynthetic Bacteria (Japanese Society of Plant Physiologists), pp. 353–372, Univ. of Tokyo Press.
9 Heath, R. L. and Hind, G. (1969) Biochim. Biophys. Acta 172, 290–299
10 Zweig, G. and Avron, M. (1965) Biochem. Biophys. Res. Commun. 19, 397
11 Mitchell, P. (1966) Research report (No. 66/1), Glynn Research Laboratories, Bodmin, Cornwall, U.K.
12 Izawa, S., Connolly, T. N., Winget, G. D. and Good, N. E. (1966) Energy Conversion by the Photosynthetic apparatus, Brookhaven Symp., Biol., Vol. 19, p. 169
13 Malkin, S. and Kok, B. (1966) Biochim. Biophys. Acta 126, 413–432
14 Gromet-Elhanan, Z. (1969) in Progress on Photosynthesis Research (Metzner, A., ed.), Vol. III, p. 1197, Tübingen
15 Katoh, S., Satoh, K., Ikegami, I., Kikimura, M. and Takamiya, A. (1971) IInd International Congress on Photosynthesis Research (Forti, G., Avron, M., Melandri, A., eds), p. 525, Stresa, Italy
16 Bennoun, P. (1970) Biochim. Biophys. Acta 216, 357–363
17 Bennoun, P. and Joliot, A. (1969) Biochim. Biophys. Acta 189, 85–94
18 Cheniae, G. M. and Martin, I. F. (1971) Plant Physiol. 47, 568–575
19 McKenna, J. M. and Bishop, N. I. (1967) Biochim. Biophys. Acta 131, 339–349
20 Cheniae, G. M. and Martin, I. F. (1970) Biochim. Biophys. Acta 197, 219–239
21 Desmet, G. M. (1975) Thesis, Leuven, Belgium
22 Cedeno-Maldonaldo, A., Swader, J. A. and Heath, R. L. (1972) Plant Physiol. 50, 698–701
23 Honeycutt, R. C. and Krogmann, D. W. (1972) Plant Physiol. 49, 376–380
24 Miles, C. D., Brandle, J. R., Daniel, D. J., Chu-Der, O., Schnare, P. D. and Uhlik, D. J. (1972) Plant Physiol. 49, 820–825
25 Blankenship, R. P. and Sauer, K. (1974) Biochim. Biophys. Acta 357, 252–266
26 Witt, H. T., Rumberg, B. and Junge, W. (1968) in Biochemie des Sauerstoffs, p. 262, Springer-Verlag, Berlin