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UPTAKE OF ATP ANALOGS BY ISOLATED PEA CHLOROPLASTS AND THEIR EFFECT ON CO_2 FIXATION AND ELECTRON TRANSPORT

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SUMMARY

1. The ATP analog, adenylyl-imidodiphosphate rapidly inhibited CO_2-dependent oxygen evolution by isolated pea chloroplasts. Both α,β- and β,γ-methylene adenosine triphosphate also inhibited oxygen evolution. The inhibition was relieved by ATP but only partially relieved by 3-phosphoglycerate. Oxygen evolution with 3-phosphoglycerate as substrate was inhibited by adenylyl-imidodiphosphate to a lesser extent than CO_2-dependent oxygen evolution. The concentration of adenylyl-imidodiphosphate required for 50 % inhibition of CO_2-dependent oxygen evolution was 50 μM.

2. Although non-cyclic photophosphorylation by broken chloroplasts was not significantly affected by adenylyl-imidodiphosphate, electron transport in the absence of ADP was inhibited by adenylyl-imidodiphosphate to the same extent as by ATP, suggesting binding of the ATP analog to the coupling factor of phosphorylation.

3. The endogenous adenine nucleotides of a chloroplast suspension were labelled by incubation with [^{14}C]ATP and subsequent washing. Addition of adenylyl-imidodiphosphate to the labelled chloroplasts resulted in a rapid efflux of adenine nucleotides suggesting that the ATP analog was transported into the chloroplasts via the adenine nucleotide translocator.

4. It was concluded that uptake of ATP analogs in exchange for endogenous adenine nucleotides decreased the internal ATP concentration and thus inhibited CO_2 fixation. Oxygen evolution was inhibited to a lesser extent in spinach chloroplasts which apparently have lower rates of adenine nucleotide transport than pea chloroplasts.

INTRODUCTION

The majority of research on intact isolated chloroplasts (i.e. capable of high rates of CO_2 fixation) has utilized chloroplasts isolated from spinach leaves. Isolated spinach chloroplasts retain sufficient endogenous adenine nucleotides to maintain high rates of CO_2 fixation in the absence of added cofactors [1]. Although there is evidence for transfer of adenine nucleotides between chloroplast and cytosol in vivo [1, 2], exogenous adenine nucleotides do not rapidly penetrate the outer membranes of isolated spinach chloroplasts [1, 3–5]. In contrast, chloroplasts isolated from young pea shoots are affected by exogenous adenine nucleotides suggesting that the rate of adenine nucleotide translocation is higher in these chloroplasts [6–9]. Inorganic pyrophosphate acts as an analog of ATP in that it is transported into pea chloroplasts in exchange for endogenous adenine nucleotides. The resulting deficiency of internal adenine nucleotides causes a strong inhibition of CO_2 fixation. The inhibitory effect of PP_i was reversed by catalytic amounts of adenine nucleotides [8, 9] suggesting that the adenine nucleotide transporter has a much higher affinity for adenine nucleotides than for PP_i. Because of this low affinity for PP_i, inhibition of CO_2 fixation by PP_i requires relatively high concentrations and the response to added PP_i is often slow. In addition, the inorganic pyrophosphatase present in chloroplast preparations can hydrolyse added PP_i to yield P_i which in turn may stimulate or inhibit CO_2 fixation depending on the concentration [8].

For these reasons, we have investigated analogs of ATP which may achieve the same result as PP_i but at lower concentrations and without the complicating effects of PP_i hydrolysis. Adenylyl-imidodiphosphate differs from ATP only in that the β, γ-oxygen bridge of the triphosphate chain is replaced by an imido group [10]. Adenylyl-imidodiphosphate is transported by the mitochondrial adenine nucleotide translocator but is not hydrolysed by the mitochondrial ATPase [10, 11]. Similarly, methylene substitution between the α,β-phosphates (α,β-methylene ATP) or between the β,γ-phosphates (β,γ-methylene ATP) yields analogs which are transported into mitochondria but are not metabolised [12]. Diadenosine (5′)-pentaphosphate is a powerful inhibitor of adenylate kinase, which involves the binding of two adenine nucleotides, but is without effect on pyruvate kinase, hexokinase and fructose-6-phosphate kinase [13]. We have found that both the imidophosphate and methylene phosphonic analogs of ATP produce similar effects to PP_i with isolated pea chloroplasts but act more rapidly and require lower concentrations.

