Plant Physiol. (1980) 66, 242–245
0032-0889/80/66/0242/04/$00.50/0

A Proposed Mechanism for the Stimulatory Effect of Bicarbonate Ions on ATP Synthesis in Isolated Chloroplasts[1]

Received for publication October 26, 1979 and in revised form March 6, 1980

WILLIAM S. COHEN AND WENDY A. MACPEEK
T. H. Morgan School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40506

ABSTRACT

The effect of bicarbonate ions on induction of Mg^{2+}-ATPase activity, on the *N*-ethylmaleimide inhibition of phosphorylation and on energy-dependent adenine nucleotide exchange has been examined with pea seedling chloroplasts. Incubation of chloroplasts with *N*-ethylmaleimide in the presence of 15 millimolar bicarbonate in the light results in enhanced inhibition of ATP synthesis when the preillumination pH is maintained between 7.0 and 7.5. Bicarbonate also enhances Mg^{2+}-ATPase activity when it is included in the light-triggering stage at pH 7.0. The conditions (medium pH, bicarbonate concentration, etc.) for demonstrating the bicarbonate-induced enhancement of the *N*-ethylmaleimide inhibition and ATPase activity are similar to those required for the direct effect of bicarbonate on phosphorylation. Bicarbonate, under the same conditions, does not affect adenine nucleotide exchange (binding or release). It is concluded that the stimulatory effect of bicarbonate on ATP synthesis may be related to its ability to alter directly the conformation of the chloroplast coupling factor under conditions (suboptimal pH) where the enzyme shows minimal activity.

ATP synthesis is stimulated by bicarbonate in chloroplasts which lack the capacity to fix CO_2 (2, 8, 10, 20, 21). It had been suggested (8) that the site of action for bicarbonate in producing this effect is at the level of the interaction between the energized thylakoid membrane and the CF_1[2]. In the present study, the effect of bicarbonate on the following light-dependent reactions: Mg^{2+}-ATPase activity (3, 4), [^{32}P]Pi-ATP exchange activity (6), inhibition of phosphorylation by maleimides (11, 13, 14, 16) and adenine nucleotide exchange (5, 9, 15, 19, 22, 25, 27) has been examined to determine if the bicarbonate induced increase in the rate of ATP synthesis is accompanied by alterations in the conformational state of the coupling factor. Bicarbonate enhances both Mg^{2+}-ATPase activity and [^{32}P]Pi-ATP exchange activity when it is included in the light triggering stage at pH 7.0. Bicarbonate also enhances the NEM inhibition of phosphorylation at suboptimal pH values and partially diminishes the ability of adenylates to protect against NEM inhibition. The reduced ability of adenylates to protect does not appear to be related to an effect of bicarbonate either on binding to or release of nucleotides from energized thylakoids.

[1] This research was supported by the National Science Foundation Grant PCM 76-17214 and a grant from the Major Equipment Fund of the University of Kentucky.

[2] Abbreviations: CF_1: chloroplast coupling factor, NEM: *N*-ethylmaleimide; Mops: morpholinopropanesulfonic acid; DTE: dithioerythritol.

MATERIALS AND METHODS

Chloroplast Isolation. Chloroplasts were isolated from pea seedlings (*Pisum sativum* var. Laxton's Progress) grown in a controlled growth facility (7). Pea shoots were ground for 5 s in a Waring Blendor in 400 mM sorbitol, 50 mM Mes-NaOH (pH 6.5), 10 mM NaCl, 2.5 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 2.5 mM ascorbic acid, and 0.2% crystalline BSA. The homogenate was strained through 16 layers of cheese cloth and the chloroplasts collected by centrifugation at 6,000*g* for 5 min. The chloroplasts were washed once (6,000*g* for 5 min.) and resuspended in a small volume of the following medium: 400 mM sorbitol, 50 mM Hepes-NaOH (pH 7.2), 10 mM NaCl, 2.5 mM $MgCl_2$, 1 mM $MnCl_2$, and 2 mM EDTA. Chl concentration was estimated according to Arnon (1).

