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ABSORPTION AND METABOLISM OF CrO_4^{2-} BY ISOLATED CHLOROPLASTS

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Key Word Index—Chromium, chromate, absorption, metabolism in chloroplasts

Abstract—When CrO_4^{2-}, a potential pollutant in the environment, reaches the chloroplasts of leaves, it is absorbed, and probably transformed into another chemical form by the chloroplasts. In chloroplasts, activated by light, CrO_4^{2-} is an electron acceptor of photosynthesis, and it competes with MV for the same electron donor substrate. No ATP production is necessary for the CrO_4^{2-} reduction, unlike for the reduction of SO_4^{2-}.

INTRODUCTION

Chromium in its various chemical forms may pollute the plant components of natural and agricultural ecosystems. In experiments with whole rice plants a small translocation of this heavy metal to the aerial part of the plant was observed [1].

A preliminary experiment [2] showed the possible uptake of Cr^{3+} and CrO_4^{2-} by isolated chloroplasts. Because Cr^{3+} precipitates at a pH above 5·5, no further attention has been paid to this form and therefore only CrO_4^{2-} has been investigated. It has been suggested [3] that CrO_4^{2-} competes with the SO_4^{2-} uptake in *Chlorella*, and though the CrO_4^{2-} was absorbed, it was apparently not metabolized [4].

RESULTS AND DISCUSSION

Absorption and transformation of CrO_4^{2-} by chloroplasts in the dark

The amount of CrO_4^{2-} disappearing from the supernatant was small. The relation between the concentration of CrO_4^{2-} in the medium and the amount absorbed by the chloroplasts is shown in Fig. 1. A maximum absorption of 40 ng CrO_4^{2-} per μg chlorophyll was observed. The question arises whether chromium in "dark" chloroplasts is present as CrO_4^{2-} or in a more reduced form. Therefore spectra of CrO_4^{2-} at different concentrations, with and without chloroplasts were compared. All data on CrO_4^{2-} in the presence of chloroplasts were normalized by the use of the extinction of the chloroplast suspension at 680 nm, since at this wavelength CrO_4^{2-} itself does not absorb light. According to Fig. 2, where the spectrum of CrO_4^{2-} (5×10^{-5} M) absorbed by chloroplasts was compared to the spectrum of CrO_4^{2-} in solution at the same concentration, the

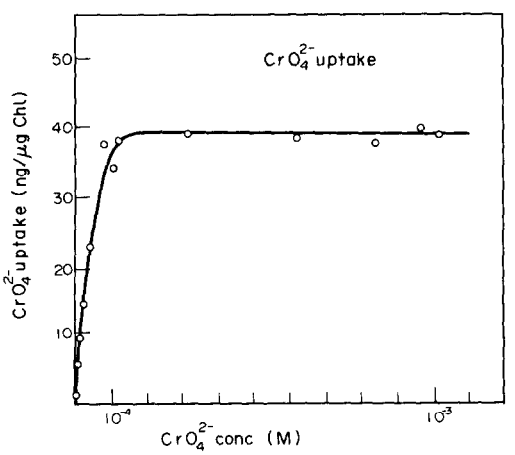

Fig 1 Amount of CrO_4^{2-} uptake after 15 min of incubation by isolated chloroplasts at different CrO_4^{2-} concentrations in the medium

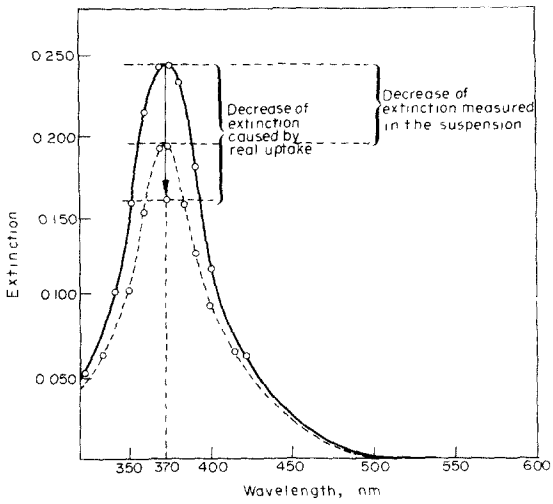

Fig. 2. Comparison between spectra of absorbed and free CrO_4^{2-}. ○– –○ free CrO_4^{2-}, ○ ○ absorbed CrO_4^{2-}. Extinction decrease and real uptake are compared at 372 nm.

extinction of CrO_4^{2-} was significantly decreased by contact with the chloroplasts. It is thus likely that on absorption, part of the CrO_4^{2-} is transformed or even reduced, although no wavelength shift is observed. The apparent absence of a wavelength shift can be explained by considering the smallness of the extinction decrease of the "absorbed" CrO_4^{2-}. Actually, the molecular extinction coefficient (ϵ) of CrO_4^{2-} is approximately 4100 while Cr (III) complexes generally have ϵ values varying between 10 and 100[5]. Therefore, a wavelength shift due to a reduction to Cr (III) is not easily detectable.

