A direct interaction between Photosystem I and the chloroplast coupling factor

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A regulation of the ATP-synthesizing complex by electron-transport rate has been found. The site of regulation could be localized within the Photosystem I region. The regulatory effect probably is produced by direct interactions between neighbouring charged protein complexes. The primary result is an increase in the percentage of those binding sites adopting a low-affinity state. This seems to lead to an enhanced leakage of protons out of the thylakoids, especially under those experimental conditions employing low nucleotide concentrations. Changes in the P/2e ratio can be observed, especially if the total ADP+ATP concentration used in the experiment is below 200 μM .

INTRODUCTION

It is a basic assumption that an electrochemical proton gradient links the redox reactions of the electron-transport chain with ATP synthesis. The simplest model is based on the assumption of a proton being transported between two bulk phases (Mitchell, 1966). Within the sequence of linear electron transport from water to acceptors like ferricyanide there are two proton-transducing sites. Site no. 1 is located at the water-splitting system of PSII and site no. 2 where the oxidation of plastoquinone at the cytochrome complex takes place. The total electron-transport system produces ATP at a yield of 1.3 ATP/2 electrons (Winget *et al.*, 1965). If either of these energy-conserving devices is inhibited, the assumption can be made that the two proton sources are unequal: they differ in their maximal P/2e ratio.

Phosphorylation coupled to the oxidation of plastoquinone shows a maximal P/2e ratio of 0.6. This value was measured both by using artificial electron donors after an inhibition of the water-splitting system (Izawa & Ort, 1974) and by oxidizing analogues of plastoquinone (Izawa & Pan, 1978). However, when PS I was inhibited by an inhibitor such as DBMIB, measurements of PS II-driven ATP synthesis with a lipophilic acceptor show P/2e ratios of the order of 0.3 (Izawa *et al.*, 1973; Trebst & Reimer, 1973). Moreover, differences in the pH-sensitivity of the P/2e ratios have been observed (Gould & Izawa, 1973).

The lower P/2e ratios observed with isolated PS II might be interpreted in terms of a impeded energy (proton) flux from the water-splitting system to the coupling factor. In their flashlight experiments, Flores & Ort (1984) showed two ways to enhance P/2e values up to 0.5: (i) by introducing an artificial pH gradient of two units before illumination and (ii) by a PS I illumination period prior to measurement. These experiments indicated that each of the two proton-translocating sites contributes 50% of ATP synthesis under conditions *in vivo*. A third

proton-generating site within electron transport from water to ferricyanide cannot be expected. However, a proton translocation by Cyt *b₆-f* complex may occur if ATP synthesis depends on PSI-driven cyclic electron transport.

This harmonious scheme was disturbed by results published in our previous paper (Loehr *et al.*, 1985). We found a variation of P/2e ratios with varying electron-transport rate. In agreement with Vinkler (1981), we also observed that changes in the electron-transport rate were paralleled by variations of the apparent affinity of the coupling factor for nucleotides. We therefore think the P/2e ratios reflect the apparent nucleotide affinity of the coupling factor.

As long as we used tightly stacked thylakoids in our experiments, we were unable to detect any effect of PS II electron transport on the coupling-factor system. The effects described above were due to PSI exclusively. In the present paper this aspect is described in greater detail. In particular we paid attention to the fact that changing the H^+/e^- ratio would bring about results similar to those described above.

EXPERIMENTAL

Isolation of thylakoids from spinach (*Spinacia oleracea*) leaves was carried out as described by Strotmann *et al.* (1976). The thylakoid stock solution contained 100 mM-sucrose, 50 mM-NaCl, 5 mM-MgCl₂ and 2 mM-Tricine buffer, pH 8.0. The chlorophyll content was adjusted to 2 mg/ml. For chlorophyll determination, Arnon's (1949) method was used. All measurements were made at 20 °C in a temperature-controlled reaction chamber as described in the previous paper (Loehr *et al.*, 1985).

The acid/base phosphorylation experiments were performed on a magnetic stirrer with top-illumination equipment. A 100 μl portion of thylakoid suspension was

Abbreviations used: Cyt, cytochrome complex; DBMIB, dibromothymoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MV, Methyl Viologen; PS I, Photosystem I; PS II, Photosystem II; TMPD, *NNN'*-tetramethyl-*p*-phenylenediamine.