MATERIALS AND METHODS

Pea seedlings (*Pisum sativum* cv. Massey Gem) were grown in vermiculite for 11–12 days in a glasshouse. Bovine serum albumin was obtained from the Commonwealth Serum Laboratories (Melbourne, Australia). Adenylyl-imidodiphosphate was obtained from Boehringer and Soehne (Mannheim, Germany) and all other biochemicals from the Sigma Chemical Co. (Saint Louis, U.S.A.).

Pea shoots (60–80 g) were ground in a Polytron blendor for 2–3 s in 200 ml of ice-cold medium containing 400 mM sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM $NaNO_3$, 20 mM NaCl, 0.5 mM P_i, 2 mM isoascorbate, 50 mM 2-(*N*-morpholino)-ethanesulphonic acid (MES) buffer and 0.4 % bovine serum albumin, adjusted to pH 6.2. The brei was squeezed through a double layer of miracloth containing a layer of cotton wool and the filtrate was centrifuged at 2000 × *g* for 30 s in an MSE Super Minor centrifuge. The chloroplast pellet was rinsed with a medium containing 400 mM sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$ 2 mM $NaNO_3$, 20 mM NaCl, 0.5 mM P_i, 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulphonic acid (HEPES) buffer and 0.4 % bovine serum albumin, adjusted to pH 6.7. The pellet was resuspended in 1 ml of the same medium using a glass rod wrapped with cotton wool. All procedures were carried out at 2° C using chilled solutions and apparatus.

Oxygen evolution was measured with a Rank oxygen electrode connected to a Rikadenki B-261 recorder. The vessel was illuminated with a slide projector giving a light intensity of $2 \cdot 10^5$ ergs $\cdot$ $cm^{-2} \cdot s^{-1}$ at the centre of the vessel. The vessel was maintained at 20 °C. Chlorophyll was determined by the method of Arnon [14].

Back-exchange of adenine nucleotides was measured according to the method of Heldt [3, 8]. The adenine nucleotide pool of the chloroplasts was labelled by incubation of the chloroplasts (1.5 mg chlorophyll/ml) for 60 min at 2 °C in a medium containing 330 mM sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 0.4 % bovine serum albumin, 20 mM Tricine buffer (pH 8.4) and 0.2 mM [^{14}C]ATP (20 Ci/mol). The chloroplasts were washed twice and resuspended in the above medium minus ATP. For assay of back-exchange, chloroplasts (80 μg chlorophyll) were added to 2 ml of a medium containing 330 mM sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$ and 50 mM HEPES (pH 7.6). The suspension was maintained at 20 °C and illuminated as for oxygen evolution measurements. Back-exchange was started by the addition of ATP or adenylyl-imidodiphosphate. Two samples (0.2 ml) were withdrawn at various time intervals and one was immediately centrifuged in a Beckman model 152 Microfuge for 1 min. Aliquots (0.1 ml) of this supernatant and of the untreated chloroplast suspension were added to 10 ml of scintillation fluid (toluene : Brydet, 2 : 1, plus 0.4 % PPO plus 0.02 % POPOP) and counted in a liquid scintillation counter (Packard). Release of labelled nucleotides was calculated from the appearance of ^{14}C label in the supernatant.

