Inactivation and reactivation of light-triggered ATP hydrolysis on the chloroplast coupling factor

Mizuho Komatsu-Takaki

Department of Chemistry, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa, 199-01, Japan

Received 30 July 1984

Decay of light-triggered ATP hydrolysis in the dark was diminished with a decrease in chloroplast concentration. The enhancing effect of NH_4Cl on ATP hydrolysis decreased with dark time. The decrease was much faster than that in ATP hydrolysis activity. The NH_4Cl effect increased with ATP preincubation time. Reactivation of ATP hydrolysis occurred with the progress of ATP hydrolysis. P_i enhanced the activation remarkably. These results suggest that ATP hydrolysis produces some energized state, which stimulates NH_4Cl effect and makes coupling factor active in the presence of P_i and that to keep coupling factor active, energy is not necessarily needed.

ATP hydrolysis *Activation* *Energized state* *P_i effect* *NH_4Cl effect* *Chloroplast*

1. INTRODUCTION

Illumination of chloroplasts in the presence of sulfhydryl reagent induces light-triggered ATP hydrolyzing activity, which decays with time in the post-illumination dark [1]. The decay has been shown to be accelerated by ADP and suppressed by P_i [2]. ADP has been suggested to be correlated with the activation of ATP hydrolysis: the activation of ATP hydrolysis in the light is associated with the release of ADP from the coupling factor (CF_0–CF_1) and the inactivation of ATP hydrolysis with the rebinding of ADP in the dark [3–6]. It is supposed that the suppressing effect of P_i on the decay of ATP hydrolyzing activity results from the inhibition of ADP rebinding by P_i [7,8]. In this connection, P_i has recently been shown to enhance the release of ADP from nonenergized chloroplasts [9].

Here, the inactivation and reactivation of light-triggered ATP hydrolyzing activity was investigated in connection with the energized state of chloroplasts.

2. MATERIALS AND METHODS

Chloroplasts were prepared from spinach leaves as in [10] and washed 4 times in a medium containing 0.1 M KCl and 2 mM *N*-tris(hydroxymethyl)methylglycine (Tricine)–KOH (pH 7.2) and finally suspended in the washing medium at a concentration equivalent to 3 mg chlorophyll (chl) per ml.

ATP hydrolysis was assayed at 27°C as follows. A reaction mixture containing 50 mM KCl, 25 mM Tricine–KOH (pH 8.3), 5 mM $MgCl_2$, 0.1 mM methyl viologen, 5 mM dithiothreitol, 10 units pyruvate kinase and chloroplasts equivalent to 150 μg of chl in a total volume of 1 ml was illuminated for 60 s with heat-filtered white light (3.6×10^5 ergs/cm^2 per s). At 10 s after turning the light off (dark 10 s), 10 μl of an ATP mixture containing 25 mM ATP, 0.25 mM P^1,P^5-di(adenosine-5′)pentaphosphate and 0.15 M phosphoenolpyruvate was added and incubated for 20 s. For measurement of ATP hydrolysis in the presence of NH_4Cl (NH_4Cl-ATP hydrolysis), 5 μl of 0.65 M NH_4Cl was added with or after ATP mixture addition and incubated for 20 s. For measurement of

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

ATP hydrolysis in the presence of P_i, 5 μl of 1 M P_i was added either at dark 10 s, with ATP or with NH_4Cl as described in each experiment. The reaction was terminated by addition of 0.25 ml of 15% perchloric acid and the mixture chilled and centrifuged. The supernatant solution was neutralized with 0.5 M Tris–KOH. The pyruvate content was assayed with lactate dehydrogenase and NADH by measuring the absorbance at 340 nm.

ATP, P^1,P^5-di(adenosine-5′)pentaphosphate, NADH, phosphoenolpyruvate, pyruvate kinase and lactate dehydrogenase were purchased from Boehringer, Mannheim.

