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Adenylate Regulation of Photosynthetic Electron Transport and the Coupling Sites of Phosphorylation in Spinach Chloroplasts

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Abstract

The regulation by adenylates of activities of various partial electron transport systems in spinach chloroplasts was studied using systems from H_2O to 2,5-dimethyl-*p*-benzoquinone, H_2O to 2,6-dichlorophenol-indophenol, reduced 2,6-dichlorophenolindophenol to methyl viologen, and H_2O to methyl viologen or ferricyanide. Adenylates regulated all of them. The ratio of the amount of esterified Pi (P) to that of electrons transported (Δe) in coupling with phosphorylation manifested that there are two phosphorylation sites: one between H_2O and 2,5-dimethyl-*p*-benzoquinone or 2,6-dichlorophenolindophenol and another between reduced 2,6-dichlorophenolindophenol and methyl viologen, under the proposed stoichiometries, *i.e.*, $P/\Delta H^+ = 0.5$ and $\Delta H^+/\Delta e = 1$, where ΔH^+ is the amount of protons pumped by electron transport (= those translocated during phosphorylation), when the basal electron transport (the part not regulated by adenylates) was excluded. The effects of pH, phlorizin, and methylamine on the adenylate regulation of electron transport, and the stimulation profile of electron transport coupled with quasiarsenylation suggested no distinction between the two phosphorylation sites.

Introduction

In the previous paper we showed that the adenylate regulation of electron transport and phosphorylation in the H_2O–Fecy* system in

* Abbreviations—Fecy: ferricyanide; CF_1: chloroplast coupling factor protein; As: arsenate; DMQ: 2,5-dimethyl-*p*-benzoquinone; DPIP: 2,6-dichlorophenol-indophenol; $DPIPH_2$: reduced 2,6-dichlorophenolindophenol; MV: methyl viologen. A partial electron transport system is abbreviated as the donor–acceptor representation.

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spinach chloroplasts could be interpreted by sequential binding of ADP to (at least) two binding sites in CF_1 [1]. That is, as CF_1 binds ADP (or ATP) at the site of the higher binding affinity, Fecy reduction is inhibited to the level of nonphosphorylating (basal) electron transport activity, and then restored from this level when phosphorylation or arsenylation is induced by ADP bound to the other site in the presence of Pi or *As*.

We studied adenylate regulation in various partial electron transport systems using various combinations of electron donors and acceptors in connection with phosphorylation, arsenylation, and quasiarsenylation activities [2] in these systems.

Materials and Methods

Chloroplasts were prepared from market spinach leaves with a 0.5 M choline medium [3, 4] as described previously [3]. The reaction mixture contained 0.1 M sucrose, 5 mM $MgCl_2$, 10 mM tris(hydroxymethyl)methylglycine (pH 8.3), chloroplasts equivalent to 20 μg/ml chlorophyll concentration determined according to the method of Arnon [5], and additional 500 μM DMQ (for the H_2O–DMQ system) or 50 μM DPIP (for H_2O–DPIP) or 1 mM ascorbate, 50 μM DPIP, 50 μM MV and 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (for $DPIPH_2$–MV) or 50 μM MV (for H_2O–MV) or 500 μM Fecy (for H_2O–Fecy). In the pH dependence experiments, a mixed buffer composed of citrate, piperazine-*N*-*N'*-bis(2-ethane sulfonic acid), tris(hydroxymethyl)methylglycine and sarcosine (10 mM each) was used instead of a single tris(hydroxymethyl)methylglycine buffer. Nucleotide, Pi, *As*, phlorizin, and methylamine hydrochloride were added to the reaction mixture as required.

The activities of these partial electron transport systems were measured polarographically with an oxygen electrode (Field oxygen analyzer, Beckman) at 15°C under actinic light (>500 nm) from a 750-W projector lamp. The error for the estimation of the activity was several per cent. For the assay of phosphorylation activity, the amount of esterified [^{32}P] Pi was determined by a modification of the method of Asada et al. [6].

