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Synthesis and Discharge of the Coupling Factor • Adenosine Diphosphate Complex in Spinach Chloroplast Lamellae

(light-dependent electron transport/arsenate/sulfate/photophosphorylation)

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ABSTRACT **The formation of a coupling factor·ADP complex is shown to be dependent on photoinduced electron transport, AMP, and P_i, and sensitive to arsenate and sulfate. The stability of the complex is unaffected by subsequent addition of arsenate, but is quite markedly sensitive to the addition of ADP. The data are discussed in relation to possible models of photophosphorylation, and in particular, to one in which coupling factor-bound, photosynthetically generated, ADP serves as a phosphoryl donor to substrate ADP.**

Earlier work in this laboratory (1) has identified the approximately 10-nm (100-Å) particle associated with the photosynthetic lamellae of spinach chloroplasts as the morphological counterpart of the Ca^{++}-dependent ATPase of Vambutas and Racker (2), and equivalent to the coupling factor of photophosphorylation. This enzyme has been dissociated from the membranes, purified to homogeneity, and found to be devoid of *any* pigment, consisting almost exclusively of protein (1, 3). Recently we have shown (4) that this purified enzyme, when supplied with any of the adenine nucleotides, can bind either ADP or ATP but *not* AMP. In addition, under appropriate conditions, the *homogeneous* enzyme can bind [^{14}C]ADP at two sites and carry out a transphosphorylation reaction (myokinase-like), by which it transfers the β-phosphate of one bound ADP molecule to the β-phosphate of the other bound ADP molecule to yield ATP and AMP. This reaction is novel since the products formed show such a high affinity for the enzyme that they *do not* appear in the micromolecular phase of the reaction mixture; for their detection, the enzyme must be destroyed after the completion of the reaction; only then are the products released to the medium. Although in this case the enzyme again has a much greater affinity for ATP than for AMP, the fact that *this* AMP can be recovered as a complex with the enzyme indicates that there are at least two different ways for enzyme–nucleotide interactions to take place *in vitro:* one is that effecting the saturation of the enzyme with *exogenously* added nucleotides (where AMP never binds to the enzyme, even in the presence of ATP), and the other is that operating in the stabilization of complexes with nucleotides derived from *within* the enzyme domain as products of the transphosphorylation reaction. The second type of interaction is not entirely equivalent to the first, and it results in complexes of higher stability.

Abbreviations: DTT: dithiothreitol; DCMU: 3-(3,4-dichlorophenyl)-1,1 dimethylurea.

Taking advantage of this stability, we have attempted to resolve the enzymatic steps leading to photosynthetic ATP formation by this enzyme when it is operating as a *membrane-associated coupling factor*, that is, while the enzyme is on the chloroplast lamellae. In an earlier communication, we reported that when this enzyme is isolated from chloroplast lamellae previously illuminated in the presence of [^{32}P]P_i and the electron transport cofactor pyocyanine, it contains up to one molecule of [^{32}P]ADP per about 300,000 daltons of protein. All the radioactivity associated with the enzyme after purification is entirely in the form of ADP (4). If during the illumination of the lamellae [^{3}H]AMP is added to the system, the enzyme is again recovered with 1 mol of [^{3}H]ADP that is esterified during illumination. The data lead to a model for the terminal step of photophosphorylation in which energy is trapped first in photosynthetically generated ADP. This ADP molecule is bound to the coupling factor and is derived from an enzyme or membrane·AMP complex, phosphate, and light energy. This enzyme-bound ADP serves in a second step as a phosphate donor to a substrate ADP for the generation of ATP. This model differs from earlier ones in introducing a dual role for ADP, both as a phosphate donor and acceptor. It also differentiates between two potentially unequal classes of ADP. One is the enzyme-bound ADP in equilibrium with membrane-associated AMP, phosphate, and the energy supply. Under normal phosphorylating conditions, this ADP fraction is constantly turning over. The second class of ADP is the exogenous substrate (possibly extending into the membrane space), which reacts transiently with the coupling factor, acting as the terminal phosphate acceptor. The overall nucleotide balance of a particular reaction mixture should reflect the extent to which these two ADP pools can interact with each other.

