Biochimica et Biophysica Acta, 423 (1976) 103–110

BBA 47020

INHIBITION OF PHOTOPHOSPHORYLATION BY ATP AND THE ROLE OF MAGNESIUM IN PHOTOPHOSPHORYLATION

MIZUHO KOMATSU and SATORU MURAKAMI

Department of Biology, College of General Education, University of Tokyo, Komaba, Meguro, Tokyo (Japan)

(Received June 30th 1975)

SUMMARY

ATP and pyrophosphate at high concentration (> 1 mM) inhibited photophosphorylation of isolated spinach chloroplasts in the normal salt medium and did not cause stimulation of electron transport. The inhibition of photophosphorylation by ATP or pyrophosphate was shown to be abolished by the addition of excess $MgCl_2$, ADP and phosphate. It has been demonstrated that the rates of photophosphorylation in the absence and presence of ATP or pyrophosphate are determined similarly by the concentrations of magnesium-ADP ($Mg \cdot ADP^-$) and magnesium-phosphate ($Mg \cdot P_i$) complexes.

It is highly probable that $Mg \cdot ADP^-$ and $Mg \cdot P_i$, but not free ADP and free phosphate, are the active form of the substrates of photophosphorylation. This is in support of the view that ATP inhibits photophosphorylation by decreasing the concentration of Mg^{2+} which is available for the formation of the complex with ADP and phosphate.

INTRODUCTION

It has been reported that ATP inhibits photophosphorylation in chloroplasts [1–4] and in chromatophores [5]. Shavit and Herscovici [1], using lettuce chloroplasts, studied the effect of ATP on photophosphorylation and electron transport. Under their experimental conditions, 50 % inhibition of photophosphorylation was obtained with 3 mM ATP. ATP added at high concentration as such inhibited coupled electron transport, whereas the basal electron transport was insensitive. These results led them to the view that ATP appears to affect the photosynthetic reactions as an energy transfer inhibitor. In a low salt medium, uncoupling of photophosphorylation by ATP at low concentration has been observed by Shavit [2] and Gross and Huffman [4]. Since the chloroplasts in the low salt medium are mostly depleted of cations and ATP has a high affinity to Mg^{2+}, it is reasonable to assume that under the conditions of low ionic strength, in the absence of excess Mg^{2+}, ATP causes uncoupling by chelating Mg^{2+} thus removing coupling factor [4]. The uncoupling effect of ATP at high concentration has not been observed in the normal salt medium. But we cannot ignore

the behavior of ATP as a chelator of Mg^{2+} in interpreting the effect of ATP on photophosphorylation. With this in mind, we have investigated the inhibitory effect of ATP on photophosphorylation under conditions of high ionic strength and found that the rate of photophosphorylation is limited by the concentrations of Mg · ADP and Mg-phosphate complexes. We shall discuss the results in terms of interaction of adenine nucleotides and phosphate with Mg^{2+}, leading to the view that ATP at high concentration inhibits photophosphorylation as a chelator of Mg^{2+}, but not as an energy transfer inhibitor. A possible participation of Mg · ADP and Mg-phosphate complexes as the substrates of photophosphorylation is also discussed.

MATERIALS AND METHODS

Fresh leaves of spinach obtained from a local market were homogenized for 15 s in a Waring blendor with a medium containing 0.4 M sucrose, 10 mM KCl and 10 mM Tricine-KOH (pH 7.8). The chloroplasts were isolated by centrifugation at $1000 \times g$ for 7 min, washed twice in the same medium by centrifugation at $3000 \times g$ for 5 min and finally suspended in the isolation medium at a chlorophyll concentration of 5 mg per ml. The chlorophyll concentration was measured by the method of Arnon [6] using the nomogram presented by Kirk [7].

