# PATHWAY OF ATP FORMATION IN PHOTOSYNTHETIC PHOSPHORYLATION[1,2]

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It has been suggested from the evidence of ATP-$P_i^{32}$[3] and $ATP^{32}$-ADP exchange, that the pathway leading to the formation of ATP in oxidative phosphorylation involves a carrier~$PO_4$ intermediate (Lehninger et al. 1958, Chiga and Plaut, 1959). Based on the indirect evidence that in order to show arsenate uncoupling in chloroplasts, ADP was required, it was suggested that in photosynthetic phosphorylation the reverse order was true and a carrier~ADP intermediate exists (Avron and Jagendorf, 1959).

An attempt is made here to give more direct evidence as to which of the two is the precursor in photosynthetic phosphorylation. The working hypothesis tested was this: If a carrier~ADP is the precursor, it should accumulate in chloroplasts in the light when $P_i$ is absent and should form ATP when $P_i$ is subsequently added in the dark. Conversely, if a carrier~$PO_4$ is the precursor, it should accumulate in the light in the absence of ADP. In preliminary experiments the test for a carrier~ADP intermediate gave consistently negative results. However, evidence was obtained for the existence of a carrier~$PO_4$ intermediate for ATP formation in photosynthetic phosphorylation.

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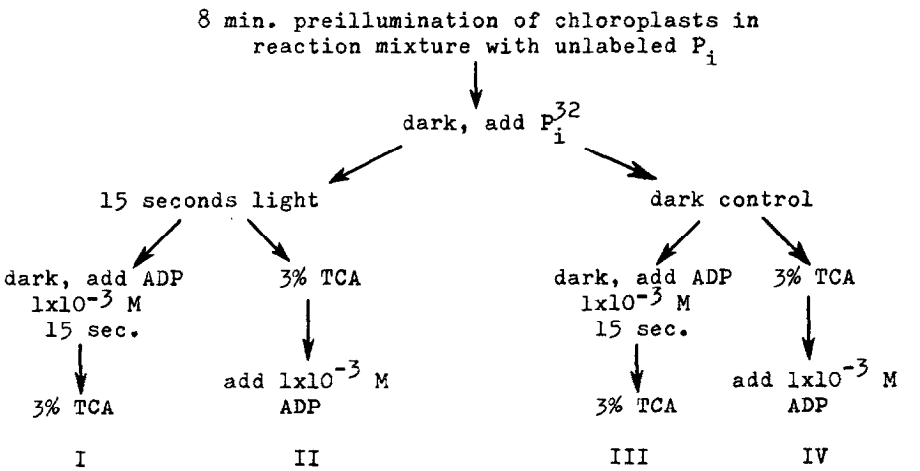
[3] Abbreviations used: $P_i$ for inorganic phosphate; TCA for trichloroacetic acid.

## Methods

Washed chloroplasts were prepared by the method previously described (Jagendorf and Avron, 1958). The reaction mixture contained $1.3 \times 10^{-2}$ M Tris HCl pH 8.0, $3.3 \times 10^{-3}$ M $MgCl_2$, $3.3 \times 10^{-2}$ M NaCl, $3 \times 10^{-5}$ M phenazine methosulfate, $3.3 \times 10^{-4}$ M $K_2HPO_4$ and chloroplasts containing 1-5 μmoles chlorophyll; final volume 12 ml. The procedure used was as follows:




The preincubation was necessary in order to phosphorylate the endogenous ADP of the chloroplasts.

Nucleotides were adsorbed on charcoal and either counted directly or eluted with 50% ethanol containing .3% conc. $NH_4OH$. The eluate was chromatographed on Whatman #1 paper with isobutyric acid : conc. $NH_4OH$ : water (66:1:33) as solvent. The spots corresponding to ADP and ATP were cut out and counted.

