BBA 45536

THE RELATION OF ELECTRON TRANSPORT AND PHOTOPHOSPHORYLATION TO CONFORMATIONAL CHANGES IN CHLOROPLASTS

NOUN SHAVIT AND MORDHAY AVRON

Biochemistry Section, Weizmann Institute of Science, Rehovoth (Israel)

(Received August 19th, 1966)

SUMMARY

1. The rate of the Hill reaction and photophosphorylation, and the ratio of ATP produced to the electron flow are shown to be strongly dependent on the solute concentration of the medium.

2. A large part, but not all, of the requirement for $MgCl_2$ or phosphate in photophosphorylation can be replaced by $SrCl_2$ or other solutes.

3. In two-stage photophosphorylation, solutes are required during the light-activation stage.

4. The presence of solutes causes marked changes in the packed volume of the chloroplasts, and their light-scattering properties. These changes are essentially complete within 1 min.

5. The effectiveness of solutes in enhancing the rate of electron transport and photophosphorylation parallels their effectiveness in inducing conformational changes in chloroplasts.

6. It is suggested that the solutes act by inducing a conformational change of the chloroplast structure which is more optimal for electron transfer and coupled phosphorylation.

INTRODUCTION

A strong dependence of the rate of electron flow and photophosphorylation in several photoreactions of chloroplasts upon the osmotic concentration of the medium has been previously reported[1]. It is well substantiated that chloroplasts undergo volume changes as the osmotic concentration of the medium is varied[2–6]. Also, several reports have appeared recently which correlated conformational changes in chloroplasts[7–10] with the formation of a high-energy, non-phosphorylated intermediate in photophosphorylation[11,12].

This communication will report several observations designed to clarify the relationship, if any, between the dependence of the rate of electron flow and photophosphorylation, the formation of a non-phosphorylated high-energy intermediate, conformational changes in chloroplasts and the solute concentration of the medium.

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; PMS, phenazinemethosulphate.

METHODS

Isolated chloroplasts and chloroplast fragments from swiss-chard leaves were prepared as previously described[13]. The final pellet of "once-washed chloroplasts" was suspended in Tris–HCl buffer (0.01 M, pH 7.8). Assays for ferrocyanide produced and ATP formed were as previously described[18,25]. Reaction mixtures for photophosphorylation assay contained in a total volume of 3 ml (in μmoles): Tris–HCl, 4; $MgCl_2$, 3; ADP, 1; orthophosphate, 4 (containing $1 \cdot 10^6$ counts/min $^{32}P_i$); PMS, 0.1 or ferricyanide, 1; and chloroplasts containing 30–60 μg chlorophyll. The pH was 7.8; illumination time, 2 min; light intensity, 180000 lux; temperature, 20°; gas phase, air.

For the two-stage photophosphorylation assay two reaction mixtures were prepared: the light-stage reaction mixture contained in a volume of 1.5 ml the following components (in μmoles): maleate buffer, 3; Tris–HCl, 1; PMS, 0.05; and chloroplasts containing 80–120 μg chlorophyll. The pH was 6.4. The reaction mixture was taken up in a chilled 2-ml syringe and illuminated for 15 sec at 90000 lux. Following the illumination, the syringe contents were immediately injected into a dark-reaction mixture[12]. The dark-reaction mixture contained in a volume of 1.5 ml (in μmoles): Tris–HCl, 6; $MgCl_2$, 3; ADP, 2; and orthophosphate, 2 (containing $2 \cdot 10^6$ counts/min $^{32}P_i$). The pH was 8.4. After mixing both reaction mixtures, the pH was 7.8; temperature, 4°. Reactions were stopped by addition of trichloroacetic acid to a final concentration of 3% (v/v).

Absorbance measurements were made at room temperature in a Zeiss spectrophotometer model PMQ II or a Cary recording spectrophotometer model 14. Chloroplasts containing 30–60 μg chlorophyll were suspended in a final volume of 3 ml containing (in μmoles): Tris–maleate (pH 7.8) or Tris–HCl (pH 7.8), 9; and PMS, 0.1. Additives, as specified for each experiment, were added in a volume of 0.01–0.2 ml. Differential absorption spectrum and absorbance changes at 510 mμ were measured with the same mixture divided between two cuvettes. ΔA/min refers to the change in "absorbance" observed during the first minute. Packed volume was determined after centrifugation for 15 min at 12000 $\times$ g in capillary tubes.