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mixed for 5 s with 200 μ l of acid step medium (consisting of 15 mM-sodium succinate+15 mM-sodium ascorbate +0.2 mM-TMPD) at pH 4.2. Then 1 ml of base step medium [consisting of 110 mM-Tricine buffer, 50 mM-NaCl, 5 mM-MgCl₂, 6 mM-[³²P]phosphate (0.2 MBq/ μ mol; Amersham-Buchler, Braunschweig, Germany) 30 μ M-DCMU, 0.2 mM-TMPD, 12 mM-glucose, hexokinase (35 units/ml) and different concentrations of ADP] was added, resulting in a pH of 8.3. After 20 s the reaction was stopped by addition of 0.1 ml of 3 M-HClO₄ and the samples were stored on ice until analysis. Organic phosphate was determined by the method of Sugino & Miyoshi (1964)

The rate of nucleotide binding was measured in an apparatus consisting of a stop-flow mixing chamber and a high-speed fraction collector. The amount of [8-¹⁴C]ADP (2.11 MBq/ μ mol; Amersham-Buchler) bound to the coupling factor was determined by using the method of Huchzermeyer (1986). Light-triggered ATPase activity was measured as described by Schumann (1981).

RESULTS

The results shown in Table 1 indicate the regulatory effect observed to be due to PS I exclusively. When driving ATP synthesis by PS II with inhibited PS I, reduced P/2e ratios were observed under low-light conditions. This decay is due to a relatively high proportion of proton leakage when employing these experimental conditions. Experiments with active+PSI in the presence of inhibited or active PS II resulted in maxima of the P/2e ratios at distinct light intensities. These were attributable to the fact that, with increasing light intensity, the phosphorylation rate reached its maximal value before that of the electron-transport rate.

We attribute these observed changes in P/2e ratios to apparent changes in substrate specificity of the coupling factor rather than to variable H/e ratios. The first reason for this assumption is the fact that different nucleotide specificities have indeed been measured (Loehr *et al.*, 1985; Huchzermeyer, 1986). A second experimental proof is shown in Fig. 1. In this experiment, acid/base-driven phosphorylation was measured. The incubation medium contained additional ascorbate, TMPD, MV and an ADP-regenerating hexokinase system. Therefore

Fig. 1. Effect of PSI-driven electron transport on the ATP yield and the apparent K_m in an acid/base experiment

An acid/base phosphorylation experiment was performed in the presence of TMPD. In the double-reciprocal plot, results from a 'dark' experiment (Δ) are compared with those from another in which the samples had been illuminated during the acid/base transition with white light at an intensity of 420 W/m² ($\circ$).

electron transport through PS I could proceed when the samples were illuminated. In agreement with publications on the matter (Izawa, 1980), we found in a prior experiment that this electron transport was incapable both of producing a transmembrane protein gradient and of driving ATP synthesis (Table 1). In the acid/base experiments the effects of different light intensities on apparent K_m values for ADP and ATP yield were measured. As shown in Fig. 1, light-dependent electron transport through PS I results in an increase in the apparent K_m values that is paralleled by decreasing ATP yields.

In Fig. 2 the effect of TMPD-mediated electron transport through PS I on the rate of ADP binding to high-affinity sites is shown. Though we found a decrease in the apparent affinity in parallel experiments, we observed increasing rates of ADP binding with increasing TMPD-mediated electron-transport rates.

Schumann (1981) showed that non-exchangeable binding of ADP is paralleled by a decrease of light-triggered ATPase activity. Non-exchangeably bound nucleotides are found under those conditions

Table 1. P/2e ratios measured with different donor/acceptor systems

The P/2e ratio was regarded as constant when its value did not change except under low light intensities; it was regarded as variable when we found a maximum between low and high light intensities. Only the maximal P/2e ratios are given in this Table. The electron-transport is given in μ mol of ferricyanide reduced (electrons transported respectively)/h per mg of chlorophyll. Further abbreviations used: Pd, phenylene-1,4-diamine; Fecy, ferricyanide; DMBQ, 2,5-dimethylbenzoquinone; asc, ascorbate; DAD, diaminodurene.