Results and Discussion

Figure 1 shows the effect of ATP on the activities of various partial electron transport systems in the full-length noncyclic electron transport system described usually as the Z scheme [7] in spinach chloroplasts. The H_2O–DMQ [8] and the H_2O–DPIP [9] systems contain photo-

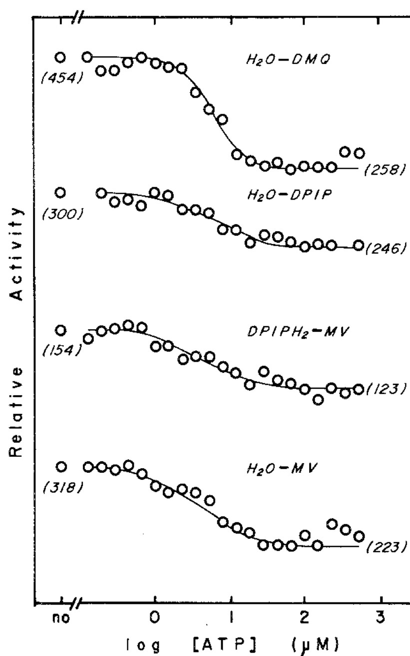

Figure 1. Effects of ATP on the activity of various partial electron transport systems. The numerals in the parentheses show the observed activity in μequiv/mgchl·h.

system II, the $DPIPH_2$–MV system [10] photosystem I, and the H_2O–MV system [11–13] both photosystems I and II. With an increase in ATP concentration (>about 1 μM), the activities of the electron transports studied here all gradually decreased and reached the lowest (basal electron transport) level at an ATP concentration of around 30 μM. These changes were similar to those obtained by the H_2O–Fecy system, but the extent of inhibition differed. The percentage inhibition was found to be 43% for H_2O–DMQ, 18% for H_2O–DPIP, 30% for H_2O–MV, and 20% for $DPIPH_2$–MV. The percentage inhibitions of the two electron transport systems that included DPIP were considerably smaller than those of the others. This smaller inhibition might be due to an uncoupling action of DPIP [14], since this apparently corresponded with the fact that the percentage inhibition of the H_2O–Fecy system decreased with an increase in the uncoupler concentration [15]. On the other hand, the ATP concentration required to induce the half maximal inhibition was similar (4–8 μM) among transport systems. ADP gave the same results as ATP (data not shown). Therefore, these results were quite similar to those of the H_2O–Fecy system [1].

The regulation mechanism of the H_2O–Fecy system was postulated in

TABLE I. Activities of phosphorylation and electron transport in various electron transport systems

Conditions	Electron transport (μequiv/ mgchl·h)	Δe (μequiv/ mgchl·h)	Esterified Pi (μequiv/ mgchl·h)	$P/\Delta e$
H_2O–DMQ				
none	600			
100 μM ATP	288			
1 mM ADP, 1 mM Pi	628	340	150	0.44
$DPIPH_2$–MV				
none	140			
100 μM ATP	100			
1 mM ADP, 1 mM Pi	168	68	30	0.44
H_2O–MV				
none	260			
100 μM ATP	189			
1 mM ADP, 1 mM Pi	339	150	124	0.83

the previous paper [1]: The activity of electron transport that pumps protons [16] to supply the amounts leaked is depressed by a decrease of the H^+ leakage [17] by a conformation change in CF_1 [18] as CF_1 binds ADP (or ATP) at the site of higher binding affinity in (at least) two nucleotide binding sites [19–21]. Therefore, the inhibition by ATP (or ADP) observed in all partial electron transport systems investigated here might be a reflection of the conformation change in CF_1. If this change in CF_1 is one of the several steps of conformation changes needed for the turnover of phosphorylation, the phosphorylation activity should be detected in these electron transport systems. As shown in Table I (also see [14]), this was true for all systems studied here.

The turnover of phosphorylation should induce an apparent restoration of electron transport from the level of the inhibition [1], since electron transport functions to resupply H^+ translocated by phosphorylation. As shown in Figs. 2A and 2B, restoration ($\Delta E.T._{coupled} = E.T._{ADP,Pi} - E.T._{ATP}$) under the phosphorylation condition was observed in every electron transport system. In the electron transport system containing only photosystem II, the restoration under the phosphorylation condition has been reported by Reeves and Hall [22] but was not observed by Gould and Izawa [23]. The present results agree with the former. When the coupling between phosphorylation and electron transport is considered to be mediated by a so-called H^+ gradient [16], the ratio ($P/\Delta e$) of the amount of esterified Pi (P) to $\Delta E.T._{coupled}$ (Δe) should correspond to the number of phosphorylation sites. Table I also shows the $P/\Delta e$ values. When two protons are

