Inorganic phosphate-enhanced ADP release on the chloroplast coupling factor

Mizuho Komatsu-Takaki

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

Received 27 February 1984

The effect of P_i on the release of tightly bound ADP on the spinach chloroplast coupling factor was studied. P_i enhanced the release of tightly bound ADP in the dark with a half maximal effect at 2 mM. The pH optimum of the P_i-enhanced ADP release was above 8.0. The P_i-enhanced ADP release was above 8.0. The P_i-enhanced ADP release occurred on the same site as the light-enhanced ADP release. P_i and light-dependently bound ADP underwent no P_i-enhanced release in the following dark. This shows that the site on which P_i-enhanced ADP release occurs differs from the site on which P_i-dependent ADP binding occurs.

Tightly bound ADP *ADP release* *ADP binding* *P_i effect* *Chloroplast*

1. INTRODUCTION

Isolated chloroplasts contain tightly bound ADP on the coupling factor 1 [1,2] and under energized conditions they release the tightly bound ADP rapidly [1–5]. In the post-illumination dark, they rebind the released ADP [6].

P_i has been reported to inhibit the rebinding of ADP [7,8]. On the contrary, P_i has recently been shown to enhance the binding of ADP [9,10] under energized conditions [11].

Here, the effect of P_i to enhance the release of tightly bound ADP under the non-energized condition will be shown and its relation to the effect of P_i to bring about ADP binding [9–11] will be discussed.

2. MATERIALS AND METHODS

Chloroplasts were prepared from spinach leaves as in [11] and washed 4 times in a medium containing 0.1 M KCl and 2 mM Tricine–KOH (pH 7.2) to remove free adenine nucleotide and free P_i and finally suspended in the washing medium at a concentration equivalent to 3 mg chlorophyll (chl) per ml.

The adenine nucleotide release in the dark was measured as follows. Up to 3 ml of reaction medium containing 50 mM KCl, 25 mM Tricine–KOH (pH 8.3), 5 mM $MgCl_2$ and 10 mM P_i and 0.3 ml of chloroplast suspension was added. After incubation at 20°C for 0–30 min in the dark, the mixture was filtered by suction through two layers of Whatman GF/A and one layer of Toyo no.2 filter. The total amount of ATP, ADP and AMP in the filtrate was measured with the luciferin-luciferase method [12] with a Aminco Chem-Glow photometer.

3. RESULTS

As shown in fig.1, incubation of chloroplasts at room temperature resulted in the slow release of tightly bound adenine nucleotide even in the complete dark and addition of P_i enhanced the release significantly. The half maximal stimulation was given by 2 mM P_i (fig.2). With a similar effect,

Abbreviation: Tricine, *N*-tris(hydroxymethyl)methylglycine

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

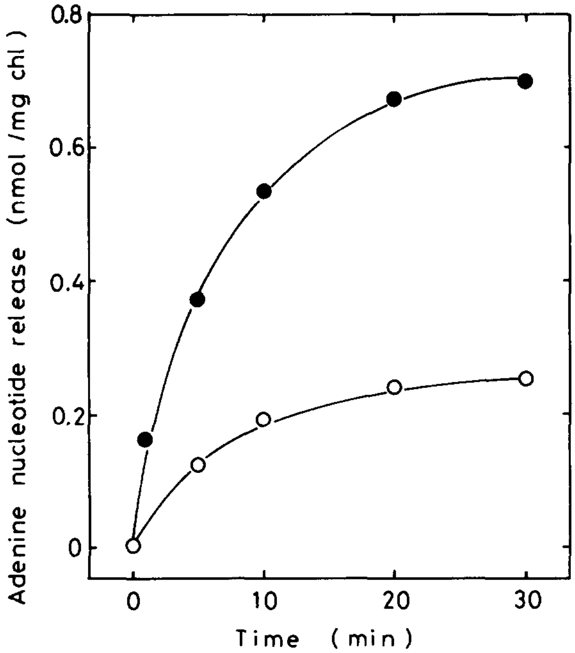

Fig.1. Adenine nucleotide release in the dark in the absence and presence of added P_i. Reaction conditions and experimental procedures were the same as those described in section 2 except that $MgCl_2$ was 3 mM and P_i was 0 (○) or 10 mM (●).