NEM Inhibition and Phosphorylation Assays. Inhibition of ATP synthesis in pea seedling chloroplasts by NEM was carried out as previously described (7). The preillumination stage contained (in 2 ml): 50 mM KCl, 25 mM Tricine-NaOH (pH 7.5), 5 mM $MgCl_2$, 15 μM pyocyanine, ±1.5 mM NEM, and chloroplasts equivalent to 90–100 μg Chl. Photophosphorylation in control and inhibited chloroplasts was assayed (7) in 1-ml reaction mixtures containing 50 mM KCl, 50 mM Tricine-NaOH (pH 8.1), 5 mM $MgCl_2$, 1 mM ADP, 5 mM K_2HPO_4 (containing 3–7 × 10^5 cpm of ^{32}P), 10 μM pyocyanine and chloroplasts equivalent to 18–20 μg Chl.

Mg^{2+}-ATPase Activity and [^{32}P]Pi-ATP Exchange Activity. Mg^{2+}-ATPase activity and [^{32}P]Pi-ATP exchange activity were assayed as described by McCarty and Racker (12). The activation mixture (in 2 ml) contained: 50 mM KCl, either 25 mM Mops-NaOH (pH 7.0) or 25 mM Tricine-NaOH (pH 8.0), 5 mM $MgCl_2$, 25 μM pyocyanine, 10 mM DTE, and chloroplasts equivalent to about 200 μg Chl. Samples were illuminated for 5 min with heat-filtered white light (4 × 10^5 ergs cm^{-2} s^{-1}) at 20 C. For assay, 0.075- or 0.10-ml aliquots of this mixture were pipetted into a solution which contained 50 mM Tricine-NaOH (pH 8.1), 5 mM $MgCl_2$, 5 mM ATP and 5 mM K_2HPO_4 (labeled with 10^6 cpm of ^{32}P) when exchange was measured. After 20 min at 37 C, trichloroacetic acid was added to a final concentration of 2% and Pi release or ^{32}P incorporation into ATP was determined as described elsewhere (7).

Binding of Labeled Nucleotides to Thylakoid Membranes. The binding mixture contained (in 3 ml): 50 mM KCl, 25 mM Mops-NaOH (pH 7.1) or 25 mM Tricine-NaOH (pH 8.1), 5 mM $MgCl_2$, 15 μM [^{3}H]ADP (1.3 × 10^5 cpm/nmol), 50 μM pyocyanine, ±15 mM $KHCO_3$ and chloroplasts equivalent to about 300 μg Chl. Samples were illuminated for 45 s with heat-filtered white light (4.5 × 10^5 ergs cm^{-2} s^{-1}) in a cuvette maintained at 19 C. After illumination the chloroplasts were collected by centrifugation (6,000*g*), washed three times (6,000*g*) with 5 ml of a medium containing 400 mM sorbitol, 50 mM Hepes-NaOH (pH 7.5), 10 mM NaCl, and finally resuspended in 0.5 ml of the sorbitol-Hepes-NaCl medium. Determination of the amount of nucleotide bound was performed in the following way; 150 μl chloroplast suspension was mixed with 50 μl ice-cold 23% $HClO_4$ in a 400-μl microcen-

trifuge tube and stored on ice for 10 min. Acid-denatured material was removed by centrifugation (90 s) at top speed in a Beckman Microfuge. Aliquots (50 μl) of the supernatant were removed, added to scintillation vials containing 3 ml Biofluor, and radioactivity was determined with a Beckman series No. 200 liquid scintillation counter.

Release of Bound Nucleotides. Prelabeled ([^{3}H]ADP) thylakoid membranes (equivalent to 20 μg Chl) were illuminated (incident light intensity = 10^5 ergs cm^{-2} s^{-1}), with a specially built setup in the Beckman Microfuge. The 200-μl reaction mixture contained 100 mM sorbitol, 20 mM Mops-NaOH (pH 7.1), 5 mM $MgCl_2$, 1 mM ADP, 1 mM K_2HPO_4, 33 μM pyocyanine, and 15 mM bicarbonate where indicated. Samples were illuminated for 15 s, after extinguishing the light the chloroplasts were immediately pelleted by accelerating the centrifuge to top speed for 60 s. The release of label into the supernatant was determined using 50-μl aliquots.

Chemicals. ^{32}P, [^{3}H]ADP, and Biofluor were obtained from New England Nuclear. Adenine nucleotides and NEM were purchased from Sigma. $KHCO_3$ solutions were prepared daily, titrated to the pH of the appropriate assay and stored in tightly stoppered containers prior to use.