In this paper, evidence will be presented to demonstrate that the formation of the enzyme·ADP complex from AMP and P_i is strictly dependent on photoinduced electron transport. The synthesis of the complex is effectively inhibited by sulfate and arsenate, although arsenate has no effect on the complex *after* it is formed. Furthermore, addition of external ADP to membranes already charged with enzyme·ADP complex causes a loss of the ADP label from the enzyme into the soluble phase.

METHODS

Chloroplast membranes were prepared as previously described (1, 3, 4) and suspended in 0.05 M Tricine–0.02 M

NaCl (pH 7.0), at 22°C, to a final chlorophyll concentration of about 400 μg/ml. 25-ml aliquots were dispensed into 150 × 200-mm flat-bottomed dishes. The composition of the reaction mixture was adjusted as indicated in the text. In general, pyocyanine was used at a concentration of 2×10^{-5} M, AMP at 10^{-4} M, P_i at 10^{-3} M, arsenate at 10^{-2} M, and ADP at 3×10^{-3} M. [^{3}H]AMP (Schwarz BioResearch, Inc.) or ^{32}P (Tracerlab, Inc.) were added as aqueous solutions in Tricine–NaCl buffer. Final chlorophyll concentration in the reaction mixture was about 360–380 μg/ml. Illumination was provided by a 500 W Sylvania "Sun Gun" tungsten–iodide lamp, mounted 45 cm from the thin layer of the sample, shielded with a $CuSO_4$ bath to absorb heat (4). After illumination, the membranes were chilled, pelleted by centrifugation, washed once in 5 mM NaCl, and extracted in the dark with 1 mM EDTA (pH 7.5–8.0). The membranes were sedimented and discarded, and the coupling factor was prepared from the EDTA extracts, as previously described (1, 3, 4), by the use of ammonium sulfate precipitation, dialysis, and sucrose-gradient centrifugation. The specific radioactivity of the *homogeneous* coupling-factor protein was determined by measurement of the radioactivity in a liquid scintillation counter (Packard 2002), and by measurement of the protein concentration, according to the method of Lowry *et al.* (5).

RESULTS AND DISCUSSION

It was shown previously that the coupling factor can be labeled by the use of either [^{3}H]AMP or [^{32}P]P_i as a tracer (4) (see also Table 3). The enzyme-bound isotope in all cases has been recovered *exclusively* in the form of ADP. This ADP is formed in a light-dependent step, and under the conditions described here becomes (or remains) associated with the coupling factor without equilibrating with the exogenous ADP pool (4). The data in Table 1 show further that deletion of the artificial electron transport cofactor, pyocyanine, and addition of the electron transport inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (5), result in almost complete (95%) inhibition of synthesis of the coupling factor·ADP complex. It is therefore clear that the formation of this complex is dependent on photoinduced electron transport.

TABLE 1. *Dependence of labeling of coupling factor on electron transport*

Sample	Additions	Specific radioactivity of purified coupling factor (cpm ADP/mg of protein)	Inhibition (%)
1	AMP + [^{32}P]P_i + pyocyanine	12,500 ± 500	0
2	AMP + [^{32}P]P_i	1,515 ± 200	88
3	AMP + [^{32}P]P_i + DCMU	550 ± 200	95

Chloroplasts were prepared as has been described (3); the salt-washed chloroplast membranes were suspended in Tricine–NaCl buffer. The concentration of AMP was 10^{-4} M; [^{32}P]P_i, 10^{-4} M; pyocyanine, 2×10^{-5} M; DCMU, 4×10^{-6} M. In sample 2, pyocyanine was omitted to minimize cyclic electron transport, and in sample 3 pyocyanine was omitted and DCMU was added, to block any noncyclic transport as well. The DCMU was added in total darkness 2 min before the addition of AMP and [^{32}P]P_i, to achieve thorough equilibration. After illumination for 1 min, the samples were chilled, and the coupling factors were prepared from the membranes as described in *Methods*, and assayed for specific radioactivity. The specific radioactivity of the [^{32}P]P_i was about 20 Ci/mol.