RESULTS

The effect of adenylyl-imidodiphosphate on CO_2-dependent oxygen evolution by pea chloroplasts is shown in Fig. 1. If ATP was included in the reaction medium from the outset, adenylyl-imidodiphosphate did not significantly affect the rate of oxygen evolution (Fig. 1A). In the absence of added ATP, addition of adenylyl-imidodiphosphate caused a rapid inhibition of oxygen evolution which was reversed by subsequent addition of ATP (Fig. 1B). The rate was further stimulated by 3-phosphoglycerate (Fig. 1B). 3-Phosphoglycerate gave only partial relief of adenylyl-imidodiphosphate inhibition but the control rate was recovered after subsequent ATP addition (Fig. 1C). Thus the effects of adenylyl-imidodiphosphate on CO_2-dependent oxygen evolution were similar to those of PP_i except that adenylyl-imidodiphosphate inhibited oxygen evolution more rapidly than PP_i [8]. The time taken for maximum inhibition by adenylyl-imidodiphosphate was usually less than 0.3 min whereas maximum inhibition by PP_i required at least 2–3 min. In addition, the relief of adenylyl-imidodiphosphate inhibition by ATP occurred more rapidly than relief of PP_i inhibition. The partial relief of adenylyl-imidodiphosphate inhibition by 3-phosphoglycerate contrasts with PP_i inhibition which was not relieved by 3-phosphoglycerate [8].

The effect of adenylyl-imidodiphosphate on oxygen evolution with different substrates is shown in Table I. With CO_2 or CO_2+ribose 5-*P* as substrates, oxygen evolution was inhibited 75–80 % by adenylyl-imidodiphosphate. 3-Phosphoglycerate-

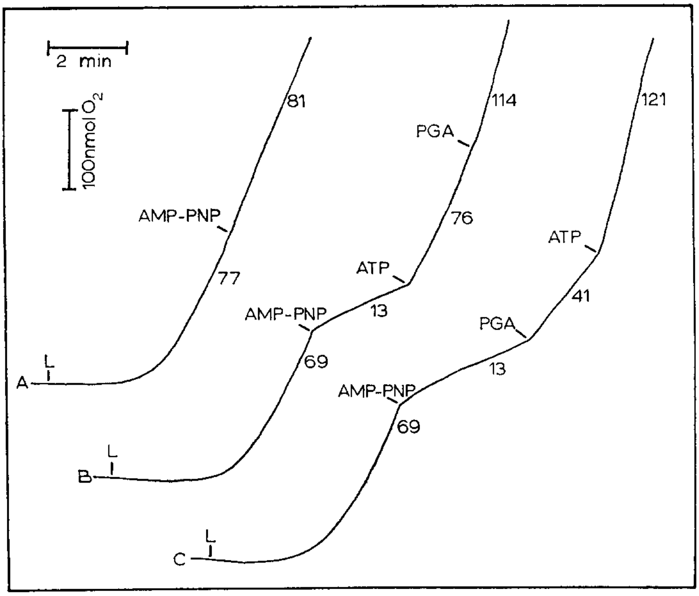

Fig. 1. Oxygen electrode traces showing the effect of adenylyl-imidodiphosphate on CO_2-dependent oxygen evolution by pea chloroplasts. Oxygen evolution was assayed in a medium containing 400 mM sorbitol, 1 mM $MgCl_2$, 1mM $MnCl_2$, 2 mM EDTA, 50 mM HEPES (pH 7.6), 4 mM $NaHCO_3$, 0.2 mM P_i and chloroplasts equivalent to 97 μg chlorophyll in a final volume of 2.2 ml. Additions were ATP (0.8 mM), adenylyl-imidodiphosphate (0.7 mM) and 3-phosphoglycerate (1 mM) as indicated. Trace A contained ATP from the outset. Numbers along the traces indicate rates of oxygen evolution in μmol O_2/mg chlorophyll per h. AMP-PNP, adenylyl-imidodiphosphate; PGA, 3 phosphoglycerate.