3. RESULTS

In the post-illumination dark, the amount of active CF_0–CF_1 decreased with time. The rate of decay depended on chloroplast concentration (fig.1A). Lowering the chloroplast concentration diminished the decay significantly and at a concentration of 30 μg chl/ml, almost all active CF_0–CF_1 was maintained even after 2 min dark incubation. Lowering the chloroplast concentration results in a decrease in the concentration of ADP originated from the endogenously bound ADP [11]. Therefore, rebinding of ADP must be very slow at low chloroplast concentrations. At dark 10 s, addition of 3 mM NH_4Cl with ATP enhanced ATP hydrolysis by a factor of 2.5. The enhancing effect of NH_4Cl decreased rapidly with dark time even at low chloroplast concentration (30 μg chl/ml). At dark 2 min, NH_4Cl lost its effect almost completely (fig.1B).

A similar situation was encountered when the decay of active CF_0–CF_1 was suppressed by addition of 5 mM P_i at dark 10 s (fig.2). In the presence of P_i, almost all active CF_0–CF_1 was maintained even after 3 min dark incubation (fig.2A). However, the enhancing effect of NH_4Cl was lost almost completely at that time (fig.2B).

These results suggest that for NH_4Cl to be effective, some energized state, which decreases with dark time, is required. The energized state seems not to be necessarily needed to maintain CF_0–CF_1 in an active state in the case where the rebinding of ADP is suppressed.

After 4 min dark incubation in the presence of P_i, almost all active CF_0–CF_1 remained but the

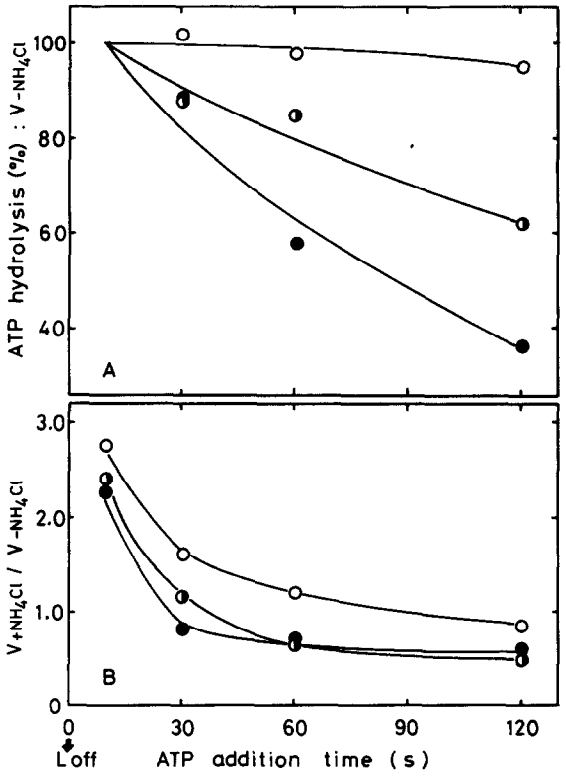

Fig.1. The decay of ATP hydrolyzing activity and of ATP hydrolysis enhancing effect of NH_4Cl in the dark. Reaction conditions and experimental procedures were as described in section 2 except that chloroplast concentration was 30 (○), 90 (◑) or 300 (●) μg chl/ml. ATP mixture was added at the time indicated and incubated for 2 min (○), 1 min (◑) or 20 s (●). ATP hydrolysis with NH_4Cl was measured by addition of NH_4Cl with ATP. A, ATP hydrolysis without NH_4Cl: 100% activity was 5.7 (○), 6.6 (◑) or 8.9 (●) nmol/mg chl per s. B, The ratios of ATP hydrolysis with NH_4Cl to those without NH_4Cl.

enhancing effect of NH_4Cl was lost completely as shown above. However, by addition of NH_4Cl after ATP preincubation, the enhancing effect was recovered and increased with the preincubation time in the presence of ATP (fig.3A). After 1 min preincubation with ATP, the rate of NH_4Cl-ATP hydrolysis which was carried out by addition of ATP at dark 4 min (dark 4 min NH_4Cl-ATP hydrolysis) reached a plateau at the same level as that of dark 10 s NH_4Cl-ATP hydrolysis (fig.3B). This result implies that some energized state, which enhances the NH_4Cl effect, is formed with the pro-

