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Inhibition and Uncoupling of the ADP-Regulated Electron Transport in Isolated Chloroplasts

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Abstract

The effects of energy transfer inhibitors, electron transport inhibitors and uncouplers on the ADP-regulated and ADP-independent activity of ferricyanide reduction in isolated spinach chloroplasts were studied.

Phlorizin and sulfate did not affect the ADP-independent ferricyanide reduction. In the ADP-regulated reduction, these reagents did not affect the ADP inhibition process but inhibited the activity restoration process due to phosphorylation.

3-(3,4-Dichlorophenyl)-1,1-dimethylurea and linolenic acid depressed both ADP-regulated and ADP-independent activity of ferricyanide reduction.

Gramicidin S and 2-amino-1-butanol depressed ADP-regulated activity and stimulated ADP-independent activity. The decrease in the ADP-regulated ferricyanide-reducing activity (restoration) due to (incomplete) uncoupling paralleled the decrease in phosphorylation activity ($P/\Delta e = 1$).

Introduction

Non-cyclic electron transport in isolated chloroplasts consists of two electron transport systems, one of which is apparently regulated by adenine nucleotides (ATP and ADP) and the other independent of them [1,2,3]. As the ADP (or ATP) concentration increased from 0.1 μM in the reaction mixture, the overall electron transport activity was first partially inhibited [1,4] probably through a 1 : 1 binding of ADP (or

ATP) by the chloroplast coupling factor (CF_1), down to the zero level for the ADP-regulated electron transport [1]. The remaining part of the activity was thus ADP- (or ATP-) independent (basal [2]). As the ADP concentration increased further, took place a 1:1 binding of the second ADP molecule by the CF_1 with which the first ADP had been associated. In the presence of *Pi* (or *As*, arsenate), this second ADP binding resulted in the turnover of phosphorylation (or arsenylation) in parallel with transport of a stoichiometric amount of electrons ($P/\Delta e = 1$). Therefore, ADP-regulated electron transport activity that had been once inhibited, was restored as phosphorylation (or arsenylation) occurred.

The profile of Fecy (ferricyanide)-reducing activity *vs.* ADP concentration in the presence of *Pi*, for example, exhibited a V-shaped profile, which corresponded to the ADP-inhibition (the first ADP binding) process at lower ADP concentrations, and to the restoration (the second ADP binding) process due to phosphorylation. The lowest level of the V-shaped profile then approximated the ADP-independent Fecy-reducing activity.

In this paper we report on the effects of energy transfer inhibitors, electron transport inhibitors and uncouplers on the activity profile.

Experimental Procedures

Chloroplasts were prepared from market spinach leaves in a choline medium by a method described previously [5]. The reaction mixture was composed of 0.1 M sucrose, 5 mM $MgCl_2$, 10 mM tricine (pH 8.3), 600 μM Fecy and chloroplasts equivalent to 20 μg/ml in the chlorophyll concentration determined by the method of Arnon [6]. ADP (from Sigma Chemical Co.), *Pi* (^{32}*Pi* for phosphorylation assay), inhibitors and uncouplers were added as required. The reaction mixture was illuminated by white light (5×10^4 lux) in a water bath at $15 \pm 0.1^\circ$C for several minutes. Fecy reduction was measured from the difference in the absorbance at 420 nm before and after actinic illumination. The esterified *Pi* was determined by a modification of the method of Asada *et al.* [7].

In the following results (Figs. 1 to 3), the rates of Fecy reduction in an ADP concentration range between 1 and 100 μM might be lower than the initial rates, especially when the phosphorylation activity was high, because of ADP exhaustion during actinic illumination. The extent of restoration of Fecy reduction is thus to be measured by the activities at the ADP concentration higher than 100 μM. The initial rates, suggested by Fecy-reducing activity under arsenylation condition (1 mM *As* instead of *Pi* [1]), are indicated in Fig. 1 by a dotted line.