## Results

The results given in Table I show that $ATP^{32}$ is formed upon the addition of ADP in the dark, dependent on the previous 15 second illumination in the presence of $P_i^{32}$. This incorporation is not light-induced exchange between endogenous ATP and $P_i^{32}$, as can be seen from treatment II, nor is it exchange with added ADP in the dark (treatment III).

Table I

The formation of $ATP^{32}$ by chloroplasts due to the addition of ADP in the dark, following a short illumination in the presence of $P^{32}_i$. The Roman figures refer to the four treatments in the diagram.

| | Treatment | | mμmole $ATP^{32}$ / μmole chlorophyll | Light-Dark |
|---|---|---|---|---|
| | | Dark addition | | |
| I | Light | ADP | 1.72 | 1.04 |
| II | | TCA | .60 | .14 |
| III | Dark Control | ADP | .68 | |
| IV | | TCA | .46 | |

In a series of over 15 experiments, the amount of light dependent $ATP^{32}$ produced varied between 1 and 2 mμmoles $ATP^{32}$/μmole chlorophyll.

In order to establish whether the label is actually in ATP, the charcoal eluate was chromatographed and the spots corresponding to ADP and ATP counted. The results are given in Table II. Scanning by a strip counter showed that all the activity was localized in these two spots.

Table II

Chromatographic separation of the samples listed in Table I into $ADP^{32}$ and $ATP^{32}$

| | label in | mμmole / μmole chlorophyll | light-dark |
|---|---|---|---|
| I | $ADP^{32}$ | 0.54 | 0.14 |
| | $ATP^{32}$ | 1.33 | 1.11 |
| II | $ADP^{32}$ | 0.33 | 0.04 |
| | $ATP^{32}$ | 0.27 | 0.14 |
| III | $ADP^{32}$ | 0.40 | |
| | $ATP^{32}$ | 0.22 | |
| IV | $ADP^{32}$ | 0.29 | |
| | $ATP^{32}$ | 0.13 | |

It can be seen from the table that the light-dependent product is nearly all $ATP^{32}$. The amount of label incorporated into ADP was about the same whether illuminated or not.

The stability of the $P^{32}$-containing intermediate was tested by keeping the reaction mixture on ice after illuminating, and delaying

the addition of ADP. The results, Table III, represent the net amount of $ATP^{32}$ formed after subtraction of the dark control. The results

Table III

Time course of degradation of active intermediate. Results are net values after subtraction of the control. 2 experiments.

| Time of ADP addition after light turned off | mμmole $ATP^{32}$ formed / μmole chlorophyll | |
|---|---|---|
| 30 sec. | 1.97 | |
| 45 | | 1.80 |
| 60 | | 1.73 |
| 105 | 1.07 | |
| 120 | | 1.49 |
| 180 | 1.01 | 1.14 |
| 300 | 0.82 | 1.04 |
| 480 | 0.49 | |

show that the intermediate is labile, with a half-life of 3-4 minutes.

Work is currently in progress attempting to stabilize and characterize the intermediate. Preliminary experiments indicate that it is extremely labile both in acid and in alkali.

In an attempt to eliminate the endogenous pool of nucleotides, broken chloroplasts and grana were substituted for whole chloroplasts. The broken chloroplasts proved just as good as the whole ones, but the grana had only about 40% of the activity left.

## Discussion

The results presented indicate the existence of a high-energy phosphate precursor of ATP in photosynthetic phosphorylation. The formation of this metastable precursor is light-dependent, and the various control treatments eliminate the possibility that it arises by an exchange reaction. The "light activation" of an exchange reaction between $P_i^{32}$ and added ADP may be excluded because all treatments received 8 minutes pre-illumination. Such a light-activated exchange should therefore have occurred in the dark control (treatment III).

The chloroplasts contain a large excess of unlabeled ATP (200-

500 mμmole/μmole chlorophyll). This seems to exclude the possibility that what is stored in the light is not a precursor but a by-product in equilibrium with the trace of $ATP^{32}$ formed during illumination.

## References

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