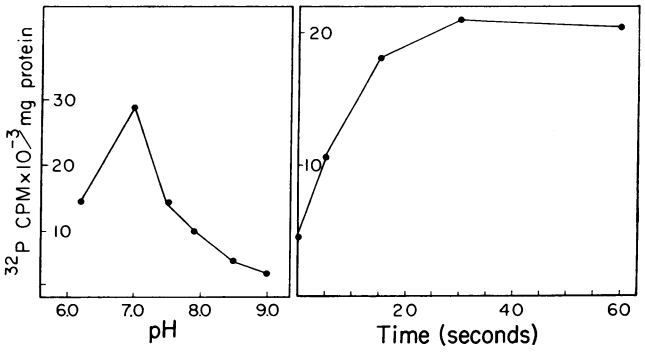


FIG. 1. (*left*) Dependence of the formation of coupling factor·ADP complex on pH of illumination mixture. Salt-washed chloroplast membranes were suspended in 0.05 M Tricine–0.02 M NaCl buffers at the indicated pH, made 2×10^{-5} M in pyocyanine, and briefly preilluminated. Then carrier-free [^{32}P]P_i (250 μCi) was added and illumination was performed as described, for 1 min. The coupling factor·ADP complexes were isolated from the membranes of each sample, and the specific radioactivity of each 13S protein peak (coupling factor fraction) was determined and plotted as a function of pH in the illumination mixture.

FIG. 2. (*right*) Dependence of formation of coupling factor·ADP complex on illumination time. Chloroplast membranes were suspended in Tricine–NaCl buffer as in the experiment of Fig. 1, and preilluminated in the presence of 2×10^{-5} M pyocyanine, to deplete any endogenous phosphate. After several minutes in the "dark," the membranes were then illuminated in 25-ml aliquots in the presence of 100 μCi of carrier-free [^{32}P]P_i for preset intervals. A shutter arrangement was used to cut off the after glow of the "Sun Gun" filament. The ^{32}P-labeled enzymes were isolated as usual, and the specific radioactivity of each coupling factor sample was plotted as a function of the illumination period. Note that there is significant incorporation of radioactivity even in the zero-time sample, which cannot be explained as yet. The operation was conducted under dim green "safelight", *not* in total darkness. It is this low illumination level that is implied by "dark".

The pH dependence of the formation of the coupling factor·ADP complex exhibits an optimum around pH 7 (Fig. 1). This is different from the pH optimum of overall photophosphorylation (net ATP synthesis), which is in the range of from pH 8 to 8.5. If the step of the formation of the coupling factor·ADP complex is truly a part of the photophosphorylation process, it may be considered that the CF_1·[^{32}P]ADP synthesis is a prerequisite for [^{32}P]ATP synthesis. If net phosphorylation is considered a process with more than one partial reaction, each with its characteristic pH optimum, then the rate-limiting pH-dependent step must be some step other than the one leading to the formation of the CF_1·ADP complex, perhaps a step involved in its utilization, such as the phosphorylation of the exogenously added substrate ADP.

The extent to which the enzyme is labeled with [^{32}P]P_i as a function of illumination time is shown in Fig. 2. A limit is reached after 30 sec of illumination. The data obtained in this experiment should not be used to calculate the rate of