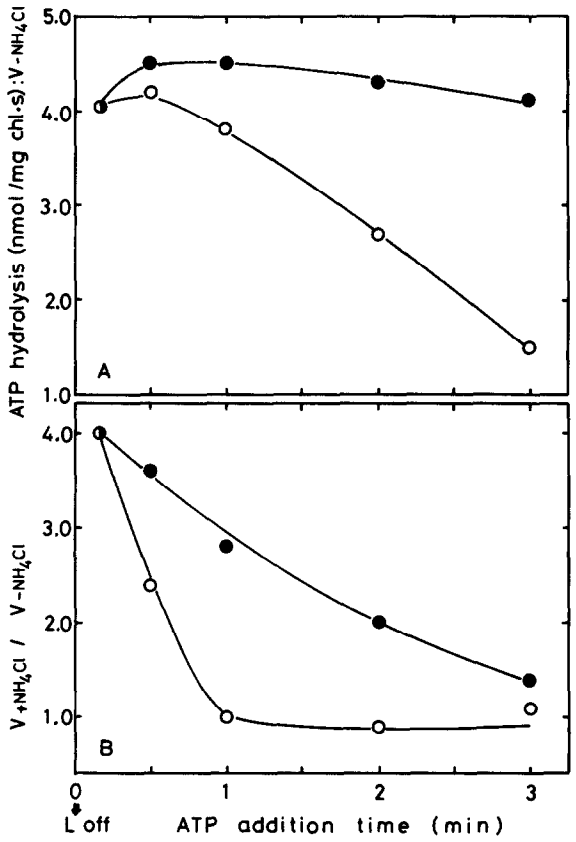

Fig.2. The decay of ATP hydrolyzing activity and of ATP hydrolysis enhancing effect of NH_4Cl in the presence and absence of P_i. P_i was added at dark 10 s (●) or with ATP (○). ATP mixture was added at the time indicated and incubated for 20 s. NH_4Cl-ATP hydrolysis was measured by addition of NH_4Cl with ATP. Chloroplast concentration was 156 μg chl/ml. A, ATP hydrolysis without NH_4Cl. B, The ratios of ATP hydrolysis with NH_4Cl to those without NH_4Cl.

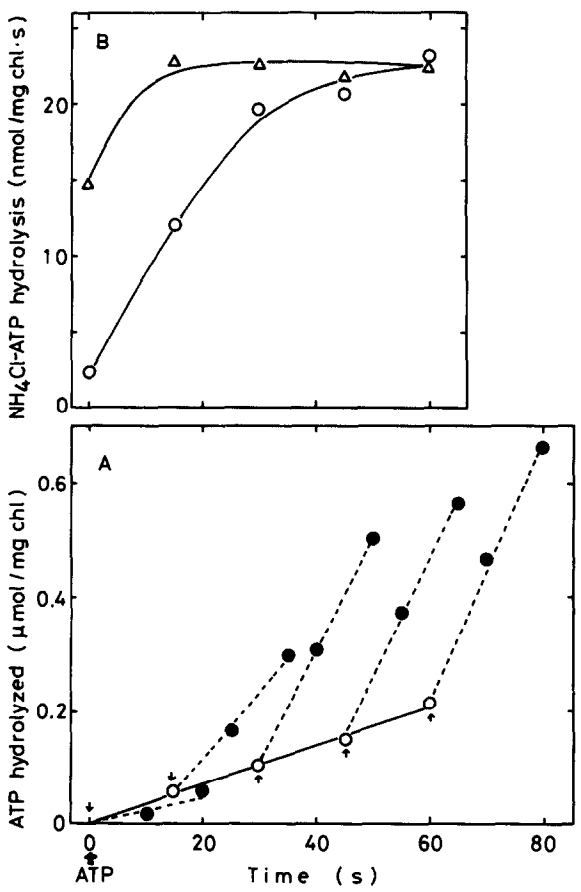

Fig.3. Dependence of NH_4Cl-ATP hydrolysis on the ATP preincubation time. P_i and ATP mixture were added at dark 10 s and at dark 4 min, respectively (○) or added at dark 10 s together (△). At the time designated with arrows, NH_4Cl was added and incubated for 10 or 20 s (●). Chloroplast concentration was 125 μg chl/ml. A, Time course of ATP hydrolysis which was started by addition of ATP at dark 4 min. B, NH_4Cl-ATP hydrolysis. In the absence of NH_4Cl, the rates of dark 10 s and dark 4 min ATP hydrolysis were 3.6 and 3.4 nmol/mg chl per s, respectively.

gress of ATP hydrolysis. As the rate of ATP hydrolysis without NH_4Cl was constant with the progress of ATP hydrolysis (fig.3A), the energized state formed seems not to change the turnover rate of ATP hydrolysis in the absence of NH_4Cl.