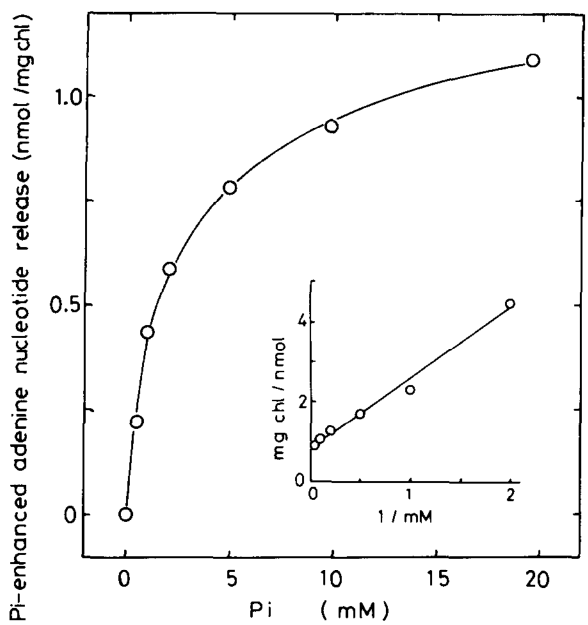

Fig.2. P_i concentration dependence of adenine nucleotide release in the dark. The experimental conditions were the same as those described in section 2 except for P_i concentration. Dark incubation time was 30 min and adenine nucleotide release in the absence of P_i was 0.3 nmol/mg chl.

arsenate also enhanced the adenine nucleotide release (not shown). Fig.3 shows the pH dependence of the adenine nucleotide release in the dark. Both in the absence and presence of added P_i, adenine nucleotide release occurs remarkably above pH 7.0 and the maximal release was observed above pH 8.0. Below pH 6.5 no release of adenine nucleotide was observed without added P_i. However, with 10 mM P_i, fairly large amounts of adenine nucleotide were seen to be released even below pH 6.5.

As is well known, illumination enhances the release of tightly bound ADP. The correlation between the light-enhanced adenine nucleotide release and the P_i-enhanced adenine nucleotide release was investigated. As shown in fig.4, illumination at the beginning of incubation brought about a rapid release of adenine nucleotide, which was followed by a slow release of adenine nucleotide in the following dark. Illumination after 20 min dark incubation with 20 mM P_i (at that time most of the tightly bound adenine nucleotide was already released) brought about a rapid release of adenine

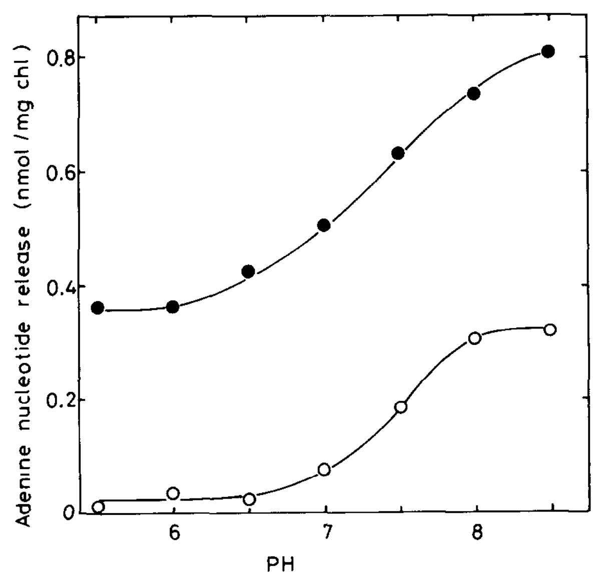

Fig.3. pH Dependence of adenine nucleotide release in the dark. Up to 3 ml of reaction medium containing 50 mM KCl, 15 mM Hepes, 15 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes), 5 mM $MgCl_2$ and 0 (○) or 10 mM (●) P_i at the pH indicated, 0.3 ml of chloroplast suspension was added and incubated for 30 min in the dark.