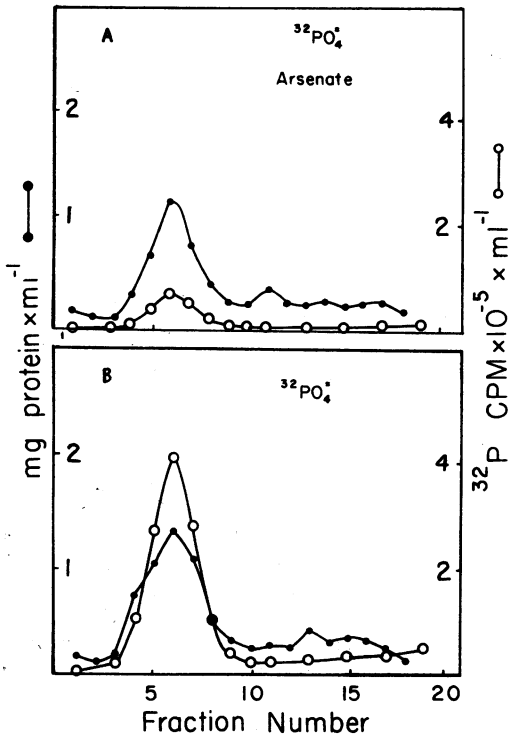


FIG. 3. Effect of arsenate on labeling of the coupling factor with [^{32}P]P_i. Salt-washed chloroplast membranes were suspended in Tricine–NaCl buffer and made 2×10^{-5} M in pyocyanine. After a 1-min illumination, 25-ml aliquots of this suspension were mixed just before illumination with 100 μCi of carrier-free [^{32}P]P_i (with or without 1 ml of 0.25 M sodium arsenate, as indicated in Fig. 3*A* and 3*B*, respectively) and AMP to a final concentration of 10^{-4} M. After a 1-min illumination, the coupling factor was isolated from the membranes as usual, and purified as shown on sucrose gradients. *Open circles* represent radioactivity, *filled circles* represent protein. All enzyme-bound radioactivity has been recovered from the enzyme as [^{32}P]ADP (4).

formation of the enzyme·ADP complex, since the chlorophyll concentration in these experiments is rather high, and light may be a rate-limiting factor. The data serve to illustrate that under the conditions used, a reproducible maximum number of enzyme·ADP complexes have been synthesized.

Table 2 shows the extent of labeling of the coupling factor with several concentrations of [^{3}H]AMP. A limit is achieved between 1.25 and 2.5×10^{-5} M [^{3}H]AMP. In all cases, the isotope is recoverable from the coupling factor *exclusively* in the form of ADP. Less extensive data with [^{32}P]P_i have shown that a limit of ^{32}P-labeling is achieved at a concentration less than or equal to 1 mM. No attempt has been made to infer precise affinity constants from these data, in view of the possible presence of endogenous nucleotides and P_i. Nevertheless, the low concentrations of AMP and P_i required to saturate the enzyme suggest that this pathway of labeling the enzyme is different from the binding of exogenous nucleotides *in vivo*, which requires much higher concentrations (unpublished data). It should be pointed out here that although the addition of Mg^{++} is required for photophosphorylation (9), in the experiments reported here the synthesis and discharge of the coupling factor·ADP complex proceed without the addition of exogenous Mg^{++}. In other experiments (not shown here), addition of Mg^{++} in these reactions had little or no effect. If Mg^{++} is acting directly at the level of the coupling factor, it is conceivable that this requirement is satisfied in the present experiments by the small amounts of endogenous (membrane-associated) Mg^{++}, since the reactions studied here are limit reactions with negligible turn-over. It has also been shown that Ca^{++} is an inhibitor of photophosphorylation (10). We have found (data not shown here) that the addition of Ca^{++} to illuminated chloroplast lamellae, which subsequently are used in experiments of the type described here, results in an inhibition of the formation of the coupling factor·ADP complex. This can be achieved only under certain conditions, and the effect appears rather complex. It will be discussed in detail elsewhere.

It has been demonstrated that sulfate inhibits photophosphorylation in a manner competitive with phosphate (11). We have studied the effect of sulfate on the synthesis of the coupling factor·ADP complex and our results are presented in Table 3. The data show that the labeling of the coupling factor is markedly inhibited by sulfate, independently of the *order* of addition of labeled AMP and P_i, and without the addition of *exogenous* ADP. The requirements for this inhibition *appear* different from those described by Ryrie and Jagendorf (12) for the sulfate-induced inhibition of photophosphorylation, presumably through an unidentified "modification" of CF_1, and for which the requirement for the addition of exogenous ADP and Mg^{++} was demonstrated (12). In view of the presence of low levels of endogenous nucleotides in our chloroplast-membrane preparations (see below), and the very low turn-over number of the reaction studied here, it is not possible with the available information to decide on the significance of this apparent difference or the relation, if any, between the two phenomena.