After 2 min dark incubation in the absence of P_i, about half of CF_0–CF_1 was inactivated at the chloroplast concentration used. The enhancing effect of NH_4Cl on dark 2 min ATP hydrolysis also increased with the progress of ATP hydrolysis (fig.4). By addition of P_i with ATP, the rate of dark 2 min NH_4Cl-ATP hydrolysis reached a plateau at a level similar to that of dark 10 s NH_4Cl-ATP hydrolysis; in the latter case, almost all CF_0–CF_1 can be assumed to be active. However, in the case where P_i was added with NH_4Cl or not added at all, dark 2 min NH_4Cl-ATP hydrolysis reached a plateau at a much lower level than that of dark 10 s NH_4Cl-ATP hydrolysis. These results imply the activation of inactivated CF_0–CF_1 with the progress of ATP hydrolysis and also the participation of P_i in the activation reaction.

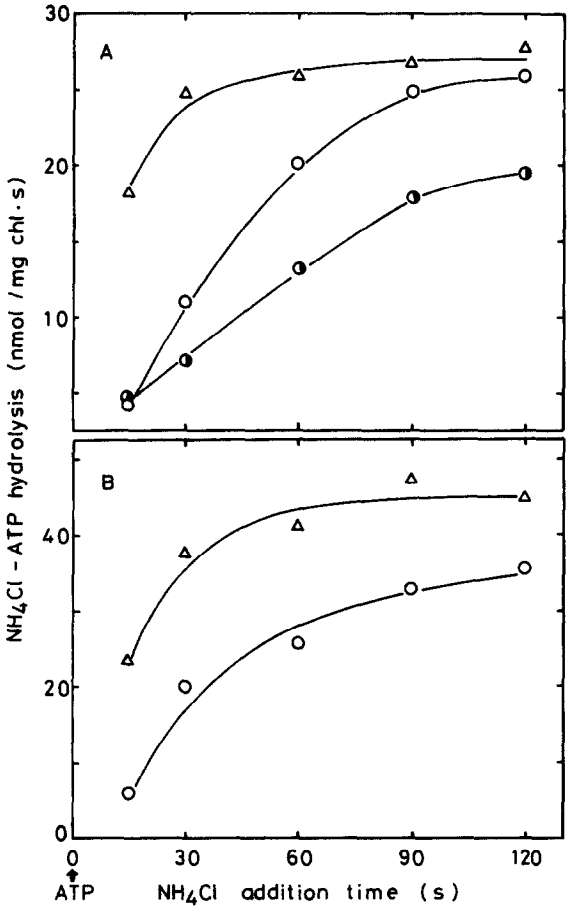

Fig.4. Dependence of NH_4Cl-ATP hydrolysis on the ATP preincubation time and the effect of P_i. ATP mixture was added at dark 10 s (△) or dark 2 min (○, ◑) and NH_4Cl was added at the time indicated and incubated for 20 s. Chloroplast concentration was 172 μg chl/ml. A, NH_4Cl-ATP hydrolysis in the presence of P_i. P_i was added with ATP (△, ○) or NH_4Cl (◑). B, NH_4Cl-ATP hydrolysis in the absence of P_i.

