# PRELIMINARY NOTES

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## Light-triggered, thiol-dependent ADP–ATP exchange activity in spinach chloroplasts

Recent work by CARMELI AND AVRON[1] and RIENITS[2] demonstrated that there is an ATP–$P_i$ exchange in the dark associated with the light-triggered, thiol-dependent ATPase of chloroplasts. It appeared probable that it would be possible to demonstrate an ADP–ATP exchange also under these conditions. An ADP–ATP exchange could represent a partial reaction of photophosphorylation if, as has been suggested[3], the light-triggered, thiol-dependent ATPase represents a part of the photophosphorylation system acting in reverse.

We have found, in confirmation of KAHN[4], that spinach chloroplasts exhibit considerable ADP–ATP exchange activity which could be largely, but not completely, removed by washing and 'breaking' them twice in 0.01 M NaCl containing 0.01 M Tris–HCl (pH 8.0). Light-triggered, thiol-dependent ADP–ATP exchange activity can be detected in washed, broken chloroplasts but not in unbroken chloroplasts where there was considerable endogenous exchange. Table I shows the effect of a variety

TABLE I

PROPERTIES OF THE ADP–ATP EXCHANGE MEASURED IN THE DARK FOLLOWING ILLUMINATION IN THE PRESENCE OF DITHIOTHREITOL

The complete reaction mixture contained 50 mM Tris–HCl (pH 8.0); 5 mM $MgCl_2$; 35 mM NaCl; 20 $\mu$M PMS; 6 mM dithiothreitol and twice-washed, broken chloroplasts to give 50 $\mu$g chlorophyll per ml in a total reaction volume of 5.0 ml. The mixture was stirred in an atmosphere of air in a thermostatted vessel at 25°, illuminated at 100000 lux for 5 min. ATP (final concn. 2.5 mM) and ADP (final concn. 0.5 mM and containing 40000 counts/min [8-$^{14}$C]ADP) were added immediately on turning off the light. Samples (1.0 ml) for analysis were removed into $HClO_4$ at 30-sec intervals for 2 min. The adenine nucleotides were separated from the deproteinised reaction mixture by column chromatography, their radioactivity determined by scintillation counting and the ADP–ATP exchange calculated.

| *Reaction conditions* | *ADP–ATP exchange ($\mu$moles [8-$^{14}$C]ATP formed per h per mg chlorophyll)* |
|---|---|
| Complete | 7.0 |
| Dithiothreitol omitted | 2.9 |
| PMS omitted | 3.8 |
| Illumination omitted | 4.2 |
| $MgCl_2$ omitted | 0.0 |
| Dithiothreitol and PMS both omitted | 2.7 |
| Dithiothreitol and illumination both omitted | 3.6 |
| Dithiothreitol present in dark phase only | 2.6 |
| PMS present in dark phase only | 4.9 |
| $MgCl_2$ present in dark phase only | 5.3 |

Abbreviation: PMS, phenazine methosulphate.

of conditions on the ability of the washed, broken spinach chloroplasts to exhibit ADP–ATP exchange. It is clear that appreciable increases in ADP–ATP exchange follow the treatments which elicit the light-triggered, thiol-dependent ATPase and ATP–$P_i$ exchange[1,2], *i.e.* dithiothreitol, phenazine methosulphate (PMS) and $Mg^{2+}$ during illumination. The increase of specific activity was linear over the 2-min period studied commencing immediately illumination ceased. These linear kinetics together with the absence of any appreciable effect of light on the rate of increase of specific activity of the ATP in the absence of dithiothreitol (Table I) eliminated the possibility that '$X_E$' formed during illumination[5] resulted in the net synthesis of ATP from the added ADP and endogenous $P_i$. There was no incorporation of radioactivity into AMP during the incubation indicating that the exchange was not due to the presence of myokinase (adenylate kinase, EC 2.7.4.3).