TABLE I

EFFECT OF ADENYLYL-IMIDODIPHOSPHATE ON OXYGEN EVOLUTION BY PEA CHLOROPLASTS WITH DIFFERENT SUBSTRATES

Oxygen evolution was measured as described in Fig. 1. Substrates were 2 mM except for $NaHCO_3$ which was 4 mM. NH_4Cl was 11 mM where indicated. After an initial steady rate of oxygen evolution was achieved, adenylyl-imidodiphosphate was added to a final concentration of 0.6 mM. When a new steady rate of oxygen evolution had been obtained, ATP was added to a final concentration of 0.8 mM.

Substrate	Rate of oxygen evolution (μmol O_2/mg chlorophyll per h)			Inhibition by adenylyl-imidodiphosphate (%)
	Initial rate	+Adenylyl-imidodiphosphate	+ATP	
CO_2	69	13	76	81
CO_2+ribose-5-*P*	105	25	116	76
3-Phosphoglycerate	107	53	133	50
Oxaloacetic acid	58	47	58	19
Oxaloacetic acid+NH_4Cl	76	76	–	0

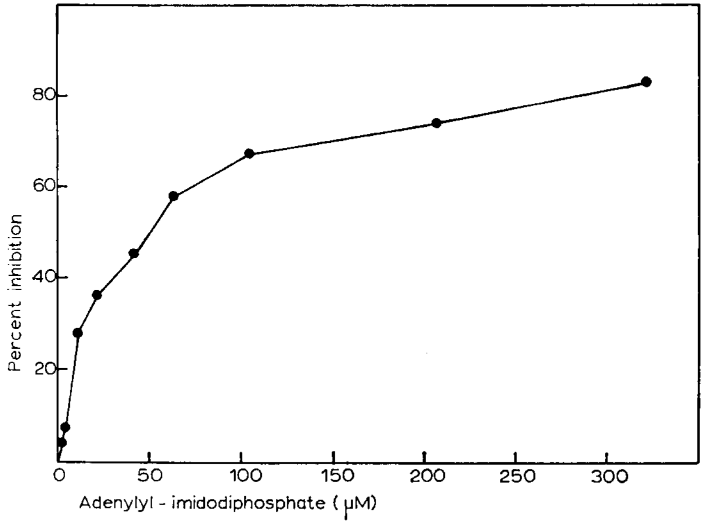

Fig. 2. Effect of adenylyl-imidodiphosphate concentration on inhibition of CO_2-dependent oxygen evolution by pea chloroplasts. Oxygen evolution was measured as described in Fig. 1. Adenylyl-imidodiphosphate was included in the reaction medium from the outset. Control rate: 69 µmol/mg chlorophyll per h.

dependent oxygen evolution was inhibited to a lesser extent. Oxaloacetat -dependent oxygen evolution, which does not directly involve any adenine nucleotide-dependent reactions [6], was not inhibited by adenylyl-imidodiphosphate if an uncoupling agent was also present (Table I). With chloroplasts isolated from young pea shoots, adenylyl imidodiphosphate (0.6 mM) inhibited CO_2-dependent oxygen evolution by 70–95 %. The effect of adenylyl-imidodiphosphate concentration is shown in Fig. 2. 50 % inhibition occurred at 50 µM adenylyl-imidodiphosphate and maximal inhibition required 0.6–0.7 mM adenylyl-imidodiphosphate. With PP_i, 50 % inhibition required much higher concentrations (1.2–1.4 mM) [8] than for adenylyl-imidodiphosphate. Both α, β- and β,γ-methylene ATP also inhibited oxygen evolution in an ATP-

TABLE II

EFFECT OF DIFFERENT ATP ANALOGS ON CO_2-DEPENDENT OXYGEN EVOLUTION BY PEA CHLOROPLASTS

Oxygen evolution was measured as described in Fig. 1. The analogs were added after a steady rate of oxygen evolution was achieved and ATP (0.8 mM) was added subsequently.