Photophosphorylation was measured according to the method of Avron [8]. The reaction mixture contained in a volume of 1.07 ml, 0.1 M KCl, 20 mM Tricine-KOH or 20 mM HEPES · KOH (pH 8.0), 0.2 mM ADP, 3 mM potassium phosphate, 2 mM $MgCl_2$, 60 μM methyl viologen, 10 μCi $^{32}P_i$ and chloroplasts equivalent to 30 μg of chlorophyll. When necessary the concentrations of ADP, P_i and $MgCl_2$ were varied as indicated in each experiment. Actinic light, from a 650 watt halogen lamp, was passed through 6 cm of water layer and Hoya cut-off filter (R64). The light intensity was $3 \cdot 10^5$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. After illumination for 5, 10 and 20 s, the reaction was stopped by addition of 50 μl of 60 % trichloroacetic acid and the reaction mixture was centrifuged. Aliquots of the supernatant were assayed for esterified $^{32}P_i$.

Adenine nucleotides (ADP and ATP), phosphate and pyrophosphate form complex with Mg^{2+} by the following reversible reactions at pH 8.0 (20 °C):

$$ADP^{3-} + Mg^{2+} \rightleftharpoons MgADP^-, \quad K = 10^{3.11}\ M^{-1}$$
$$ATP^{4-} + Mg^{2+} \rightleftharpoons MgATP^{2-}, \quad K = 10^{4.00}\ M^{-1}$$
$$HPO_4^{2-} + Mg^{2+} \rightleftharpoons MgHPO_4, \quad K = 10^{1.62}\ M^{-1}$$
$$P_2O_7^{4-} + Mg^{2+} \rightleftharpoons MgP_2O_7^{2-}, \quad K = 10^{5.41}\ M^{-1}$$
$$MgP_2O_7^{2-} + Mg^{2+} \rightleftharpoons Mg_2P_2O_7, \quad K = 10^{2.34}\ M^{-1}$$
$$HP_2O_7^{3-} + Mg^{2+} \rightleftharpoons MgHP_2O_7^-, \quad K = 10^{3.06}\ M^{-1}$$
$$P_2O_7^{4-} + H^+ \rightleftharpoons HP_2O_7^{3-}, \quad K = 10^{8.93}\ M^{-1}$$

The values for the association constants (K) presented above were taken from the literature [9–12]. Using these association constants and the conservation equations of adenine nucleotides, phosphate, pyrophosphate and magnesium, the concentrations of magnesium-ADP complex ($Mg \cdot ADP^-$) and magnesium-phosphate complex ($Mg \cdot P_i$) were calculated. In the case where Tricine was used as a buffer, the binding of Mg^{2+} to Tricine was also considered by using the value of $10^{1.2}\ M^{-1}$ as an associa-

tion constant [13]. The thylakoid membrane system has a rather high affinity to Mg^{2+}, but the size of binding site [14] is so much smaller than the $MgCl_2$ concentration added that the binding of Mg^{2+} to the thylakoid membrane can be excluded from our consideration.

ADP and ATP were purchased from Boehringer, Mannheim.

RESULTS

Inhibitory effect of ATP and the phosphorylation reagents

The effect of ATP on photophosphorylation is shown in Fig. 1. 50 % inhibition was observed at 8 mM ATP under our experimental conditions. ATP in the same concentration range inhibited the coupled electron transport and had only a slight effect on the basal electron transport as reported by Shavit and Herscovici [1]. A similar effect was observed with pyrophosphate at a concentration comparable to that for ATP (Fig. 1). ADP also inhibited the photophosphorylation but it was less effective than ATP: as high as 20 mM, ADP inhibited only 30 % of the phosphorylation activity in the presence of 2 mM $MgCl_2$ but ATP did so by nearly 80 %.

Fig. 2 shows the inhibitory effect of 5 mM ATP on photophosphorylation in the presence of various amounts of $MgCl_2$. It is noticeable that the inhibitory effect of ATP was marked at a $MgCl_2$ concentration lower than 1 mM but was overcome completely when the $MgCl_2$ concentration was increased up to 10 mM. Inhibition of photophosphorylation by 5 mM pyrophosphate was also overcome by 10 mM $MgCl_2$.