System	Acceptor	Donor	Rate	P/2e
PS II	Pd + Fecy	Water	669	0.41, constant
	DBMIB + Fecy	Water	417	0.35, constant
	DMBQ + Fecy	Water	342	0.37, constant
	MV	Pd + asc	2043	0.39, variable
PS I	MV	TMPD + asc	1283	No phosphorylation
	MV	DAD + asc	2248	0.49, variable
	MV	Duroquinone	736	0.57, variable
PS I + Cyt	MV	Water	663	1.28, variable
PS II + Cyt + PSI	Fecy	Water	623	1.36, variable

Fig. 2. Effect of PS I-driven electron transport on the rate of ADP binding during inactivation of ATPase activity

The incubation mixture was essentially the same as that listed in Table 2. After activation of ATPase activity, 5 μM -[8- ^{14}C]ADP was added and the rate of its binding to thylakoid-bound coupling factor 1 was monitored. In the first experiment (Δ), nucleotide binding in the dark was measured. In the second one ($\circ$), the ATPase experiment was performed with continuous illumination.

in which thylakoids lack energy to initiate the catalysis at coupling factor 1. The surprising result of our binding studies induced us to measure the effect of TMPD-mediated electron transport on light-triggered ATPase activity. Table 2 shows that PS I electron transport results in an inhibition of ATPase activity. This effect is diminished by adding valinomycin + KCl. The effect of uncouplers could not be analysed, since low concentrations of all tested uncouplers result in a stimulation of

ATPase activity, and high concentrations are inhibitory. A clear-cut interpretation of the results of uncoupler experiments is therefore difficult.

DISCUSSION

Our experimental results indicate that there is a regulation of enzymic properties of the coupling-factor complex induced by the electron-transport rate through PS I. As a model, one could imagine a change in the surface charge of the PS I complex inducing a conformational change in some region of the neighbouring coupling-factor system. In a previous paper (Loehr *et al.*, 1985) we showed that the effect of the PS I electron-transport rate on the coupling factor is abolished by adding gramicidin. The gramicidin effect was not found with a number of different hydrophilic uncouplers. In terms of our model this means that some ions bound to surface charges of the membrane proteins must be involved in the assumed protein–protein interaction.

The changes in P/2e ratios we attribute to a phenomenon investigated by McCarty's group (see review by McCarty, 1979). They showed that the leakage of protons out of the thylakoids is increased, with increasing portions of coupling factors free from nucleotides. Of course the probability of coupling factors being free from nucleotides increases with their decreasing affinity to nucleotides. In agreement with this assumption, we observed that the maximum P/2e under PS I conditions became more pronounced if the ADP concentration was reduced.

An analysis of the nucleotide-binding kinetics and light-triggered ATPase activity leads to the conclusion that the observed changes in apparent nucleotide affinity reflect changes in the proportion of high- and low-affinity states of the binding sites. Increasing the percentage of binding sites which adopt their low-affinity state under PS I conditions results in a decreasing apparent affinity for nucleotides. According to Boyer & Kohlbrenner's (1981) binding-change model, during the ATP-catalysis cycle at least three different states of the binding sites must be postulated: low-affinity, high-affinity and non-exchangeable binding. The conformational change transforming the third state back to the low-affinity one

Table 2. Effect of TMPD-mediated electron transport in light-triggered ATPase activity

Thylakoids were illuminated for 3 min in a medium containing 30 mM-Tricine buffer, pH 8.0, 5 mM-phosphate, 5 mM-MgCl₂, 50 mM-KCl, 0.2 mM-MV, 2.5 mM-ascorbate, 0.2 mM-TMPD and thylakoids corresponding to 40 μg of chlorophyll/ml. Then ATP was added and, where indicated, the light was switched off and 30 μM -DCMU was added. To monitor ATPase activity, the ATP concentration was measured at intervals of 5 s. Where indicated the incubation mixture contained, in addition, 1 μM -valinomycin.