Analog	mM	Rate of oxygen evolution (µmol/mg chlorophyll per h)			Inhibition (%)
		−	+	+ATP	
Adenylyl-imidodiphosphate	0.6	69	13	76	81
α,β-Methylene ATP	0.6	61	0	61	100
β,γ-Methylene ATP	0.6	68	0	72	100
Diadenosine pentaphosphate	0.2	61	61	–	0

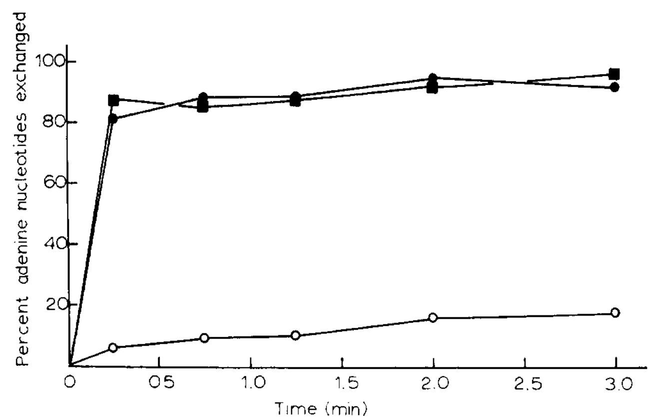

Fig. 3. Adenine nucleotide exchange by pea chloroplasts. Back-exchange of labelled adenine nucleotides was measured as described in Materials and Methods. ○–○, control; ■–■, 0.7 mM adenylyl-imidodiphosphate; ●–●, 0.8 mM ATP.

reversible manner (Table II). Diadenosine pentaphosphate [A(5′)P_5(5′)A] did not affect oxygen evolution (Table II).

In order to verify that adenylyl-imidodiphosphate was transported into the chloroplasts and to establish the kinetics of its uptake, back-exchange of labelled adenine nucleotides was measured. When no additions were made, a slow efflux of labelled nucleotides from the chloroplasts was observed (Fig. 3). The addition of ATP or adenylyl-imidodiphosphate resulted in a rapid efflux of labelled nucleotides

TABLE III

EFFECT OF ADENYLYL-IMIDODIPHOSPHATE AND ATP ON COUPLED ELECTRON TRANSPORT BY BROKEN PEA CHLOROPLASTS

Oxygen evolution was assayed in a medium containing 100 mM sucrose, 10 mM P_i, 5 mM $MgCl_2$, 50 mM HEPES (pH 7.6), 1.3 mM ferricyanide and chloroplasts equivalent to 78 µg chlorophyll in a total volume of 2.1 ml. ADP and NH_4Cl were added to final concentrations of 0.15 and 10 mM, respectively. ATP (0.8 mM) or adenylyl-imidodiphosphate (0.7 mM) was included in the reaction medium as indicated. State 2, Hill reaction rate (−ADP); State 3, phosphorylating rate (+ADP), State 4, rate after exhaustion of added ADP. PCR, photosynthetic control ratio. ADP/O ratios were determined as described previously [6].

	Rate of oxygen evolution (μmol O_2/mg chlorophyll per h)		
	Control	+ATP	+adenylyl-imidodiphosphate
State 2	36	25	20
State 3	95	81	81
State 4	32	32	32
+NH_4Cl	162	144	144
PCR	2.97	2.53	2.53
ADP/O	1.51	1.30	1.41