In net phosphorylation (ATP synthesis), arsenate is reported to act as a competitive inhibitor with phosphate (6). According to the model that we have proposed (4), one might

TABLE 2. *Labeling of coupling factor as a function of [^{3}H]AMP concentration*

	[^{3}H]AMP (μM)	Condition	Mol of [^{3}H]ADP/mol of coupling factor
Expt. I	12.5	Light	0.39
	25	Light	0.65
	80	Light	0.63
	160	Light	0.83
	240	Light	0.65
Expt. II	320	Light	0.61
	400	Light	0.73
	40	Dark	0.06
	50	Dark	0.05

Salt-washed chloroplast membranes were suspended in Tricine–NaCl buffer as described in *Methods*. The concentration of P_i was 1 mM, and the concentration of pyocyanine was 2×10^{-5} M. The concentration of [^{3}H]AMP was varied as shown. After a 1-min illumination period, the membranes were sedimented, and the coupling factor was isolated from the membranes of each sample. The number of mol of [^{3}H]ADP per mol of coupling factor was calculated from the measured specific radioactivity of the coupling factor isolated in sucrose gradients and the specific radioactivity of the [^{3}H]AMP, which was 2.2×10^7 cpm/μmol in Expt. I and 10^6 cpm/μmol in Expt. II.

expect an arsenate-sensitive step in ADP formation. Fig. 3 shows sucrose-gradient profiles of the coupling factor isolated from membranes illuminated with AMP, [^{32}P]P_i, and pyocyanine, in the presence or absence of arsenate. In this experiment, arsenate inhibited the labeling by 87%. That is, an arsenate-sensitive step does exist in the formation of the enzyme·ADP complex, as predicted by the model.

The data in Table 4 show further that once the coupling factor·ADP complex has been formed, arsenate does not cause the discharge of the bound ADP. This confirms the inference that the arsenate-sensitive step occurs before CF_1·ADP formation. It also suggests that by the time arsenate is added, there is little or no turnover, and it raises the possibility that the presence of [^{32}P]ADP on the coupling factor is due to some block in the normal metabolic pathway imposed by the experimental design. Most significantly, however, the addition of ADP alone at this point results in a 75% discharge of the ^{3}H- or ^{32}P-labeled material from the coupling factor (Table 4, samples 3 and 6). In other experiments, a discharge of 86% was observed. The depletion of bound ADP induced by the addition of unlabeled ADP was completely unaffected by the presence of arsenate (Table 4, samples 4 and 7). The addition of ADP in this kind of experiment results not only in the discharge of the coupling factor, but also, of course, in the release of labeled ATP from the particulate phase into the supernatant solution obtained after the membranes have been spun down. With this particular experimental design, however, it is impossible to tell just where the terminal phosphate of the ATP is coming from: ADP on the enzyme, P_i, or something else.

In recent, unpublished experiments, we have found that the discharge reaction can be brought about in total darkness in the presence of arsenate, using ADP, GDP, or ATP, but not AMP, as discharging reagents. Although the mechanism of the discharge reaction is not known for any of these reagents, it is clear from recent experiments that exogenously added [^{3}H]ADP can be partially recovered in association with the coupling factor only if added during or immediately after illumination of the membranes. In view of the wide variety of reactions now attributed to the coupling-factor protein (ATPase, ADP kinase, transphosphorylation), it would be premature to conclude more than that the [^{32}P]ADP is probably located at the active site of phosphorylation, and that molecules known to interact with this site cause the release of that label.