This extinction decrease of CrO_4^{2-} at 372 nm, due to the contact with chloroplasts was also compared (Fig. 2) to the extinction decrease used for calculating the amount of CrO_4^{2-} uptake. This extinction decrease represents about 60% of the extinction decrease due to uptake. It could be suggested from these data that part of the absorbed CrO_4^{2-} is transformed into another chemical form, and part of it is present as free CrO_4^{2-} in the chloroplasts.

Interaction of CrO_4^{2-} with illuminated chloroplasts

The E_0' of CrO_4^{2-}/Cr^{3+} in an alkaline medium is given to be about −0.13 V [6]. The light-activated chloroplasts generate a redox power of at least −0.40 V [7]. Because of these data, the influence of CrO_4^{2-} on the electron transport in chloroplasts was investigated. This was done by measuring the influence of CrO_4^{2-} on the intensity of variable fluorescence of illuminated chloroplasts, known to be related to the reduction of Q by photosystem II [8] and to the oxidation of Q^- by an electron acceptor [9].

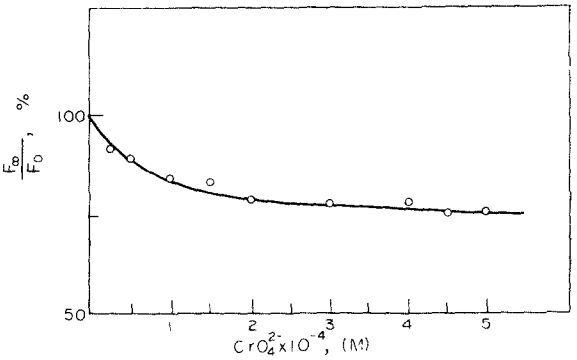

Fig. 3. Relation between the concentration of CrO_4^{2-} and the decrease of the ratio F'/F_0.

According to the present results the fluorescence ratio F'/F_0 depended on the same CrO_4^{2-} concentration range as the uptake (Fig. 3). Furthermore, the "Hill reagent" capacity of CrO_4^{2-} was studied and it was shown that O_2 production by illuminated chloroplasts depended upon the concentration of CrO_4^{2-}. The O_2 evolution in the presence of 10^{-3} M $Fe(CN)_6^{3-}$ was used as a reference (Fig. 4).

The results thus show that the concentrations of CrO_4^{2-} of metabolic interest coincide with those for uptake. CrO_4^{2-} is clearly an electron acceptor for the photosynthetic electron transport chain, devoid of any natural or other acceptor. Nevertheless, when methylviologen (MV) was added to the medium as a substitute for $NADP^+$, the natural final acceptor in electron transport

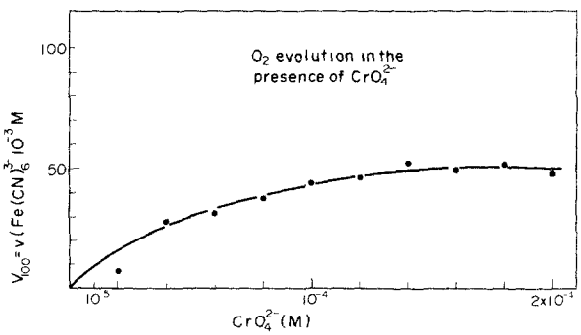

Fig. 4. Rate of O_2 evolution in the presence of CrO_4^{2-}. The O_2 evolution in the presence of 10^{-3} M $Fe(CN)_6^{3-}$ is used as 100% reference.

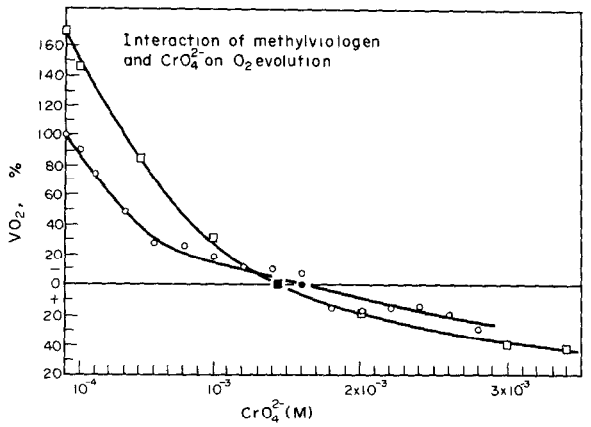

Fig 5. Interaction of methylviologen and CrO_4^{2-} on the rate of O_2 evolution Above the zero line, O_2 uptake, below, O_2 evolution is shown. O——O, in absence of $MeNH_3Cl$, □——□ in presence of $MeNH_3Cl$.