and the effect of adenylyl-imidodiphosphate was indistinguishable from that of ATP within the time resolution of the experiment. This result is in agreement with the rapid inhibition of oxygen evolution observed in Fig. 1. Like PP_i, adenylyl-imidodiphosphate inhibits oxygen evolution by entering the chloroplast in exchange for internal adenine nucleotides thus depleting the ATP concentration in the chloroplasts and inhibiting CO_2 fixation. The reversal by ATP suggests that exogenous ATP subsequently enters the chloroplasts in exchange for internal PP_i or adenylyl-imidodiphosphate restoring the internal ATP levels and thus allowing CO_2 fixation. Once inside the chloroplast, PP_i may be hydrolysed to 2 P_i whereas adenylyl-imidodiphosphate would not be metabolised. We investigated the possibility that, in addition to inhibition of CO_2 fixation by depletion of internal ATP, adenylyl-imidodiphosphate might also inhibit photophosphorylation or the enzymes of the CO_2 fixation pathway. Oxygen evolution by broken pea chloroplasts with ferricyanide as the electron acceptor was measured to assess photophosphorylation activity. Addition of ATP or adenylyl-imidodiphosphate resulted in a partial inhibition of State 2 (—ADP) rates of electron transport (Table III). State 3, State 4 and uncoupled rates of electron transport were inhibited to a much lesser extent by the nucleotides or were not affected. The ADP/O ratio was decreased by the addition of ATP but was only slightly decreased by adenylyl-imidodiphosphate (Table III). The decrease in ADP/O ratio with ATP suggests hydrolysis of the ATP by an ATP-consuming reaction present in pea chloroplast preparations thereby increasing the effective ADP concentration [6]. Inhibition of State 2 rates by both adenylyl-imidodiphosphate and ATP is consistent with binding of the nucleotides to the chloroplast coupling factor resulting in decreased proton efflux from the granal compartment and therefore decreased electron transport [6, 15, 16]. It is apparent that adenylyl-imidodiphosphate, like ATP, can bind to the coupling factor but that this binding does not significantly affect phosphorylation. The two ATP-utilizing reactions of CO_2 fixation, 3-phosphoglycerate kinase and ribulose-5-phosphate kinase, were inhibited 50 % in the presence of equal concentrations (2.2 mM) of adenylyl-imidodiphosphate and ATP (data not shown).

As adenylyl-imidodiphosphate was a more effective inhibitor of CO_2 fixation than PP_i, it was hoped that it might also inhibit CO_2 fixation in spinach chloroplasts or chloroplasts isolated from older pea plants which are not significantly affected by PP_i [8]. With chloroplasts from both of these tissues, adenylyl-imidodiphosphate inhibition was only 30–40 % suggesting that adenine nucleotide transport is lower in these tissues [7, 8].

DISCUSSION

For comparison, the characteristics of the effects of adenylyl-imidodiphosphate and of PP_i on isolated pea chloroplasts are shown in Table IV. The inhibition by adenylyl-imidodiphosphate occurs more rapidly than PP_i inhibition in agreement with the faster uptake rate indicated by adenine nucleotide back-exchange. The concentration of adenylyl-imidodiphosphate required for 50 % inhibition ($50\,\mu M$) is of the same order as the concentration of ATP required for stimulation of PP_i-inhibited chloroplasts ($25\,\mu M$). These values probably reflect the affinity and rate of transport of the three compounds by the adenine nucleotide transporter which appears to be ATP $>$ adenylyl-imidodiphosphate $\gg PP_i$. The lack of inhi-

TABLE IV

COMPARISON OF THE EFFECTS OF ADENYLYL-IMIDODIPHOSPHATE AND PP_i ON VARIOUS ACTIVITIES OF PEA CHLOROPLASTS

The results for PP_i are taken from ref. 8. $t_{\frac{1}{2}}$ = time taken for 50 % exchange.

	PP_i	Adenylyl-imidodiphosphate
Percent inhibition CO_2-O_2 evolution	93 %	81 %
Percent inhibition 3-phosphoglycerate	80 %	50 %
Time to inhibit CO_2-O_2 evolution	2–3 min	0.3 min
Concentration for 50 % inhibition	1.4 mM	50 μM
Time for ATP relief	1.2 min	0.3 min
ATP concentration for 50 % relief	25 μM	–
$t_{\frac{1}{2}}$ adenine nucleotide exchange	1 min	< 2 s