TABLE 3. *Effect of sulfate on labeling of the coupling factor*

Sample	Additions to chloroplast membranes: Step 1	Additions to chloroplast membranes: Step 2	Recovery of enzyme-bound label, mol of radionucleotide / mol of coupling factor: [^{3}H]ADP	Recovery of enzyme-bound label, mol of radionucleotide / mol of coupling factor: [^{32}P]ADP
1	—	[^{32}P]P_i + [^{3}H]AMP	0.40	0.49
2	[^{32}P]P_i + SO_4	[^{3}H]AMP	0.22	0.26
3	[^{3}H]AMP + SO_4	[^{32}P]P_i	0.19	0.16

Chloroplast membranes were prepared and suspended in Tricine–NaCl–pyocyanine, as previously described. The illumination period was arbitrarily divided into two 30-sec steps separated by a dark interval of about 20 sec. Reagents were added in the dark in 1-ml volumes of Tricine–NaCl buffer to the 25-ml chloroplast suspensions. The final concentration of [^{32}P]P_i was 1 mM; of [^{3}H]AMP, 3.2×10^{-5} M; and of Na_2SO_4, 10^{-2} M. The specific radioactivity of [^{32}P]P_i was 8.0×10^{12} cpm/mol, and of [^{3}H]-AMP 9×10^{12} cpm/mol. Preparation and assay of radionucleotide coupling factor complexes were performed as described in *Methods*. Counting efficiency for ^{32}P was 95%, and for ^{3}H 25%.

At least three alternative mechanisms can be visualized to account for the fact that added ADP causes a disappearance of [^{32}P]ADP from the coupling factor: (*a*) the enzyme-bound [^{32}P]ADP exchanges with the unlabeled ADP; (*b*) the added ADP induces the incorporation of [^{32}P]P_i from the pool into the enzyme-bound [^{32}P]ADP, which then is released from the *membrane-bound* enzyme as [β,γ-^{32}P]ATP; (*c*) the enzyme-bound [^{32}P]ADP serves as a donor of phosphate to the unlabeled substrate ADP, resulting in the synthesis of [γ-^{32}P]-ATP and unlabeled AMP, which are then released from the coupling factor. (Presumably, the constraints on release of

TABLE 4. *Depletion of enzyme-bound ADP by addition of exogenous ADP to illuminated membranes*

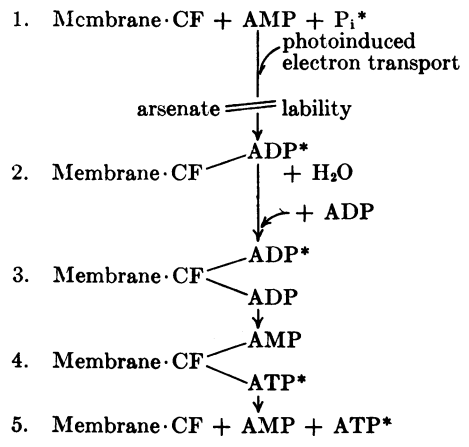
Sample	Additions (M): Step 1: [^{3}H]AMP	Step 1: [^{3}H]AMP	Step 1: [^{31}P]P_i	Step 1: [^{32}P]P_i	Step 2: AsO_4	Step 2: ADP	Moles of isotope bound/mole of CF_1: ^{3}H	Moles of isotope bound/mole of CF_1: ^{32}P
1	—	10^{-4}	10^{-3}	—	—	—	0.56	—
2	—	10^{-4}	10^{-3}	—	10^{-2}	—	0.53	—
3	—	10^{-4}	10^{-3}	—	—	3×10^{-3}	0.18	—
4	—	10^{-4}	10^{-3}	—	10^{-2}	3×10^{-3}	0.16	—
5	10^{-4}	—	—	10^{-3}	10^{-2}	—	—	0.59
6	10^{-4}	—	—	10^{-3}	—	3×10^{-3}	—	0.16
7	10^{-4}	—	—	10^{-3}	10^{-2}	3×10^{-3}	—	0.15

Salt-washed chloroplast membranes were suspended in Tricine–NaCl buffer as described in *Methods*, and made 2×10^{-5} M in pyocyanine. Final concentrations of other reagents in the illumination mixture are given in the table, together with the extent of labeling of the coupling factor in each sample, determined as described in *Methods*. The illumination period was divided into two steps. In the first step, AMP and phosphate were present, in order to form the enzyme·ADP complex. In the first four samples, [^{3}H]AMP was used as a tracer, and in the last three samples [^{32}P]P_i was used. After illumination for 60 sec, arsenate alone, ADP alone, or arsenate *and* ADP were added (step 2). Illumination was continued for an additional 15 sec. The membranes were then chilled and the coupling factors were isolated from them as already described. The specific radioactivity of the [^{3}H]AMP was 7.6×10^6 cpm/μmol; of [^{32}P_i]P, 1.41×10^7 cpm/μmol.

these products are relaxed in the membrane-bound state of the coupling factor.)