RESULTS

Preliminary experiments, in which light-dependent conformational changes of membrane-bound CF_1 were measured at pH 7.2 by hydrogen exchange (3, 4) into the protein, indicated that 20 mM $KHCO_3$ enhanced light-dependent tritium incorporation 2-fold (W. S. Cohen and I. J. Ryrie, unpublished). A simpler and more rapid technique, the irreversible NEM inhibition of phosphorylation (11, 13, 14, 16), was chosen more recently to examine the effect of bicarbonate ions on the conformation of CF_1. Chloroplasts are illuminated in the presence of NEM and a cofactor of electron transport. After illumination a thiol reagent (DTE) is added to remove unreacted NEM, and an aliquot of the NEM-treated chloroplasts is then assayed for phosphorylation activity. This method has the advantage of permitting an examination of the effect of bicarbonate on the conformational state of CF_1 (addition to preillumination stage), while minimizing the direct effect of bicarbonate on ATP synthesis (phosphorylation stage).

Bicarbonate included with NEM in the preillumination stage enhanced NEM inhibition 1.79-fold at pH 7.2, whereas at pH 8.2 it had no effect (Table I). Bicarbonate in the phosphorylating medium affected ATP synthesis in a similar fashion as a function of medium pH. In 11 different experiments, enhancement varied between 1.1- and 1.8-fold at pH 7.0–7.5. Bicarbonate (15 mM) at pH 7.5 also enhanced the NEM and light-dependent inhibition of phosphorylating electron transport with ferricyanide as acceptor (data not shown).

Like the direct effect of bicarbonate on phosphorylation (8), the enhancement of the NEM inhibition requires 15–30 mM bicarbonate for maximal activity (Table II). Malate (5 mM), which also stimulates phosphorylation in isolated chloroplasts, did not affect the level of NEM inhibition at pH 7.2 (data not shown).

The enhancement of the NEM inhibition by bicarbonate at suboptimal pH values was even more pronounced when small amounts of adenine nucleotides (ADP or ATP) were included in the preillumination stage (Table III). This effect was most striking at nucleotide concentrations of 10 μM or above (Fig. 1). Arsenate

Table I. *Effect of Preillumination Stage pH on the Bicarbonate Enhancement of the NEM Inhibition*

Assays were performed as described under "Materials and Methods" except that the preillumination stage was buffered with either 25 mM Mops-NaOH (pH 7.2) or 25 mM Tricine-NaOH (pH 8.2). The direct effect of bicarbonate on phosphorylation was assayed in a reaction mixture similar to the preillumination mixture with the addition of 1 mM ADP and 5 mM [^{32}P]KH_2PO^4. When added, bicarbonate was present at a final concentration of 15 mM. The numbers in parentheses represent the per cent inhibition of phosphorylation by NEM.

pH	$KHCO_3$	Preillumination – NEM	Preillumination + NEM	Direct Effect of Bicarbonate on ATP Synthesis in Absence of Preillumination
			μmol ATP/mg Chl·h	
	–	619	519 (16.1)	351
7.2	+	619	441 (28.8)	533
	–	587	117 (80.1)	656
8.2	+	587	126 (78.6)	696

Table II. *Enhancement of the NEM Inhibition of Phosphorylation as a Function of Bicarbonate Concentration*

The conditions were similar to those described under "Materials and Methods." The control rate of ATP synthesis (preilluminated minus NEM, HCO_3, etc) was 578 μmol/mg Chl·h.

HCO_3^- Added to Preillumination Stage	Inhibition of Phosphorylation by NEM
mM	%
0	22.3
7.5	29.3
15.0	35.4
30.0	40.6

Table III. *Effect of Adenylates on the Bicarbonate-induced Enhancement of the NEM Inhibition*

The conditions were similar to those described in the legend to Table I. The control rates of ATP synthesis were 555 and 590 μmol/mg Chl·h (preilluminated minus NEM) in experiments I and II, respectively. When added, bicarbonate was present at 15 mM. The numbers in parentheses represent the degree of enhancement by bicarbonate.

Experiment	Condition	Inhibition of Phosphorylation	
		Preillumination in Absence of Bicarbonate	Preillumination in Presence of Bicarbonate
		%	
	NEM	35.6	40.1(1.13)
I	NEM + 10 μM ADP	10.4	25.5(2.45)
	NEM	33.3	42.5(1.28)
II	NEM + 15 μM ATP	8.5	18.8(2.18)

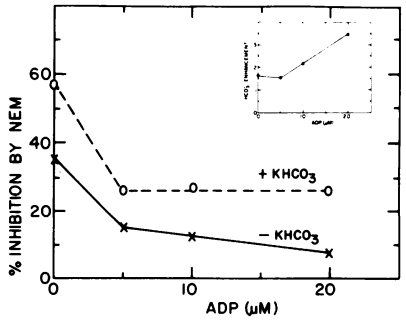

FIG. 1. Enhancement of the NEM inhibition of phosphorylation by bicarbonate as a function of ADP concentration in the preillumination stage. The pH of the preillumination stage was 7.5, and the control rate of ATP synthesis (preilluminated minus NEM) was 738 μmol ATP/mg Chl·h.