Fig. 3 shows the inhibitory effect of 3.5 mM pyrophosphate on photophosphor-

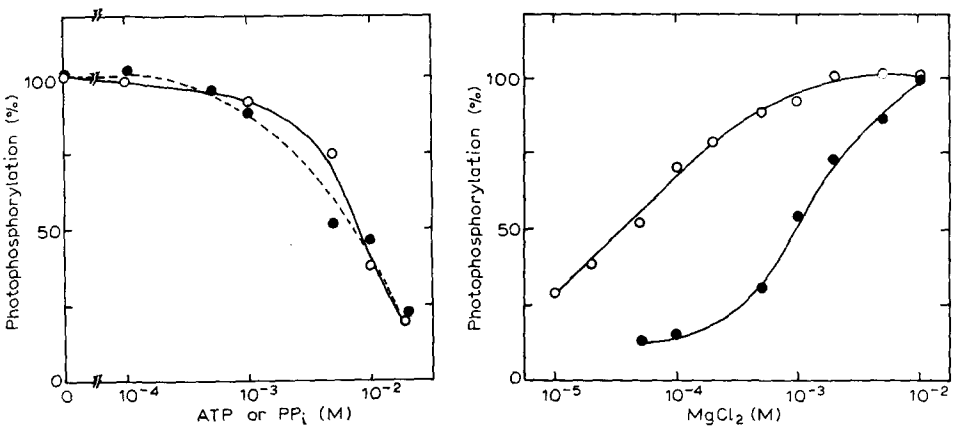

Fig. 1. Inhibition of photophosphorylation by ATP and pyrophosphate. The reaction conditions were as described in Materials and Methods. Buffer used was 20 mM Tricine-KOH (pH 8.0). Indicated concentrations of ATP (○ — ○) or sodium pyrophosphate (● — ●) were added. Each point was the average of three separate experiments. Control activities were 70–120 μmol $P_i \cdot mg^{-1}$ chlorophyll $\cdot h^{-1}$.

Fig. 2. Effect of ATP on the rates of photophosphorylation in the presence of various amounts of $MgCl_2$. The reaction conditions were the same as in Fig. 1 except that $MgCl_2$ concentrations were varied as indicated. Each point was the average of three separate experiments. The rates at 100 % activity were 80–110 μmol $P_i \cdot mg^{-1}$ chlorophyll $\cdot h^{-1}$. (○ — ○), no addition; (● — ●), 5 mM ATP was added.

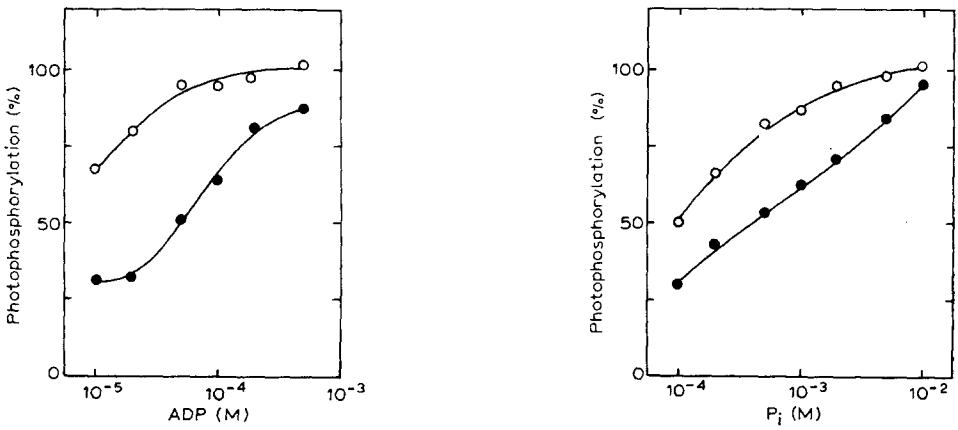

Fig. 3. Effect of pyrophosphate on the rates of photophosphorylation in the presence of various amounts of ADP. The reaction mixture contained 0.1 M KCl, 20 mM HEPES · KOH (pH 8.0), 20 mM potassium phosphate, 3 mM $MgCl_2$, 60 μM methyl viologen and where indicated, 3.5 mM sodium pyrophosphate. ADP concentration was varied as indicated. Other conditions were as described in Materials and Methods. Each point was the average of three separate experiments. The rates at 100 % activity were 140–180 μmol $P_i \cdot mg^{-1}$ chlorophyll $\cdot h^{-1}$. (○ – ○), no addition; (● – ●), 3.5 mM sodium pyrophosphate was added.