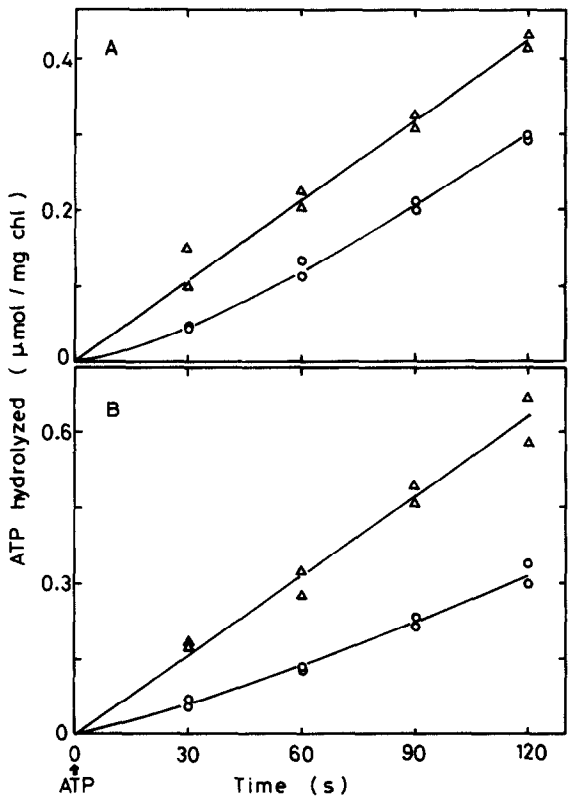

Fig.5. Time courses of ATP hydrolysis without NH_4Cl in the presence and absence of P_i. ATP mixture was added at dark 10 s (△) or dark 2 min (○). Chloroplast concentration was 172 μg chl/ml. A, P_i added with ATP. B, P_i not added.

The time courses of ATP hydrolysis without NH_4Cl, which were obtained by addition of ATP at dark 10 s or at dark 2 min, with or without P_i, are shown in fig.5. The rate of dark 10 s ATP hydrolysis was constant throughout the reaction, irrespective of P_i. However, the rate of dark 2 min ATP hydrolysis increased with the progress of ATP hydrolysis. In the presence of P_i, the increase was much more remarkable than that in the absence of P_i. In the case where P_i was added at dark 10 s and almost all active CF_0–CF_1 were maintained in the following dark, the rate of dark 2 min ATP hydrolysis did not change throughout the reaction (not shown, see fig.3A).

The ratios of the rate of dark 2 min ATP hydrolysis to that of dark 10 s ATP hydrolysis with or without P_i are shown in fig.6. Assuming that all CF_0–CF_1 was active at dark 10 s, dark incubation in the absence of P_i decreased the amount of active CF_0–CF_1 to below 40%. With the progress of ATP hydrolysis, the inactivated CF_0–CF_1 was reactivated and after 2 min, the amount of active CF_0–CF_1 recovered to 90 and 60%, in the presence and absence of P_i, respectively.

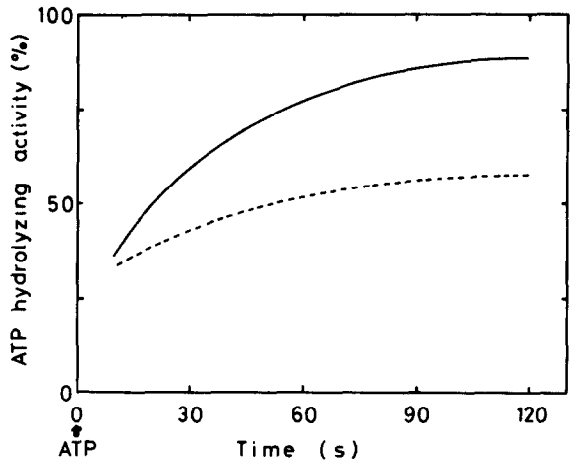

Fig.6. Increase in the ATP hydrolyzing activity with the progress of ATP hydrolysis. The ratios of the rates of ATP hydrolysis started by addition of ATP at dark 2 min to those started by addition of ATP at dark 10 s in the presence (——) and absence (– – –) of P_i were calculated from the data shown in fig.5A and B, respectively.

4. DISCUSSION

The decrease in the amount of active CF_0–CF_1 in the post-illumination dark has been suggested to be caused by the rebinding of ADP to the regulatory site on the active CF_0–CF_1 [4,5]. This suggestion was supported by the following result; lowering the chloroplast concentration, i.e., lowering the concentration of ADP which originated from endogenously bound ADP [11], decreased the rate of CF_0–CF_1 inactivation significantly (fig.1A).

Even if the active CF_0–CF_1 was maintained completely by lowering chloroplast concentration or by addition of P_i at dark 10 s, the enhancing effect of NH_4Cl on ATP hydrolysis [12] decreased rapidly with the dark time after illumination (figs 1,2). This result suggests that some energized state, which decreases with time in the dark, is required for NH_4Cl to increase the turnover rate of ATP hydrolysis and is not necessarily needed for the maintenance of active CF_0–CF_1 in the case where ADP-rebinding was suppressed.