Results and Discussion

Figure 1 shows the effect of phlorizin (1 mM) on the ADP-regulated Fecy reduction. As reported [1], in the absence of phlorizin (control), Fecy reduction was inhibited at low ADP concentrations (0.1 to 10 μM) and restored at higher ADP concentrations as *Pi* was present. By contrast, in the presence of 1 mM phlorizin, the inhibition process was almost coincident with that of the control but no restoration process was observed.

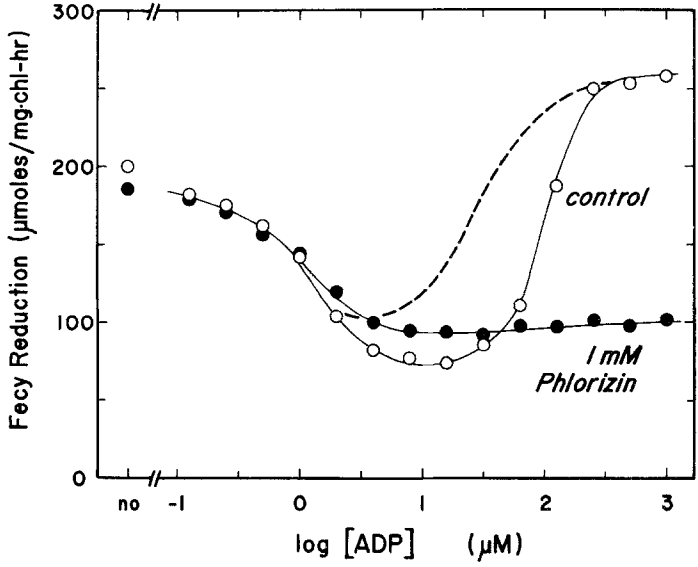

Figure 1. Effects of phlorizin on ADP-regulated Fecy reduction. Fecy reduction (pH 8.3, 15° C) was measured in the presence of 1 mM *Pi* and ADP at the concentration shown on the abscissa, with (1 mM) or without (control) phlorizin. The observed values plotted at ADP 1-100 μM do not represent the initial rates of Fecy reduction, as explained in *Experimental Procedures*. The broken line shows the Fecy-reducing activity coupled to arsenylation and approximates the initial rates for the control.

It has been known that phlorizin inhibits phosphorylation and the coupled electron transport (= restoration) in chloroplasts [2] except that ADP is absent upon actinic illumination [8]. This result shows that the binding of the first ADP to CF_1 could occur regardless of the presence of phlorizin but the following process of phosphorylation was inhibited by phlorizin. It would inhibit either binding of the second ADP by the CF_1 which had bound the first ADP, or turnover of phosphorylation (including *Pi* binding by CF_1), or the mechanism for driving the turnover

(proton translocation and/or electron transport *etc.*). As shown in Fig. 1, the lowest level of the ADP-regulated Fecy-reducing activity in the presence of phlorizin was slightly higher than that of the control and close to the level of the control obtainable in the absence of *Pi* [1]. This may correspond to the fact [9] that phlorizin inhibits photophosphorylation incompletely competitively with *Pi*.

Na_2SO_4 at 10 mM showed a profile quite similar to that obtained with phlorizin. Sulfate inhibits photophosphorylation [10] competitively with *Pi* [11, 12] and causes a modification of CF_1 structure [13] when present in photophosphorylating chloroplasts. The result implies that the functional interaction between CF_1 and sulfate (and possibly *Pi* also) occurs after CF_1 binds the first ADP and alters its conformation [14].

Figure 2 shows the effect of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), an electron transport inhibitor, on Fecy reduction. With an increase in the DCMU concentration, the activity was depressed in both ADP-regulated and ADP-independent Fecy reduction. The degree of the activity depression appeared to be larger in the ADP-regulated Fecy reduction than the ADP-independent one. This may correspond to the presence of residual activity of Fecy reduction even at sufficiently high DCMU concentrations [15].