(1 mM) neither potentiated nor diminished the enhancement (data not shown).

The reduced ability of adenine nucleotides to protect against inhibition by NEM when bicarbonate is present could be related to an alteration in the binding properties of the nucleotides, thus the effect of bicarbonate on energy-dependent adenine nucleotide exchange was examined. Neither light-induced binding or release of labeled ADP is altered significantly by bicarbonate (Tables IV and V). Pyocyanine-catalyzed phosphorylation was stimulated 1.4- to 1.5-fold in chloroplasts used in the binding and release studies.

In a previous communication (8), we reported that bicarbonate did not have an effect on the induction of light-triggered Mg^{2+}-ATPase activity. In those studies bicarbonate was added to the activation stage only at pH 8.0. In the present investigation the experimental protocol for assaying bicarbonate effects on the light- and thiol-dependent Mg^{2+}-ATPase was similar to the one employed to monitor bicarbonate effects on the NEM inhibition. Bicarbonate was added to the light-triggering stage, either at pH 7.0 or 8.0, and ATP hydrolysis was assayed at pH 8.1 in the dark. Under these conditions, an enhancement of ATPase activity could be observed when bicarbonate was present in the light-triggering stage at pH 7.0 and a small amount of NH_4Cl (1 mM) was included in the assay stage (Table VI). Light-triggered [^{32}P]Pi-ATP exchange activity was also enhanced (20–30%) when bicarbonate was included in the activation stage at pH 7.0 and exchange activity was assayed at pH 8.1. (data not shown). Bicarbonate-induced increases in exchange activity did not exhibit a requirement for NH_4Cl in the assay medium.

Table IV. *Effect of Bicarbonate on the Energy-dependent Binding of Labeled ADP to Thylakoid Membranes*

Binding was assayed as described under "Materials and Methods." The Chl concentration in the illumination stage was 99 μg/ml Chl in experiment I and 106 μg/ml Chl in experiment II. When added, bicarbonate was present at a final concentration of 15 mM.

Experiment	pH	[^{3}H]ADP Bound		
		−$KHCO_3$	+$KHCO_3$	+$KHCO_3$ / −$KHCO_3$
		nmol/mg Chl		
I	7.1	0.82	0.80	1.0
I	8.1	0.73	0.74	1.0
II	7.1	0.92	0.98	1.1

Table V. *Effect of Bicarbonate on the Energy-dependent Release of ADP from Prelabeled Thylakoid Membranes*

Release of [^{3}H]ADP was assayed as described under "Materials and Methods." The Chl concentration was 21.1 μg/ml Chl in experiment I and 21.5 μg/ml Chl in experiment II. 0.59 nmol of [^{3}H]ADP and 0.95 nmol [^{3}H]ADP were bound to the thylakoid membranes in experiments I and II, respectively.

Experiment	Addition	[^{3}H]ADP Released	+$KHCO_3$ / −$KHCO_3$
		nmol/mg Chl	
I	—	0.55	
	15 mM $KHCO_3$	0.53	1.0
II	—	0.76	
	15 mM $KHCO_3$	0.72	1.0

Table VI. *Effect of Bicarbonate on the Activation of Mg^{2+}-ATPase Activity*

ATP synthesis and Mg^{2+}-ATPase activity were assayed as described under "Materials and Methods." When added, bicarbonate and NH_4Cl were present at 15 and 1 mM, respectively. The numbers in parentheses represent the degree of enhancement by bicarbonate.