Fig. 4. Effect of ATP on the rates of photophosphorylation in the presence of various amounts of phosphate. The reaction mixture contained 0.1 M KCl, 20 mM HEPES–KOH (pH 8.0), 1 mM ADP, 5 mM $MgCl_2$ and 60 μM methyl viologen. Potassium phosphate concentration was varied as indicated. Other conditions were as described in Materials and Methods. Each point was the average of three separate experiments. The rates at 100 % activity were 70–100 μmol $P_i \cdot mg^{-1}$ chlorophyll $\cdot h^{-1}$. (○ – ○), no addition; (● – ●), 5 mM ATP was added.

ylation in the presence of various amounts of ADP. Pyrophosphate inhibited more than 50 % of the control in the presence of 50 μM or less ADP. However, the activity of photophosphorylation was restored to nearly 90 % of the control by increasing the concentration of ADP up to 0.5 mM. (For this experiment, ATP was not used since ATP available from commercial sources contained 2–3 % ADP.)

The inhibitory effect of ATP on photophosphorylation activity was also found to be affected by the phosphate concentration in the reaction mixture (Fig. 4). The phosphorylation activity was significantly inhibited by 5 mM ATP in the lowest range of phosphate concentration employed. However, the inhibition was decreased with the increase of phosphate concentration and became almost negligible in the presence of 10 mM phosphate.

Photophosphorylation and $Mg \cdot ADP^-$ and $Mg \cdot P_i$ complexes

Since ATP has a high affinity to Mg^{2+}, forming $MgATP^{2-}$, it decreases the concentration of free Mg^{2+} in the reaction mixture. ADP and phosphate also form complexes, $Mg \cdot ADP^-$ and $Mg \cdot P_i$. Thus ATP (or pyrophosphate) added to the reaction mixture at high concentration reduces the amount of $MgADP^-$ and MgP_i through reducing the free Mg^{2+} concentration in the reaction mixture. As shown above, photophosphorylation activity inhibited by ATP (or pyrophosphate) was restored by a sufficient amount of $MgCl_2$, ADP and phosphate. This suggests that the recovery of photophosphorylation activity by increasing the concentrations of phos-

phorylation reagents was due to increase in the concentrations of $Mg \cdot ADP^-$ and $Mg \cdot P_i$ which may be active forms of the substrates for photophosphorylation. If this is the case, the activity of photophosphorylation must depend on either or both $Mg \cdot ADP^-$ and $Mg \cdot P_i$ concentrations similarly in the presence and absence of ATP (or pyrophosphate).

Using the conservation equations and the association constants found in Materials and Methods, the concentrations of $Mg \cdot P_i$ in the experiments presented in Fig. 3 were calculated to be 1.3–1.2 mM in the absence of pyrophosphate and 96–90 μM in the presence of pyrophosphate. Assuming the $Mg \cdot P_i$ concentration to be sufficient in both cases, photophosphorylation in the absence and presence of pyrophosphate would depend on the concentration of $Mg \cdot ADP^-$ in a similar way. The rates of photophosphorylation presented in Fig. 3 were replotted against the concentrations of $Mg \cdot ADP^-$ in the reaction mixture (Fig. 5). It should be noted that, in fact, the activities in the absence and presence of 3.5 mM pyrophosphate show a similar dependence on the concentrations of $Mg \cdot ADP^-$. $Mg \cdot ADP^-$ saturated the activity at 0.1 mM and gave 50 % activity at 4.5 μM. A small difference between the activities in the absence and presence of pyrophosphate might be due to the difference of $Mg \cdot P_i$ concentration: the concentration of $Mg \cdot P_i$ in the presence of pyrophosphate was not sufficient for maximum activity (Fig. 6).