In the presence of 2.5 mM ATP maximum rates of the exchange were obtained with 0.5 mM ADP. In the presence of 0.5 mM ADP the ATP concentration giving maximum exchange was 2.5 mM ATP. Very little activity was obtained at 1.0 mM. As this was the concentration used by CARMELI AND AVRON[6] it may explain why they did not detect ADP–ATP exchange in their lettuce chloroplasts (but which exhibited ATPase and ATP–$P_i$ exchange similar to spinach chloroplasts).

Fig. 1 shows that there was a marked effect of $P_i$ when present during both light and dark phases, and lack of effect when present in the dark phase only. SKYE, SHAVIT AND BOYER[7] have recently shown that the light-triggered, thiol-dependent ATPase responds similarly to $P_i$ additions, as does the ATP–$P_i$ exchange[6].

In the absence of either ADP or ATP there is considerable dark decay of the ADP–ATP exchange, although as shown in Table II this is largely prevented if $P_i$ is present during illumination. The dark decay in the absence of ATP and the effect of $P_i$ resemble the behaviour of the ATP–$P_i$ exchange[2,6]. The decay observed in the absence of ADP seems limited to the ADP–ATP exchange and does not seem to have been observed with ATPase or ATP–$P_i$ exchange.

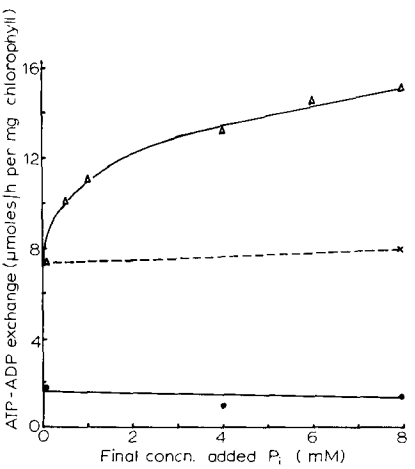


Fig. 1. Effect of added inorganic phosphate on the ADP–ATP exchange activities. Reaction conditions as for complete reaction of Table I except 4-min illumination given. $P_i$ added as indicated to give final concentrations shown on figure. ×---×, $P_i$ added after illumination; △—△, $P_i$ present during illumination; ●—●, dithiothreitol omitted and $P_i$ present during illumination.

The ADP–ATP exchange is in general less affected by uncouplers and inhibitors of photophosphorylation than either the light-triggered, thiol-dependent ATPase or the ATP–$P_i$ exchange. For example, treatment with EDTA which completely prevented the development of the ATPase had no effect upon the ADP–ATP exchange.

TABLE II

DARK DECAY OF ADP–ATP EXCHANGE

Reaction conditions were as for Table I except that illumination was for 4 min. ATP and ADP were added in the dark at the times shown after illumination ceased. Samples were taken for separation and assay 60 and 90 sec after illumination ceased. The ADP–ATP exchange values in the absence of dithiothreitol were subtracted from the total ADP–ATP exchange values to give the net ADP–ATP exchange brought about by light and dithiothreitol.

| *Additions in the dark following illumination* | | *Net ADP–ATP exchange (μmoles [8-$^{14}$C]ATP formed per h per mg chlorophyll)* |
|---|---|---|
| *(a) at 0 sec* | *(b) at 20 sec* | |
| *No added $P_i$* | | |
| ATP, ADP | | 10.5 |
| ATP | ADP | 5.8 |
| ADP | ATP | 5.7 |
| | ATP, ADP | 3.0 |
| *$P_i$ (to 8 mM) added prior to illumination* | | |
| ATP, ADP | | 14.8 |
| ATP | ADP | 13.4 |
| ADP | ATP | 12.4 |

Atebrin ($7 \cdot 10^{-6}$ M) added at the end of illumination in one experiment stimulated the ADP–ATP exchange by 52 % but inhibited the ATPase by 77 % and the ATP–$P_i$ exchange by 94 %.

The ADP–ATP exchange which is light triggered and thiol dependent is considered to be a partial reaction of the light-triggered, thiol-dependent ATPase and together with ATP–$P_i$ (refs. 1, 2, 6), the $P_i$–HOH and ATP–HOH exchange[7] to offer another reaction by which to investigate the mechanism of the ATPase and also photophosphorylation in chloroplasts.

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