Activation Stage pH	Present in: Activation Stage $KHCO_3$	Present in: Assay Stage NH_4Cl	Mg^{2+}-ATPase Activity	Direct Effect on ATP Synthesis
			μmol/mg Chl·h	
7	−	−	146	
	+	−	155(1.06)	
	−	+	135	402
	+	+	182(1.35)	568(1.42)
8	−	−	159	
	+	−	155(0.98)	
	−	+	334	729
	+	+	368(1.10)	798(1.06)

DISCUSSION

The stimulation of ATP synthesis by bicarbonate in isolated chloroplasts is not accompanied by large effects on the rate of phosphorylating electron flow (2, 21), on the magnitude of the transmembrane proton concentration gradient (A. T. Jagendorf, personal communication), or on the apparent K_m values for the phosphorylation substrates (ADP or Pi [8]). A correlation exists, though, between the effect of phosphorylation inhibitors on the stimulation of ATP synthesis by bicarbonate and their effect on energy-dependent conformational changes of the coupling factor. Uncoupling agents (*e.g.* amines) inhibit both the bicarbonate stimulation (2; W. S. Cohen, unpublished) and the conformational changes (5, 16, 24) whereas energy transfer inhibitors (*e.g.* Dio-9, deoxyphlorizin) (5, 8, 24) do not. Thus, a possible site of action for the bicarbonate effect might be at the level of the conformational state of CF_1.

Activation of Mg^{2+}-ATPase activity (and [^{32}P]Pi-ATP exchange), the ability of NEM to inhibit phosphorylation, and light-induced ^{3}H-exchange all involve energy-dependent changes in the conformation of the coupling factor (3, 4, 6, 15, 18, 23, 24). The conditions required for obtaining the bicarbonate-induced enhancement of these activities are rather similar to those required for the direct effect of bicarbonate on phosphorylation, *i.e.* medium pH (2, 8, 19, 20), bicarbonate concentration (2, 8, 20, 21), lack of a period of prior CO_2 depletion (2, 20), etc. Thus, the ability of bicarbonate to stimulate ATP synthesis at pH 7.0 appears to be correlated with its ability to alter the conformational state of CF_1.

The requirement for a small amount of NH_4Cl in the assay medium to observe maximal ATPase activity at pH 8.1 probably reflects a rate limitation to ATP-driven proton accumulation in the dark that is relieved by the uncoupler (12). The observation that bicarbonate enhances ATPase activity only when NH_4Cl is included in the assay medium may possibly be explained by the coupling factor's "pH 8-like" properties at pH 7.0 after activation in the presence of bicarbonate.

The partial protection from the NEM inhibition afforded by adenylates indicates that adenylates can modify the conformation of CF_1 such that the site that NEM reacts with is now less accessible (14). ADP (or ATP) can still modify the conformation of CF_1 in the presence of bicarbonate but not to the same extent

as in its absence. This could explain the enhanced bicarbonate effect on the level of the NEM inhibition at higher ADP concentrations. The ability of bicarbonate to partly nullify the protective effects of adenylates should be contrasted with the action of ions like phosphate and arsenate (14) which enhance the protection by adenylates or with selenate or sulfate ions which have little or no effect (A. Grebanier, personal communication).

The decrease in the ability of adenine nucleotides to protect against inhibition by NEM when bicarbonate is present could be related either to a decrease in the ability of nucleotides to bind to CF_1 or to an increased release of bound nucleotides (17). However, in chloroplasts in which phosphorylation is stimulated by bicarbonate there is little or no effect on binding or release of adenylates (nucleotide exchange). In other experiments we observed that bicarbonate had no effect on the total amount of ATP bound to pea thylakoid membranes under energized conditions (W. MacPeek and W. S. Cohen, unpublished). These data seem to indicate a direct effect of bicarbonate on the conformation of CF_1 rather than an indirect effect mediated by tightly bound nucleotides.