[ATP] (μM)	Rate of ATP hydrolysis ($\mu\text{mol of ATP} \cdot \text{h}^{-1} \cdot \text{mg of chlorophyll}^{-1}$)			
	ATPase activity measured in the dark		ATPase activity measured under continuous illumination	
	No addition	+ Valinomycin	No addition	+ Valinomycin
10	33.8	30.0	11.3	25.3
20	52.9	49.1	20.2	41.9
30	66.2	60.2	27.7	52.4
50	84.5	77.3	39.5	68.1
75	99.2	90.8	50.8	82.7
100	111.0	110.2	65.4	93.6

is obviously rate-limiting. In accordance with these considerations we found in our experiments a stimulated rate of labelling of coupling factor by incorporating [8-¹⁴C]ADP at an increased percentage of binding sites adopting their low-affinity state.

The observed regulatory effect of PS I on the coupling-factor complex *in vivo* is probably not as effective as other known mechanisms, such as the thioredoxin system (McKinney *et al.*, 1979; Mills *et al.*, 1980; Shahak, 1982). A regulation of the P/2e ratio noteworthy with respect to physiological aspects was only found with nucleotide concentrations below 200 μ M. It should be emphasized that by nucleotide concentration we mean the total of the concentrations of ADP plus ATP in the experiment. Similar experiments have been performed using GDP, GTP, IDP, and ITP. These nucleotides caused the same effects as adenine nucleotides when concentrations 10-fold higher than those mentioned above were used.

From these results we think that the maximal decrease in the ratio by light-stimulated electron-transport rate *in vivo* is unlikely to exceed 10%. Nevertheless, the observed regulatory effect indicates a very close relationship between PS I and the coupling-factor complex. This result is in good agreement with previous observations (Lam & Malkin, 1983).

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REFERENCES

- Arnon, D. I. (1949) *Plant Physiol.* **24**, 1–15
- Boyer, P. D. & Kohlbrenner, W. E. (1981) in *Energy Coupling in Photosynthesis* (Selman, B. R. & Selman-Reimer, S., eds.), pp. 231–240, Elsevier/North-Holland, Amsterdam
- Flores, S. & Ort, D. R. (1984) *Biochim. Biophys. Acta* **766**, 289–302
- Gould, J. M. & Izawa, S. (1973) *Biochim. Biophys. Acta* **314**, 211–223
- Huchzermeyer, B. (1986) *Biochem. Soc. Trans.* **14**, 45–46
- Izawa, S. (1980) *Methods Enzymol.* **69**, 413–434
- Izawa, S. & Ort, D. R. (1974) *Biochim. Biophys. Acta* **357**, 127–143
- Izawa, S. & Pan, R. L. (1978) *Biochem. Biophys. Res. Commun.* **83**, 1171–1177
- Izawa, S., Gould, J. M., Ort, D. R., Felker, P. & Good, N. E. (1973) *Biochim. Biophys. Acta* **305**, 119–128
- Lam, E. & Malkin, R. (1983) *Arch. Biochem. Biophys.* **224**, 456–463
- Loehr, A., Willms, I. & Huchzermeyer, B. (1985) *Arch. Biochem. Biophys.* **236**, 832–840
- McCarty, R. E. (1979) *Trends Biol. Sci.* **4**, 28–30
- McKinney, D. W., Buchanan, B. B. & Wolusiuk, R. A. (1979) *Biochem. Biophys. Res. Commun.* **86**, 1178–1184
- Mills, J. D., Mitchell, P. & Schürmann, P. (1980) *FEBS Lett.* **112**, 173–177
- Mitchell, P. (1966) *Biol. Rev.* **41**, 445–502
- Schumann, J. (1981) in *Energy Coupling in Photosynthesis* (Selman, B. R. & Selman-Reimer, S., eds.), pp. 223–230, Elsevier/North-Holland, Amsterdam
- Shahak, Y. (1982) *Plant Physiol.* **70**, 87–91
- Strotmann, H., Bickel, S. & Huchzermeyer, B. (1976) *FEBS Lett.* **61**, 194–198
- Sugino, Y. & Miyoshi, Y. (1964) *J. Biol. Chem.* **239**, 2360–2364
- Trebst, A. & Reimer, S. (1973) *Biochim. Biophys. Acta* **305**, 129–139
- Vinkler, C. (1981) *Biochem. Biophys. Res. Commun.* **99**, 1095–1100
- Winget, G. D., Izawa, S. & Good, N. E. (1965) *Biochem. Biophys. Res. Commun.* **21**, 438–443

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