[7] a considerable shift of the metabolic activity of CrO_4^{2-} with respect to the concentration was observed (Fig. 5). In the presence of 5×10^{-5} M MV an O_2 uptake was observed due to a kind of Mehler reaction [10]. As a result of the supply of increasing amounts of CrO_4^{2-} the O_2 consumption was shifted towards an O_2 production. Apparently CrO_4^{2-} competes with MV for the same electron donor substrate, because compared to the data in Fig. 4 approximately ten times more CrO_4^{2-} was needed to achieve an O_2 production. When the electron transport rate was increased by the uncoupler $MeNH_3Cl$, there was no effect on the competition between MV and CrO_4^{2-}, since no further shift was observed.

It can thus be concluded that a common electron donor pool exists for MV and CrO_4^{2-}. The experiments with $MeNH_3Cl$ furthermore prove that no active ATP production catalyzes the CrO_4^{2-} reduction, in contrast to what is generally accepted for SO_4^{2-} reduction.

One must comment on the striking effect of CrO_4^{2-} on the maximal rate of electron transport. It never reached the same level as in the presence of $Fe(CN)_6^{3-}$ (Fig. 4). Normally, the rate should be the same in both cases since the CrO_4^{2-} competes with MV for a site beyond the rate limiting step of photosynthesis [11]. There may be several possible explanations for the lower effectiveness of CrO_4^{2-}. A derivative of CrO_4^{2-} may be produced in the preceding dark incubation period, or the velocity constant for the reduction of CrO_4^{2-} in the light by its immediate preceding donor may itself become the rate-limiting step of the overall electron transport process. This hypothesis receives some support from the finding that CrO_4^{2-} depressed the variable F′ only to a small extent, i.e. a rather high substrate concentration of the electron donor in the chloroplast was necessary for the CrO_4^{2-} reduction.

From the experiments described in Figs. 4 and 5, it became evident that CrO_4^{2-} may act as an electron acceptor in chloroplasts, and moreover that it competes with MV. Consequently similar experiments were done with the well-known 'Hill' acceptor $Fe(CN)_6^{3-}$ and different concentrations were tested (Fig. 6). The effect on O_2 evolution was comparable to that observed for CrO_4^{2-} in Fig. 4.

A maximal rate was reached at 10^{-3} M $Fe(CN)_6^{3-}$. In contrast, however, to what was found with CrO_4^{2-}, MV could not change the initial, $Fe(CN)_6^{3-}$ dependent, rate of O_2 evolution. This means, that initially all the electrons produced by active chloroplasts are channelled to $Fe(CN)_6^{3-}$ and do not reach MV, while CrO_4^{2-} even at the lowest concentrations competes with MV. From these observations it can be concluded that $Fe(CN)_6^{3-}$ acts on the electron transport before MV, while CrO_4^{2-} has probably the same electron donor as MV.

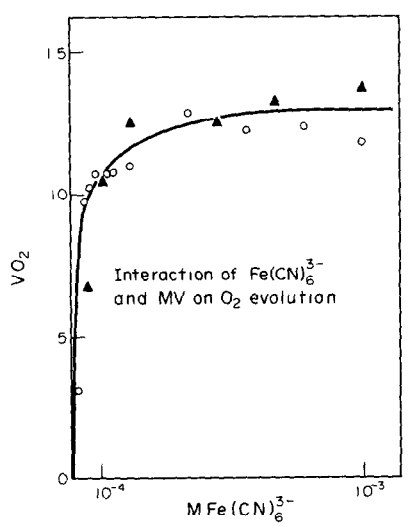

Fig 6. Rate of O_2 evolution in the presence of $Fe(CN)_6^{3-}$ O——O with $Fe(CN)_6^{3-}$, ▲——▲ $Fe(CN)_6^{3-}$ and MV

EXPERIMENTAL

Chloroplasts were isolated from spinach leaves (*Spinacea oleracea* L. cv. Verbeterd Breedblad). Plants were grown in climate controlled rooms (day 22°, night 15°, daylength 13 hr, light-intensity of 33000 lux, and relative humidity of 65–70%) for 3 weeks using Hoagland-Arnon I soln. The isolation and measuring medium for the chloroplasts contained TES pH 7·6, 10^{-2} M $MgCl_2$, 10^{-2} M KCl and 0·35 M sucrose. Chloroplasts were obtained from an homogenate centrifuge filtered through 4 layers of nylon cloth, and centrifuged at 4000 *g* for 4 min. Chlorophyll content was calculated by the method described by Bruinsma [12]. Uptake of CrO_4^{2-} by the chloroplasts was measured by spectrophotometry combined with centrifugation. Chloroplasts were incubated for 15 min, in the same medium as the one described above, centrifuged at 20000 *g* and the decrease of the extinction of the CrO_4^{2-} in the supernatant measured at 372 nm in the spectrophotometer. O_2 measurements were made with a YSI Clarck electrode. Fluorescence induction was excited at 475 nm and observed at 680 nm. All the experiments were done at 20°.

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