Acknowledgments—The authors thank Drs. Richard McCarty and Andre Jagendorf for helpful discussions.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. BATRA PP, AT JAGENDORF 1965 Bicarbonate effects on the Hill reaction and photophosphorylation. Plant Physiol 40: 1074–1079
3. BAKKER-GRUNWALD T 1977 ATPase. *In* A Trebst, M Avron, eds, Encyclopedia of Plant Physiology, New Series, Vol V. Springer-Verlag, Berlin, pp 369–373
4. BAKKER-GRUNWALD T, K VAN DAM 1974 On the mechanism of activation of the ATPase in chloroplasts. Biochim Biophys Acta 347: 290–298
5. BICKEL-SANDKOTTER S, H STROTMANN 1976 Effects of external factors on photophosphorylation and exchanges of CF_1 bound nucleotides. FEBS Lett 65: 102–106
6. CARMELI C 1977 Exchange Reactions. *In* A Trebst, M Avron, eds, Encyclopedia of Plant Physiology, New Series, Vol V, Springer-Verlag, Berlin, pp 492–500
7. COHEN WS 1978 The coupling of electron flow to ATP synthesis in pea chloroplasts stored in the presence of glycerol at −70°C. Plant Sci Lett 11: 191–197
8. COHEN WS, AT JAGENDORF 1974 Further studies on the bicarbonate stimulation of photophosphorylation in isolated chloroplasts. Plant Physiol 53: 220–223
9. HARRIS DA 1978 The interactions of coupling ATPases with nucleotides. Biochim Biophys Acta 463: 245–273
10. JAGENDORF AT 1971 Proton flux and ATP synthesis. *In* G Forti, M Avron, A Melandri, eds, Proc 2nd Int Congr Photosynthesis Dr W Junk, The Hague, pp 1057–1064
11. MCCARTY RE 1977 The reaction of coupling factor 1 in chloroplast thylakoids with N-substituted maleimides. *In* DO Hall, J Coombs, TW Goodwin, eds, Proc 4th Int Congr Photosynthesis. Biochemical Society, London, pp 571–579
12. MCCARTY RE, E RACKER 1968 Partial resolution of the enzymes catalyzing photophosphorylation. III. Activation of adenosine triphosphatase and ^{32}P labeled orthophosphate-adenosine triphosphate exchange in chloroplasts. J Biol Chem 243: 129–137
13. MCCARTY RE, J FAGAN 1973 Light stimulated incorporation of N-ethylmaleimide into coupling factor 1 in spinach chloroplasts. Biochemistry 12: 1503–1507
14. MAGNUSSON RP, RE MCCARTY 1976 Influence of adenine nucleotides on the inhibition of photophosphorylation in spinach chloroplasts by N-ethylmaleimide. J Biol Chem 250: 2593–2598
15. MAGNUSSON RP, RE MCCARTY 1976 Light induced exchange of nucleotides into coupling factor 1 in spinach chloroplast thylakoids. J Biol Chem 251: 7417–7422
16. MCCARTY RE, PR PITTMAN, Y TSUCHIYA 1972 Light dependent inhibition of photophosphorylation by N-ethylmaleimide. J Biol Chem 247: 3048–3051
17. NELSON N, H NELSON, E RACKER 1972 Partial resolution of the enzymes catalyzing photophosphorylation. XI. Magnesium-adenosine triphosphate properties of heat-activated coupling factor I from chloroplasts. J Biochem 244: 6506–6510
18. PORTIS AR, RP MAGNUSSON, RE MCCARTY 1975 Conformational changes in coupling factor 1 may control the rate of electron flow in spinach chloroplasts. Biochem Biophys Res Commun 64: 377–384
19. O'KEEFE D, RA DILLEY 1977 The role of the membrane potential in the tight binding of adenine nucleotides of chloroplast CF_1. FEBS Lett 81: 105–110
20. PUNNETT T 1965 Influence of growth conditions on the enhancement of photophosphorylation by carbon dioxide. Plant Physiol 40: 1283–1284
21. PUNNETT T, RV IYER 1964 The enhancement of photophosphorylation and the Hill reaction by carbon dioxide. J Biol Chem 229: 2335–2339
22. REIMER S, BR SELMAN 1978 Tentoxin-induced energy-independent adenine nucleotide exchange and ATP'ase activity with chloroplast coupling factor 1. J Biol Chem 253: 7249–7255.
23. RYRIE IJ, AT JAGENDORF 1971 An energy-linked conformational change in the coupling factor protein in chloroplasts. Studies with hydrogen exchange. J Biol Chem 246: 3771–3774
24. RYRIE IJ, AT JAGENDORF 1972 Correlation between a conformational change in the coupling factor protein and the high energy state in chloroplasts. J Biol Chem 247: 4453–4459
25. SHAVIT N 1977 Bound nucleotides and conformational changes in photophosphorylation. *In* A Trebst, M Avron, eds, Encyclopedia of Plant Physiology, New Series, Vol V. Springer-Verlag, Berlin, pp 350–357
26. STROTMANN H, S BICKEL, B HUCHZERMEYER 1976 Energy-dependent release of adenine nucleotides tightly bound to chloroplast coupling factor CF_1. FEBS Lett 61: 194–198
27. WITT HT, E SCHLODDER P GRABER 1977 Conformational change, ATP generation, and turnover rate of the chloroplast ATP'ase analyzed by energization with an external electrical field. *In* L Packer, ed, Bioenergetics of Membranes. Elsevier, Amsterdam, pp 447–457