bition by adenylyl-imidodiphosphate in the presence of ATP (Fig. 1A) may indicate that the carrier has a higher affinity for ATP than for adenylyl-imidodiphosphate and thus ATP prevents adenylyl-imidodiphosphate uptake. This would be in agreement with the results obtained for adenine nucleotide transport in mitochondria where the translocation activity with adenylyl-imidodiphosphate was only 40 % of that with ATP [10]. However, it could also indicate that adenylyl-imidodiphosphate, in the presence of ATP, cannot deplete the chloroplast pool of ATP to inhibitory levels, i.e. ATP and adenylyl-imidodiphosphate are exchanged for each other in both directions. Hence reversal by ATP is more rapid with adenylyl-imidodiphosphate than with PP_i reflecting more rapid exchange of ATP for its analog (Fig. 3) than for PP_i [8] which, apart from having a lower affinity for the transporter, may undergo some internal hydrolysis and so limit the rate of ATP influx. The rapid reversal of adenylyl-imidodiphosphate inhibition by ATP suggests that this is probably a more accurate reflection of the rate of ATP entry than that suggested by the slower reversal of PP_i inhibition [8].

Both of the methylene ATP analogs inhibited CO_2-dependent oxygen evolution to a similar extent as adenylyl-imidodiphosphate (Table II). As the inhibition was reversed by ATP, it appears that the methylene ATP analogs inhibit oxygen evolution in the same manner as adenylyl-imidodiphosphate and PP_i. Diadenosine pentaphosphate was not inhibitory suggesting that it was not transported by the adenine nucleotide transporter.

3-Phosphoglycerate-dependent oxygen evolution was less sensitive to adenylyl-imidodiphosphate than oxygen evolution with CO_2 or CO_2+ribose-5-*P* (Table I). If adenylyl-imidodiphosphate exchanged for internal 3-phosphoglycerate, via the phosphate transporter, the internal level of sugar phosphates would decrease and oxygen evolution could be stimulated by added 3-phosphoglycerate. This is unlikely because ATP is not transported by the phosphate transporter [17] so adenylyl-imidodiphosphate should also not exchange for sugar phosphates. Nevertheless, 3-phosphoglycerate stimulates, and partially recovers, oxygen evolution inhibited with adenylyl-imidodiphosphate (Fig. 1). This is in contrast to the results obtained with PP_i [8]. This stimulation by 3-phosphoglycerate could be explained by a phosphate deficiency in the chloroplasts [8] but this is also unlikely as phosphate was present in the reaction medium. We suggest two possible explanations for the 3-phosphoglycerate stimulation. Firstly, although ribulose-5-phosphate kinase has a higher affinity for ATP than does 3-phosphoglycerate kinase [18], we have found the activity of 3-phosphoglycerate kinase to be 4–5 times higher than that of ribulose-5-phosphate kinase in ruptured pea chloroplasts. A similar difference has been reported for spinach and sunflower chloroplasts [19]. When the chloroplasts are inhibited by adenylyl-imidodiphosphate, i.e. when ATP is limiting, the rate of ribulose-5-phosphate kinase may become the rate-limiting step in CO_2 fixation. Addition of 3-phosphoglycerate would therefore stimulate oxygen evolution. It is also possible that adenylyl-imidodiphosphate preferentially inhibits ribulose-5-phosphate kinase when the adenylyl-imidodiphosphate/ATP ratio inside the chloroplasts is high. Alternatively, differences in transport characteristics for adenylyl-imidodiphosphate and for PP_i may explain the 3-phosphoglycerate effects. It is obvious from the kinetics of inhibition of oxygen evolution and the kinetics of adenine nucleotide back-exchange [8] that the adenine nucleotide transporter has a much lower affinity for, and rate of transport of, PP_i than ATP. Thus PP_i would enter in exchange for internal adenine nucleotides but because of the resultant low internal concentration of PP_i, efflux of PP_i, in exchange for ATP or for PP_i, would be slow. In addition, internal hydrolysis of PP_i may further lower its concentration. In contrast, adenylyl-imidodiphosphate and ATP appear to have similar affinities for, and rates of transport by, the adenine nucleotide transporter. Entry of adenylyl-imidodiphosphate in exchange for internal adenine nucleotides would subsequently result in competition between internal adenylyl-imidodiphosphate and ATP for efflux. It is therefore unlikely that adenylyl-imidodiphosphate would decrease the level of internal adenine nucleotides to the same extent as would PP_i. The PP_i inhibition probably reflects a true depletion of internal adenine nucleotides whereas with adenylyl-imidodiphosphate the inhibition may be partially a result of adenine nucleotide depletion and partially a result of inhibition of the two kinases by adenylyl-imidodiphosphate. Thus 3-phosphoglycerate cannot stimulate oxygen evolution in PP_i-inhibited chloroplasts, which have very low levels of adenine nucleotides, but 3-phosphoglycerate can stimulate adenylyl-imidodiphosphate-inhibited chloroplasts, which are not so severely depleted of adenine nucleotides, by stimulating phosphoglycerate kinase activity.