The concentrations of $Mg \cdot ADP^-$ in the experiments shown in Fig. 4 were 0.85–0.80 mM in the absence of ATP and 0.38–0.35 mM in the presence of 5 mM ATP. It is evident from Fig. 5 that the concentration of $Mg \cdot ADP^-$ was not limiting

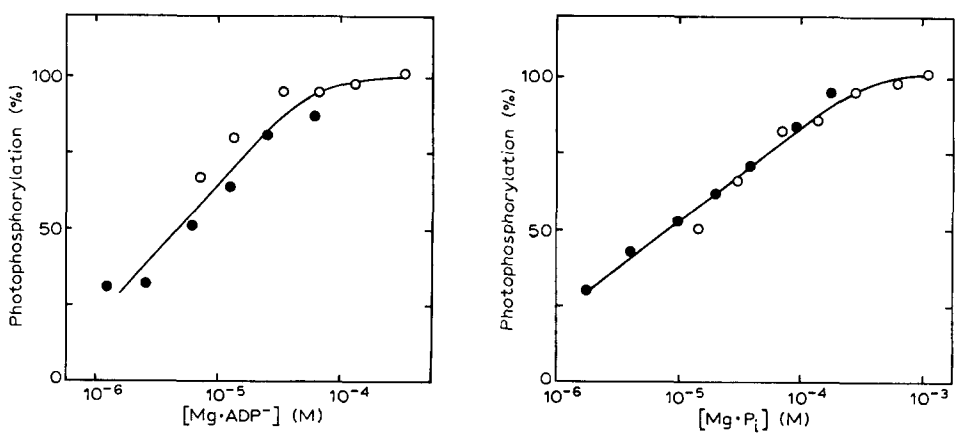

Fig. 5. Dependence of the rates of photophosphorylation on $Mg \cdot ADP^-$ concentrations. The data shown in Fig. 3 were replotted against the concentrations of $Mg \cdot ADP^-$, which were calculated as described in Materials and Methods. (○—○), no addition; (●—●), 3.5 mM sodium pyrophosphate was added. In the absence of pyrophosphate, the concentration of $Mg \cdot P_i$ varied from 1.3 mM to 1.2 mM with the concentrations of ADP added (10 μM–0.5 mM), whereas in the presence of pyrophosphate it varied from 96 μM to 90 μM.

Fig. 6. Dependence of the rates of photophosphorylation on $Mg \cdot P_i$ concentrations. The data shown in Fig. 4 were replotted against MgPi concentrations. (○—○), no addition; (●—●), 5 mM ATP was added. In the absence of ATP, the concentration of $Mg \cdot ADP^-$ varied from 0.85 mM to 0.80 mM with the concentrations of P_i added (0.1–10 mM), whereas in the presence of ATP it varied from 0.38 mM to 0.35 mM.

the rate of reaction in this experiment. In Fig. 6 the rates of photophosphorylation presented in Fig. 4 were replotted against the concentrations of $Mg \cdot P_i$ in the reaction mixture. In this case also, the rates of photophosphorylation in the absence and presence of 5 mM ATP were dependent on the concentrations of $Mg \cdot P_i$ in exactly the same manner. $Mg \cdot P_i$ saturated the activity at 0.4 mM and gave 50 % activity at 9 μM.

In the experiments presented in Fig. 1, the concentrations of $Mg \cdot ADP^-$ and $Mg \cdot P_i$ might be insufficient for maximum activity of photophosphorylation in the concentration range of ATP above 1 mM, thus limiting the rate of photophosphorylation. In the presence of ATP above 1 mM, the concentrations of $Mg \cdot ADP^-$ and $Mg \cdot P_i$ were found to be less than 0.1 mM. There is a similar situation in the experiments for Fig. 2. For the experiments with 5 mM ATP, the concentrations of $Mg \cdot ADP^-$ and $Mg \cdot P_i$ were both less than 0.1 mM in the concentration range of $MgCl_2$ below 6 mM and for the experiments without ATP, both $Mg \cdot ADP^-$ and $Mg \cdot P_i$ concentrations were below 0.1 mM in the concentration range of $MgCl_2$ below 1 mM. Under such experimental conditions the photophosphorylation rate must be a function of both $Mg \cdot ADP^-$ and $Mg \cdot P_i$ concentrations. The relation between the concentrations of $Mg \cdot ADP^-$ and $Mg \cdot P_i$ and the rate of photophosphorylation (v) can be shown by the following equation:

$$v = k[Mg \cdot ADP^-]^a \cdot [Mg \cdot P_i]^b \quad \text{(where } k \text{ is a constant)}$$