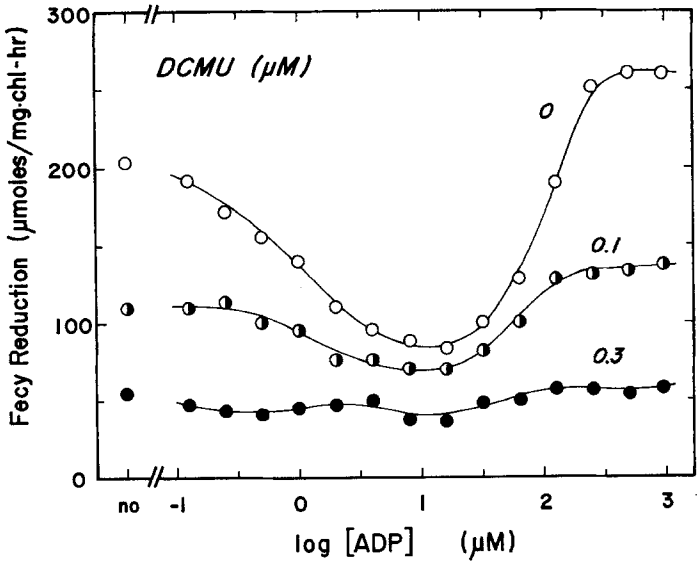

Figure 2. Effects of DCMU on Fecy-reducing activity. Fecy reduction (pH 8.3, 15° C) was measured in the presence of 1 mM *Pi* and ADP at the concentration shown on the abscissa, and DCMU indicated on the figure. The plotted values, especially for DCMU = 0 at ADP 1-100 μM, do not represent the initial rates, as explained in *Experimental Procedures*.

Linolenic acid (e.g. 50 μM) showed an inhibitory effect on Fecy reduction similar to that of DCMU. Since linolenic acid inhibits electron transport [16] most probably on the oxidizing side of photosystem II [17] and DCMU on the other side of it [18], it was readily expected that both reagents inhibit electron transport from water to Fecy irrespective of ADP regulation.

Figure 3 represents the effect of gramicidin S, an uncoupler [15], on Fecy reduction. As the concentration of gramicidin S increased, the extent of both inhibition and restoration, i.e. the ADP-regulated Fecy-reducing activity, became smaller while the ADP-independent activity became apparently larger.

Figure 4 shows the effects of 2-amino-1-butanol, on the Fecy-reducing activities under the following conditions; phosphorylation conditions (in the presence of 1 mM ADP and 1 mM Pi; Fecy $_{ADP, Pi}$) and maximally inhibitory conditions (in the presence of 100 μM ADP; Fecy$_{ADP}$). The effect on the activity of phosphorylation coupled to Fecy$_{ADP,Pi}$ was also measured. The difference in the Fecy-reducing activity under these two conditions, ΔFecy $_{coupled}$ = Fecy$_{ADP,Pi}$ – Fecy$_{ADP}$, is also shown in Fig. 4 by a broken line. Since Δe value is equivalent to ΔFecy$_{coupled}$, the P/Δe value of about 0.9 is obtained from Fig. 4. This value fluctuated within a range of 0.85 and 1.0 depending on the uncoupler concentration, experimental accuracy and chloroplast preparations.