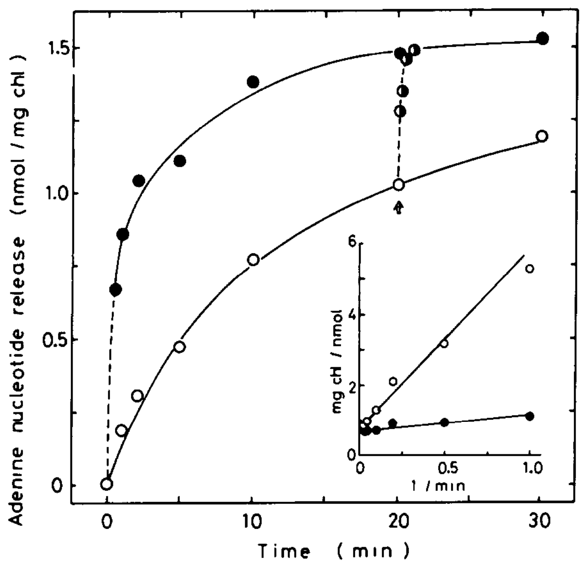

Fig.4. P_i-enhanced and light-enhanced adenine nucleotide release. Up to 3 ml of reaction medium containing 50 mM KCl, 25 mM Tricine–KOH (pH 8.3), 0.1 mM methyl viologen, 3 mM $MgCl_2$, 10 mM glucose, 20 mM P_i and 10 units/ml of hexokinase and 0.3 ml of chloroplast suspension was added. The mixture was either incubated in the dark for 0–30 min (○), illuminated for 1 min with white light (3×10^5 erg/cm^2 per s) and then incubated in the dark (●), or incubated in the dark for 20 min and then illuminated for 5–60 s (◑). After incubation, the mixture was filtered and the filtrate was heated at 90°C for 10 min. The content of adenine nucleotide in the heat-treated filtrate was measured as described in section 2.

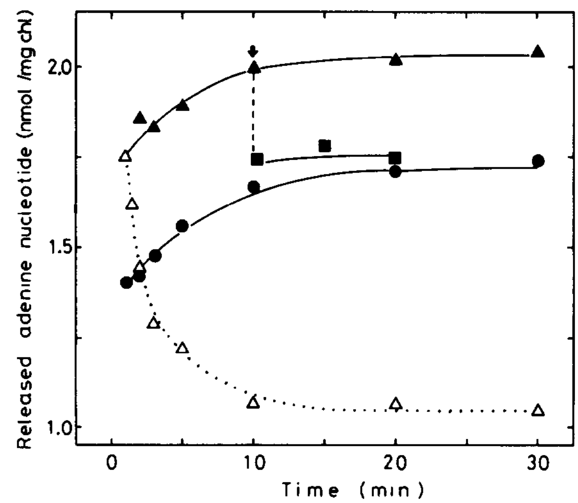

Fig.5. Adenine nucleotide release in the post-illumination dark. The component of reaction medium was the same as that shown in fig.4 except that hexokinase was 17 units/ml and P_i was 0 (△) or 20 mM (●). The reaction mixture was illuminated for 1 min and incubated in the dark. (▲) 10 s after turning the light off, 65 μl of 1 M P_i was added to the mixture which lacked P_i in the preillumination stage, and then incubated in the dark. At the time indicated, it was again illuminated for 10 s and further incubated in the dark (■). After filtration, the filtrate was heated for 10 min at 90°C.

nucleotide. But the amount was rather small compared to that obtained by illumination at the beginning of incubation. However, the sum of released adenine nucleotide brought about by 20 min dark incubation followed by 1 min illumination was equal to that brought about by 1 min illumination followed by 20 min dark incubation. Both of the double reciprocal plots of the released adenine nucleotide brought about by illumination followed by dark incubation with 20 mM P_i and by dark incubation only with 20 mM P_i against incubation time gave straight lines with the same intercept. These results suggest that P_i-enhanced adenine nucleotide release occurs on the same site as light-enhanced ADP release.