To resolve these alternatives, it is necessary to alter the experimental design in order to quantitate both the formation of [^{32}P]ADP and the transfer, if any, of the ^{32}P label onto the added, substrate ADP. Experiments of this type have been performed in this laboratory, and will be reported in another paper (manuscript in preparation). The new experiments appear to favor, but do not as yet unequivocally prove, the third alternative.

To summarize, the data presented here and in the previous communication (4) can be accounted for by a number of alternative mechanisms, of which the following seems the most likely:



The overall balance of this reaction should be:

$$\text{Membrane}\cdot\text{CF} + \text{AMP} + \text{P}_i^* + \text{ADP} \xrightarrow{\text{Light}} \text{Membrane}\cdot\text{CF} + \text{AMP} + \text{ATP}^* + \text{H}_2\text{O}.$$

It should be pointed out that the product of step 1, i.e., membrane·CF·ADP* may be formed via an alternative, less direct route. The activated [^{32}P]P$_i$ could be used to esterify *endogenous* ADP to produce [γ-^{32}P]ATP, which is then used by the membrane·coupling factor complex in a reverse myokinase-type reaction to phosphorylate an enzyme-bound AMP, yielding enzyme-bound [^{32}P]ADP and free ADP. So far, we have not obtained evidence for this alternative, but due to the presence of endogenous nucleotides in the chloroplast-membrane preparations, our findings cannot rule it out either. Within the context of our present understanding of the system, we like to believe that if this alternative is operating at all, it does not lie within the main path of the synthesis of the membrane·CF·ADP* complex, at least under the present experimental conditions.

The transition from step 3 to step 4 is meant to indicate that one site contains the donor phosphate, in the form of ADP, and the other site contains the acceptor, substrate ADP.

The above model is compatible with the findings of workers using ^{18}O-exchange analysis (7, 8), the bridge oxygen between the β and γ phosphates of the product ATP can be portrayed as deriving from the acceptor ADP molecule.

The model makes no implications, beyond what is explicitly indicated in it, although each transition portrayed in it may very well be composed of further partial reactions; future experiments will undoubtedly necessitate its revision.

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1. Howell, S. H., and E. N. Moudrianakis, *Proc. Nat. Acad. Sci. USA*, **58**, 1261 (1967).
2. Vambutas, V. K., and E. J. Racker, *J. Biol. Chem.*, **240**, 2660 (1965).
3. Karu, A. E., and E. N. Moudrianakis, *Arch. Biochem. Biophys.*, **129**, 655 (1969).
4. Roy, H., and E. N. Moudrianakis, *Proc. Nat. Acad. Sci. USA*, **68**, 464 (1971).
5. Jagendorf, A. T., and M. M. Margulies, *Arch. Biochem. Biophys.*, **90**, 184 (1960).
6. Krogmann, D. W., A. T. Jagendorf, and M. Avron, *Plant Physiol.*, **34**, 272 (1959).
7. Avron, M., and N. Sharon, *Biochem. Biophys. Res. Commun.*, **2**, 336 (1960).
8. Schultz, A. R., and P. D. Boyer, *Arch. Biochem. Biophys.*, **93**, 335 (1961).
9. Avron, M., A. T. Jagendorf, and M. Evans, *Biochim. Biophys. Acta*, **26**, 262 (1957).
10. Jagendorf, A. T., and M. Avron, *Arch. Biochem. Biophys.*, **80**, 246 (1959).
11. Asada, H., R. Deura, and Z. Kasai, *Plant Cell Physiol.*, **9**, 143 (1968).
12. Ryrie, I. J., and A. T. Jagendorf, *J. Biol. Chem.*, **246**, 582 (1971).