The value of a was obtained from the data presented in Fig. 5, where $Mg \cdot ADP^-$ concentration was limiting the rate while $Mg \cdot P_i$ concentration was not. Plotting the logarithm of photophosphorylation rates against the logarithm of $Mg \cdot ADP^-$ concentrations gave a straight line with a slope of 0.41 and therefore a was determined to be 0.41. By using the data presented in Fig. 6, where the $Mg \cdot P_i$ concentration limited the rate while $Mg \cdot ADP^-$ concentration was not limiting it, the value of b was also determined as 0.23. The rates of photophosphorylation presented in Fig. 1

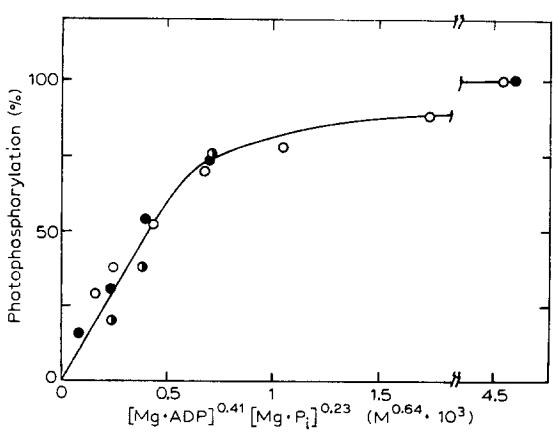

Fig. 7. Dependence of the rates of photophosphorylation on $Mg \cdot ADP^-$ and $Mg \cdot P_i$ concentrations. The data shown in Figs. 1 and 2 were replotted against the product of $[Mg \cdot ADP^-]^{0.41}$ and $[Mg \cdot P_i]^{0.23}$. Full explanation is given in the text. (◑—◑), various amounts of ATP were added (experiments presented in Fig. 1). (○—○) and (●—●), various amounts of $MgCl_2$ were added in the absence and presence of 5 mM ATP, respectively (experiments presented in Fig. 2).

and Fig. 2 were replotted against $[Mg \cdot ADP^-]^{0.41}[Mg \cdot P_i]^{0.23}$ (Fig. 7). The rate of photophosphorylation in the presence of varied concentrations of ATP (experiments presented in Fig. 1) and the rates in the presence of varied amounts of $MgCl_2$ with or without ATP (experiments presented in Fig. 2) were strictly dependent on both the concentrations of $Mg \cdot ADP^-$ and $Mg \cdot P_i$ in a similar manner. The rate of photophosphorylation was linearly proportional to the product of $[Mg \cdot ADP^-]^{0.41}$ and $[Mg \cdot P_i]^{0.23}$ in the concentration range of less than $5 \cdot 10^{-4}\ M^{0.64}$. This result shows that photophosphorylation is directly supported by the concentrations of $Mg \cdot ADP^-$ and $Mg \cdot P_i$, but not free ADP and free phosphate.

DISCUSSION

In this study we confirmed the inhibitory effect of ATP on photophosphorylation which has been reported by Shavit and Herscovici [1] with lettuce chloroplasts and Horio et al. [5] with *Rhodospirillum rubrum* chromatophores. The inhibition was observed only at higher concentrations of ATP than 1 mM under photophosphorylation conditions in normal salt medium. Thus the inhibitory effect of ATP studied here apparently differs from the effect of ATP at much lower concentrations (1–10 μM) on electron transport under non-phosphorylating conditions [15] and on light-induced formation of a proton gradient [16–18]. These effects of ATP at low concentration are possibly due to alteration of the proton permeability of thylakoid membranes, perhaps through binding of ATP to the functional site of the coupling factor [16]. It has been also reported that ATP added at low concentration stimulates electron transport and inhibits photophosphorylation of the chloroplasts in a low ionic strength medium [2, 4]. A possible model to interpret this uncoupling effect of ATP was given by Gross and Huffman [4]. Taking account of a strong chelating action of ATP to Mg^{2+} and a low Mg^{2+} content in the reaction mixture, they proposed that ATP caused uncoupling of photophosphorylation at low ionic strength medium by chelating Mg^{2+}, accompanying the release of coupling factor from the thylakoid membranes.