The enhancing effect of NH_4Cl on ATP hydrolysis increased with the progress of ATP hydrolysis (fig.3). This suggests that some energized state (probably ΔpH) is produced in the progress of ATP hydrolysis [13,14] and enhances the effect of NH_4Cl. As the rate of ATP hydrolysis without NH_4Cl is constant throughout the reaction, it may be that presence of NH_4Cl enables active CF_0–CF_1 to utilize the energized state to increase the turnover rate of ATP hydrolyzing reaction.

In the case where not all CF_0–CF_1 was active, the rate of ATP hydrolysis increased with the progress of the reaction and the increase was significantly enhanced by P_i (figs 5,6). It is most probable that ATP hydrolysis on the active CF_0–CF_1 produces some energized state, which is utilized to energize inactive CF_0–CF_1. The activation of ATP hydrolysis and ATP–P_i exchange with the progress of ATP hydrolysis has been reported previously [15–18]. P_i has been shown to inhibit the rebinding of ADP to the active CF_0–CF_1 [7,8] and to enhance the release of ADP from the nonenergized CF_0–CF_1 [9]. P_i probably enhances the release of ADP from the CF_0–CF_1 which is energized through the ATP hydrolysis reaction and the release of ADP results in an active CF_0–CF_1. The P_i-enhanced activation of CF_0–CF_1 (fig.6) was much faster than the P_i-enhanced ADP release from nonenergized CF_0–CF_1 [9]. It may be that P_i-enhanced ADP release from the energized CF_0–CF_1 is much faster than that from the nonenergized CF_0–CF_1. In mitochondria, P_i has been reported to enhance ATP hydrolysis [19,20]. This enhancing effect of P_i may be connected with the above-mentioned effect of P_i.

REFERENCES

[1] Petrack, B., Craston, A., Sheppy, F. and Farron, F. (1965) J. Biol. Chem. 240, 906–914.
[2] Carmeli, C. and Lifshitz, Y. (1972) Biochim. Biophys. Acta 267, 86–95.
[3] Shoshan, V. and Selman, B.R. (1979) J. Biol. Chem. 254, 8801–8807.
[4] Bar-Zvi, D. and Shavit, N. (1980) FEBS Lett. 119, 68–72.
[5] Dunham, K.R. and Selman, B.R. (1981) J. Biol. Chem. 256, 212–218.
[6] Bar-Zvi, D. and Shavit, N. (1982) Biochim. Biophys. Acta 681, 451–458.
[7] Schumann, J. and Strotmann, H. (1981) in: Photosynthesis (Akoyunoglou, G. ed.) vol.2, pp.881–892, Balaban Int. Sci. Serv., Philadelphia.

[8] Dunham, K.R. and Selman, B.R. (1981) J. Biol. Chem. 256, 10044–10049.
[9] Komatsu-Takaki, M. (1984) FEBS Lett. 170, 121–124.
[10] Komatsu, M. and Murakami, S. (1976) Biochim. Biophys. Acta 423, 103–110.
[11] Harris, D.A. and Slater, E.C. (1975) Biochim. Biophys. Acta 387, 335–348.
[12] Carmeli, C. (1969) Biochim. Biophys. Acta 189, 256–266.
[13] Carmeli, C. (1970) FEBS Lett. 7, 297–300.
[14] Bakker-Grunwald, T. and Van Dam, K. (1973) Biochim. Biophys. Acta 292, 808–814.
[15] McCarty, R.E. and Racker, E. (1968) J. Biol. Chem. 243, 129–137.
[16] Bakker-Grunwald, T. and Van Dam, K. (1974) Biochim. Biophys. Acta 347, 290–298.
[17] Davenport, J.W. and McCarty, R.E. (1981) in: Photosynthesis (Akoyunoglou, G. ed.) vol.2, pp.859–865, Balaban Int. Sci. Serv., Philadelphia.
[18] Shahak, Y. and Pick, U. (1983) Arch. Biochem. Biophys. 223, 393–406.
[19] Mitchell, P. and Moyle, J. (1970) FEBS Lett. 9, 305–308.
[20] Bertina, R.M. and Slater, E.C. (1975) Biochim. Biophys. Acta 376, 492–504.