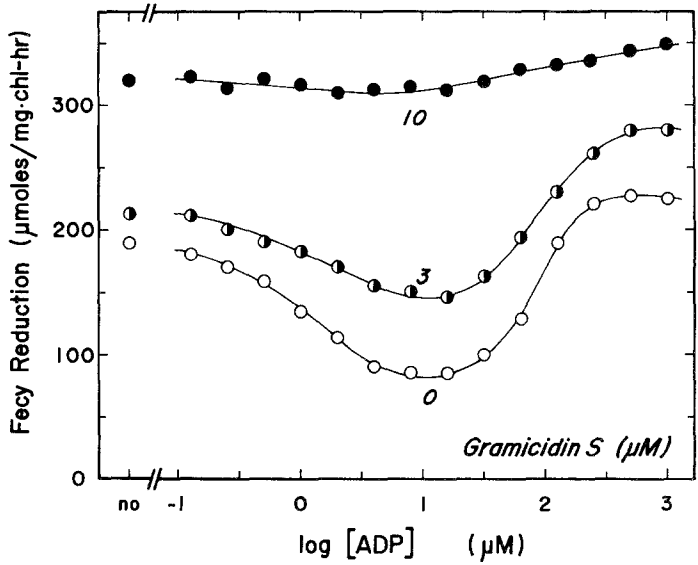

Figure 3. Effects of gramicidin S on Fecy-reducing activity. Fecy reduction was measured as in Fig. 2 except DCMU was replaced by gramicidin S. The plotted values, especially for gramicidin S = 0 at ADP 1-100 μM, do not represent the initial rates, as explained in *Experimental Procedures.*

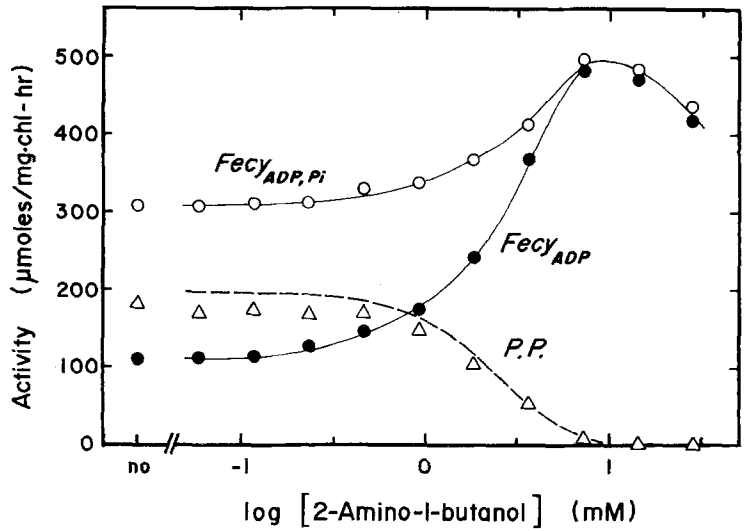

Figure 4. Uncoupling of Fecy reduction and phosphorylation by 2-amino-1-butanol. Fecy reduction (pH 8.3, 15° C) was measured in the presence of both 1 mM *Pi* and 1 mM ADP (○) or 100 μM ADP only (●). Phosphorylation (△) was measured under the former condition of Fecy reduction. The difference in Fecy-reducing activity under two different conditions is shown by the broken line.

ATP would be formed by CF_1 concomitant with translocation of protons outward from the thylakoid sacs [19]. The ADP-regulated electron transport would function to pump protons inward to complement the amounts translocated by CF_1. Then, there should be a quantitative relationship among esterified P*i*, translocated (= pumped) protons ($P/\Delta H^+ = 0.5$ [20]) and transported electrons ($\Delta H^+/\Delta e = 2$ [21]). Under satisfactory conditions, the experimental stoichiometry has been found to be $P/\Delta e = 1$ [1, 2, 3] for the full length of non-cyclic electron transport. The result in Fig. 4 would indicate that as far as CF_1 can synthesize ATP even under partially uncoupled conditions, the stoichiometry still exists.

Izawa and Good [22] reported that when the effect of methylamine hydrochloride on Fecy-reducing activity was measured, the difference in the activities measured in the presence of ADP (1 mM) with or without *Pi* (10 mM) decreased much greater than the phosphorylation activity, as the methylamine concentration increased. This meant that the $P/\Delta e$ value became greater than 1. With methylamine hydrochloride, we obtained the $P/\Delta e$ value ranging between 0.9 and 1.0 up to the amine concentration of 1 mM. The value became larger at higher uncoupler concentrations and approached 1. This discrepancy would be explained

by many factors, such as the intensity of actinic light [15] and the concentrations of ADP and/or Pi.

Our results obtained with amines would indicate that, irrespective of the level of ADP-independent Fecy-reducing activity, the level inhibited most by ADP ($\approx 100\ \mu M$) is the zero level of ADP-regulated Fecy-reducing activity.

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