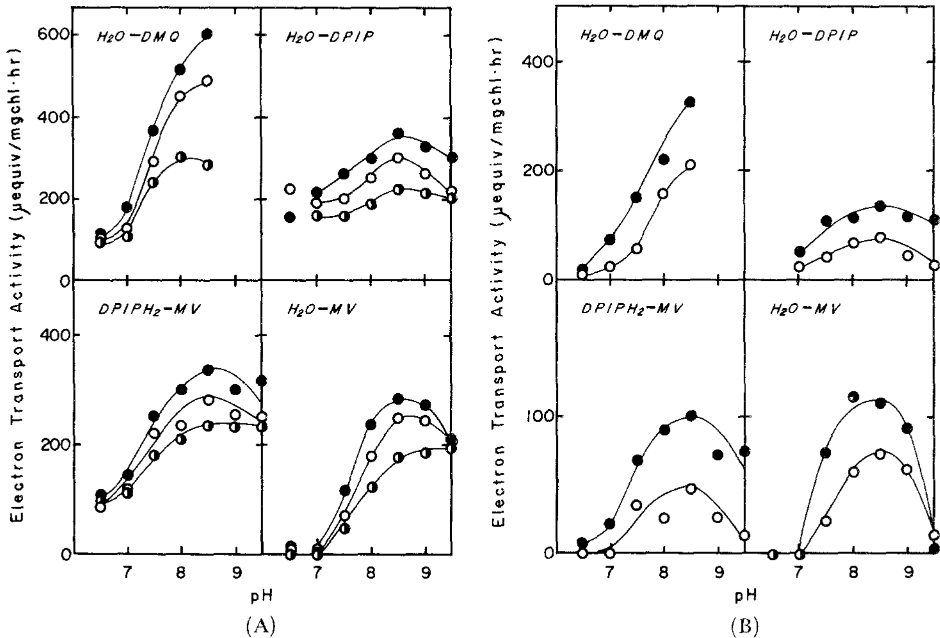

Figure 2. The pH dependence of adenylate regulation of the activity in various partial electron transport systems. (A) ○, in the absence of both adenylate and Pi (E.T.$_{none}$); ◐, in the presence of 100 μM ATP only (E.T.$_{ATP}$); ●, in the presence of both 1 mM ADP and 1 mM Pi (E.T.$_{ADP,Pi}$). (B) ○, Δ E.T.$_{inhibited}$ = E.T.$_{none}$ − E.T.$_{ATP}$ (shown in Fig. 2A); ●, Δ E.T.$_{coupled}$ = E.T.$_{ATP,Pi}$ − E.T.$_{ATP}$ (shown in A). For the H_2O–DMQ system, the results obtained at pH below 8.5 are shown, since the activity could not be measured steadily, possibly due to spontaneous oxidation of reduced DMQ above this pH value.

translocated across the thylakoid membrane to synthesize one molecule of ATP from ADP and Pi [24], the $P/\Delta H^+$ value equals 0.5. When one electron passes through one electron transport chain to release one H^+ at one phosphorylation site [24, 25], the $\Delta H^+/\Delta e$ value equals 1. If this is the case, the $P/\Delta e$ value becomes 0.5 for one electron transport chain per phosphorylation site. As Table I shows, the $P/\Delta e$ values for the H_2O–DMQ and the $DPIPH_2$–MV systems are close to this value. The $P/\Delta e$ value for the H_2O–MV system is comparable to $P/\Delta e = 1.0$, which is expected for an electron transport system containing two phosphorylation sites and close to the value obtained for the H_2O–Fecy system [1]. These results support well the scheme of two phosphorylation sites in series in the full-length noncyclic electron transport [14, 26–28], one each before and after the location where DMQ or DPIP (or $DPIPH_2$) accepts (or donates) an electron. These results strongly

TABLE II. Effects of phlorizin or methylamine on adenylate regulation in various electron transport systems[a]

Conditions	(μequiv/mgchl·h) 1 mM phlorizin −	+	10 mM CH_3NH_2 −	+
H_2O–Fecy				
1 mM Pi	341	349	201	327
100 μM ATP, 1 mM Pi	190	205	119	333
1 mM ADP, 1 mM Pi	379	215	245	326
H_2O–DMQ				
1 mM Pi	454	461	251	750
100 μM ATP, 1 mM Pi	240	263	210	760
1 mM ADP, 1 mM Pi	488	270	357	755
H_2O–DPIP				
1 mM Pi	143	151	225	349
100 μM ATP, 1 mM Pi	127	134	182	325
1 mM ADP, 1 mM Pi	353	135	243	331
H_2O–MV				
1 mM Pi	237	218	312	629
100 μM ATP, 1 mM Pi	182	168	197	627
1 mM ADP, 1 mM Pi	234	143	382	600
$DPIPH_2$–MV				
1 mM Pi	104	115	127	474
100 μM ATP, 1 mM Pi	94	94	123	472
1 mM ADP, 1 mM Pi	142	106	185	480

[a]The experiment with phlorizin was carried out with a different chloroplast preparation from that with methylamine.

imply that phosphorylation couples with electron transport stoichiometrically when the basal electron transport (the part not regulated by adenylates) is excluded from calculation.