The amount of adenine nucleotide released by illumination depends on whether P_i is present or not during illumination; in the former case, P_i-dependent ADP binding occurs [11]. In the former case, adenine nucleotide release continued in the dark after illumination. In the latter case, not release, but rebinding occurred in the post-illumination dark as has been reported in [6]. However, addition of P_i at 10 s after turning the light off brought about release of adenine nucleotide in the dark after illumination (fig.5). The amount of adenine nucleotide released by P_i-containing light incubation followed by dark incubation was always smaller than that released by P_i-lacking light incubation followed by P_i-containing dark incubation by 0.3 nmol/mg chl and no decrease in the difference was observed even after 30 min incubation. However, 10 s illumination of the sample which underwent the latter treatment brought about rapid decrease in the amount of released adenine nucleotide to a level similar to that obtained by the former treatment. This shows that P_i and light-dependently bound

ADP does not release in the dark in the presence of P_i and that the site on which P_i-dependent ADP binding occurs differs from the site on which P_i-enhanced ADP release occurs.

4. DISCUSSION

P_i enhanced the release of tightly bound adenine nucleotide in the dark. As is well known, tightly bound ADP is released by illumination [1–4]. The adenine nucleotide binding site on which P_i-enhanced adenine nucleotide release occurs seems to be the same site as that on which light-enhanced ADP release occurs in the presence of P_i (fig.4). ADP bound on the site on which P_i-dependent ADP binding occurs was not released at all in the dark after illumination even in the presence of P_i at high concentration (fig.5). This shows that the site on which P_i-dependent ADP binding occurs differs from the site on which P_i-enhanced ADP release occurs. In the absence of P_i, illumination seems to bring about release of ADP located on both these different sites (fig.5).

P_i inhibits the rebinding of ADP in the post-illumination dark ([7,8] and fig.5). In relation to this inhibitory effect of P_i, the above-mentioned enhancing effect of P_i on ADP release is of particular interest. The rate of P_i-enhanced ADP release from the non-energized chloroplast (fig.1), however, is too small to explain the inhibitory effect of P_i on ADP rebinding in the post-illumination dark. With respect to this, research is now in progress.

Recently, authors in [13], during an experiment of ATP formation without energy, observed the production of '^{32}P contaminant' during dark incubation of chloroplasts in the presence of P_i at high concentrations under completely uncoupled conditions. Their ^{32}P contaminant production resembles the P_i-enhanced ADP release shown above in respect of pH dependence, time course, P_i concentration dependence and the lack of energy requirement. The ^{32}P contaminant most probably originates from the ADP released during dark incubation with P_i.

ACKNOWLEDGEMENT

The author wishes to thank Professor H. Strotmann for his critical reading of the manuscript.

REFERENCES

[1] Harris, D.A. and Slater, E.C. (1975) Biochim. Biophys. Acta 387, 335–348.

[2] Strotmann, H., Bickel, S. and Huchzermeyer, B. (1976) FEBS Lett. 61, 194–198.

[3] Bickel-Sandkötter, S. and Strotmann, H. (1976) FEBS Lett. 65, 102–106.

[4] Magnusson, R.P. and McCarty, R.E. (1976) J. Biol. Chem. 251, 7417–7422.

[5] Gräber, P., Schlodder, E. and Witt, H.T. (1977) Biochim. Biophys. Acta 461, 426–440.

[6] Strotmann, H. and Bickel-Sandkötter, S. (1977) Biochim. Biophys. Acta 460, 126–135.

[7] Dunham, K.R. and Selman, B.R. (1981) J. Biol. Chem. 256, 10044–10049.

[8] Schumann, J. and Strotmann, H. (1981) in: Photosynthesis (Akoyunoglou, G. ed) vol.2, pp.881–892, Balaban Int. Sci. Serv., Philadelphia.

[9] Shoshan, V. and Strotmann, H. (1980) J. Biol. Chem. 255, 996–999.

[10] Bickel-Sandkötter, S. (1983) Biochim. Biophys. Acta 723, 71–77.

[11] Komatsu-Takaki, M. (1983) J. Biochem. 94, 1095–1100.

[12] Stanley, P.E. and Williams, S.G. (1969) Anal. Biochem. 29, 381–392.

[13] Feldman, R.I. and Sigman, D.S. (1983) J. Biol. Chem. 258, 12178–12183.