It is apparent from this and previous work [7–9] that the rate of adenine nucleotide transport in chloroplasts from young pea shoots is higher than that for spinach chloroplasts. Preliminary experiments in this laboratory suggest that the rate of ATP uptake by chloroplasts isolated from young pea shoots is in excess of 20 μmol/mg chlorophyll per h. Recently, Huber and Edwards [20] reported rates of ATP transport of 30–40 μmol/mg chlorophyll per h in mesophyll chloroplasts of a C_4 plant, *Digitaria sanguinalis.* These rates are in excess of that reported for spinach chloroplasts (5 μmol/mg chlorophyll per h) [3]. The use of chloroplasts isolated from young pea shoots, in which adenine nucleotide transport occurs at relatively high rates, and of ATP analogs which closely mimic ATP in their transport properties, should prove useful tools for further investigation of adenine nucleotide transport in chloroplasts.

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REFERENCES

1 Heber, U. and Santarius, K. A. (1970) Z. Naturforsch. 25b, 718–728
2 Santarius, K. A. and Heber, U. (1965) Biochim. Biophys. Acta 102, 39–54
3 Heldt, H. W. (1969) FEBS Lett. 5, 11–14
4 Heber, U. (1974) Ann. Rev. Plant Physiol. 25, 393–421
5 Walker, D. A. (1974) Chloroplast and Cell (Northcote, D. H., ed.), Med. Tech. Publ. Int. Rev. Sci. Biochem., Ser. 1, Vol. 11, pp. 1–49, Butterworths, London
6 Robinson, S. P. and Wiskich, J. T. (1976) Biochim. Biophys. Acta 440, 131–136
7 Robinson, S. P. and Wiskich, J. T. (1976) Plant Physiol. 58, 156–162
8 Robinson, S. P. and Wiskich, J. T. (1977) Plant Physiol., in the press
9 Stankovic, Z. S. and Walker, D. A. (1977) Plant Physiol., in the press
10 Klingenberg, M., Grebe, K. and Scherer, B. (1971) FEBS Lett. 16, 253–256
11 Melnick, R. L., Tavares de Sousa, J., Maguire, J. and Packer, L. (1975) Arch. Biochem. Biophys. 166, 139–144
12 Duée, E. D. and Vignais, P. V. (1968) Biochem. Biophys. Res. Commun. 30, 420–427
13 Lienhard, G. E. and Secemski, I. I. (1973) J. Biol. Chem. 248, 1121–1123
14 Arnon, D. I. (1949) Plant Physiol. 24, 1–15
15 Telfer, A. and Evans, M. C. W. (1972) Biochim. Biophys. Acta 256, 625–637
16 Cantley, L. C. and Hammes, G. G. (1975) Biochemistry 14, 2968–2975
17 Heldt, H. W. and Rapley, L. (1970) FEBS Lett. 10, 143–148
18 Lavergne, D., Bismuth, E. and Champigny, M. L. (1974) Plant Sci. Lett. 3, 391–397
19 Delaney, M. E. and Walker, D. A. (1976) Plant Sci. Lett. 7, 285–294
20 Huber, S. C. and Edwards, G. E. (1976) Biochim. Biophys. Acta 440, 675–687