The circumstances encountered in the experiments presented here are different from those in a low salt medium. The inhibition of photophosphorylation caused by ATP or pyrophosphate at high concentration was not accompanied by stimulation of electron transport and was abolished by the addition of excess amount of $MgCl_2$. ADP or phosphate were also effective in abolishing ATP-induced inhibition of photophosphorylation. Considering the high affinity of ATP as a chelator to Mg^{2+}, these results strongly suggest that ATP inhibits the activity of photophosphorylation by decreasing the concentration of Mg^{2+} which are in some way involved in the phosphorylation reaction. Magnesium ions are believed to participate in the terminal steps of photophosphorylation as an essential requirement [19]. They are also required for stabilization of coupling factor [20, 21] and affect high-energy states of the thylakoid membranes [19, 22]. The former of these seems to be concerned with the present study. Since, as is shown here, the rates of photophosphorylation in the absence and presence of ATP at high concentration are determined in a similar manner by the concentrations of $Mg \cdot ADP$ and Mg-phosphate complexes, it can be concluded that $Mg \cdot ADP$ and Mg-phosphate complexes, but not free ADP and free phosphate, are the substrates for the reaction of photophosphorylation.

It has been noted that for maximum activity of photophosphorylation much more phosphate is required than the amount necessary for esterification of ADP [8, 23]. This can be understood from the fact that phosphate has a much lower affinity to Mg^{2+} than ADP, being in support of the model proposed for the role of magnesium in photophosphorylation.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Dr. Sakae Katoh for his helpful discussions.

REFERENCES

1 Shavit, N. and Herscovici, A. (1970) FEBS Lett. 11, 125–128
2 Shavit, N. (1972) Proc. 2nd Int. Congr. Photosynth. Res. (Forti, G., Avron, M. and Melandri, A. eds), Vol. 2, pp. 1221–1231, W. Junk, The Hague
3 Walz, D., Schuldiner, S. and Avron, M. (1971) Eur. J. Biochem. 22, 439–444
4 Gross, E. L. and Huffman, R. (1972) Biochem. Biophys. Res. Commun. 47, 260–266
5 Horio, T., Yamashita, J. and Nishikawa, K. (1963) Biochim. Biophys. Acta 66, 37–49
6 Arnon, D. I. (1949) Plant Physiol. 24, 1–15
7 Kirk, J. T. O. (1968) Planta 78, 200–207
8 Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272
9 Martell, A. E. and Schwarzenbach, G. (1956) Helv. Chim. Acta 39, 653–661
10 Tabor, H. and Hastings, A. B. (1943) J. Biol. Chem. 148, 627–632
11 Lambert, S. M. and Watters, J. I. (1957) J. Am. Chem. Soc. 79, 4262–4265
12 Lambert, S. M. and Watters, J. I. (1957) J. Am. Chem. Soc. 79, 5606–5608
13 Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S. and Singh, R. M. M. (1966) Biochem. 5, 467–477
14 Gross, E. L. and Hess, S. C. (1974) Biochim. Biophys. Acta 339, 334–346
15 Avron, M., Krogmann, D. W. and Jagendorf, A. T. (1958) Biochim. Biophys. Acta 30, 144–153
16 McCarty, R. E., Fuhrman, J. S. and Tsuchiya, Y. (1971) Proc. Natl. Acad. Sci. U.S. 68, 2522–2526
17 Telfer, A. and Evans, M. C. W. (1972) Biochim. Biophys. Acta 256, 625–637
18 Portis, A. R. J. and McCarty, R. E. (1974) J. Biol. Chem. 249, 6250–6254
19 Shavit, N. and Avron, M. (1967) Biochim. Biophys. Acta 131, 516–525
20 Jagendorf, A. T. and Smith, M. (1962) Plant Physiol. 37, 135–141
21 Avron, M. (1963) Biochim. Biophys. Acta 77, 699–702
22 Gross, E., Dilley, R. A. and San Pietro, A. (1969) Arch. Biochem. Biophys. 134, 450–462
23 Jagendorf, A. T. and Avron, M. (1958) J. Biol. Chem. 231, 279–290