The adenylate regulation of the H_2O–Fecy system has been studied concerning pH dependence [1], the effects of an energy transfer inhibitor and an uncoupler [15], and the effect of the coexistence of AMP and *As* [2]. The pH dependence and these effects were thus studied in relation to the partial electron transport systems and compared with those of the H_2O–Fecy system.

As shown in Fig. 2B, the pH dependence of the extent of the inhibition ($\Delta E.T._{inhibited} = E.T._{none} - E.T._{ATP}$) and of the restoration were similar with the optimum pH around 8.5. These profiles resembled those of the H_2O–Fecy system [1], and the optimum pH was identical.

The effects of energy transfer inhibitor, phlorizin [29] or uncoupler, methylamine [30] on the adenylate regulation of electron transport are shown in Table II. In every electron transport system, phlorizin (1 mM) abolished the restoration, but did not affect the inhibition of electron

TABLE III. Effects of arsenylation or quasiarsenylation on electron transport in various electron transport systems

Conditions	(μequiv/mgchl·h) 1 mM phlorizin −	+
H_2O–Fecy		
1 mM *As*	332	341
100 μM AMP, 1 mM *As*	329	328
100 μM ATP, 1 mM *As*	179	208
100 μM ATP, 100 μM AMP, 1 mM *As*	307	200
1 mM ADP, 1 mM *As*	373	193
H_2O–DMQ		
1 mM *As*	678	664
100 μM AMP, 1 mM *As*	708	675
100 μM ATP, 1 mM *As*	495	547
100 μM ATP, 100 μM AMP, 1 mM *As*	682	508
1 mM ADP, 1 mM *As*	868	511
H_2O–DPIP		
1 mM *As*	329	338
100 μM AMP, 1 mM *As*	351	323
100 μM ATP, 1 mM *As*	298	317
100 μM ATP, 100 μM AMP, 1 mM *As*	342	316
1 mM ADP, 1 mM *As*	388	311
H_2O–MV		
1 mM *As*	218	205
100 μM AMP, 1 mM *As*	212	202
100 μM ATP, 1 mM *As*	150	135
100 μM ATP, 100 μM AMP, 1 mM *As*	229	138
1 mM ADP, 1 mM *As*	252	139
$DPIPH_2$–MV		
1 mM *As*	138	138
100 μM AMP, 1 mM *As*	134	129
100 μM ATP, 1 mM *As*	120	125
100 μM ATP, 100 μM AMP, 1 mM *As*	164	129
1 mM ADP, 1 mM *As*	169	133

transport by ATP (or ADP). By the addition of methylamine, the electron transport activities were stimulated to the same level, regardless of the presence of ADP or ATP or Pi. These results were consistent with those for the H_2O–Fecy system reported previously [1, 15].

The electron transport inhibited by ATP (100 μM) was restored by adding both AMP (100 μM) and *As* (1 mM). But the restoration was barely observed upon removal of any one of these three components. This apparent restoration with the coexistence of ATP, AMP, and *As* was found, studied and termed "quasi-arsenylation" [2]. As shown in Table III, in every electron transport system studied here, the restoration of electron transport accompanied by arsenylation (ADP–*As* system) or

quasiarsenylation (ATP–AMP–*As* system) was observed. The extent of restoration under an arsenylation condition was always larger than that under a quasiarsenylation condition. Also the restoration under both conditions was abolished by phlorizin (1 mM) as entirely as under a phosphorylation condition. These results were also consistent with those found in the H_2O–Fecy system [2], in which the mechanism of quasiarsenylation was interpreted through the transphosphorylation mechanism [19, 31].

These results indicate that the characteristics of two phosphorylation sites located in the full-length noncyclic electron transport are hardly distinguishable and suggest that there is a phosphorylation mechanism containing transphosphorylation in both sites.

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