RESULTS

Electron transport and photophosphorylation

In order to be able to measure the effect of added solutes on the rates of electron flow and phosphorylation the concentrations commonly used for the ingredients of the reaction mixture were reduced. Under these conditions, addition of a solute brought about a several-fold increase in the rate of reaction (Fig. 1). The observed extent of stimulation depended upon the particular chloroplast preparation and the initial concentration of solute in the reaction mixture. Usually, enhancements of 5- to 10-fold were observed. The increase in the rate of electron flow with ferricyanide as electron acceptor is shown in Fig. 1. A similar increase with other electron acceptors (DCIP, NADP) has been previously observed[1]. Fig. 1 also shows that increasing the concentrations of $MgCl_2$, $SrCl_2$, $CaCl_2$, NaCl or sucrose in the reaction mixtures also had a pronounced effect on the rates of phosphorylation with PMS or that coupled to the reduction of ferricyanide. Moreover, the ATP/$2e^-$ ratio at different solute concentrations showed an increase from values of 0.3–0.5 to 1.0–1.3 (Fig. 1).

It should be emphasized that this increase in the efficiency of ATP formation was attained by the mere addition to the reaction mixture of solutes such as sucrose, NaCl, or $SrCl_2$ which by themselves do not enhance ATP formation. Even $CaCl_2$, which has been shown to act as an inhibitor of phosphorylation in a manner competitive with Mg^{2+} (ref. 14), can be seen to stimulate phosphorylation at concentrations up to 4–5 mM, when the ratio of Ca^{2+}/Mg^{2+} was no higher than 5 (Fig. 1). The inhibitory effect was observed only at higher concentrations. No inhibition of electron flow by Ca^{2+} ions in the absence of phosphate-acceptor system was observed, in agreement with the findings of JAGENDORF AND AVRON[14].

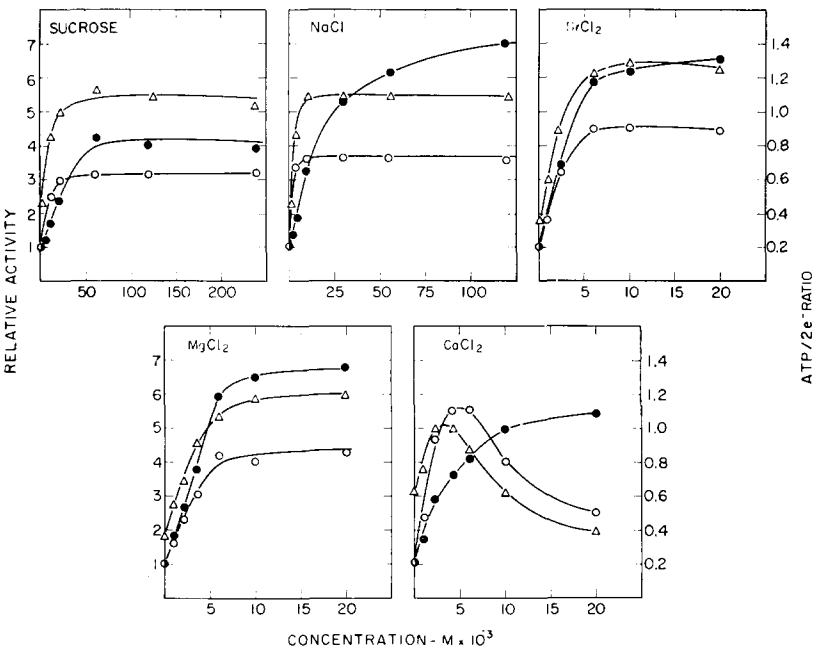


Fig. 1. The effect of the concentration of solutes on the rates of ferricyanide reduction, of phosphorylation coupled to ferricyanide reduction, and of PMS-catalysed phosphorylation. Experimental details as described in METHODS. ○, PMS-catalysed phosphorylation (relative activity of 1 equivalent to 150–200 μmoles ATP formed/mg chlorophyll per h); ●, ferricyanide reduction in the absence of $MgCl_2$, ADP and phosphate (relative activity of 1 equiv to 30–60 μmoles ferricyanide reduced/mg chlorophyll per h); △, ATP/$2e^-$ ratio calculated for phosphorylation with ferricyanide as the electron acceptor.

Sucrose has been found less effective than NaCl in the stimulation of electron flow with ferricyanide (Fig. 1). However, the extent of stimulation of phosphorylation catalysed by PMS was similar to that elicited by NaCl, but was attained at a somewhat higher concentration. The same behavior can be observed with respect to the efficiency of ATP formation, as illustrated in the ATP/$2e^-$ ratio plot.

Mg^{2+} ions are known to participate in the steps leading to ATP formation. However, from the above experiments, it became apparent that some components generally used for assay of the photophosphorylation reaction might be assigned a dual function. In the case of Mg^{2+} ions, one function is the participation as a cofactor in the last steps of ATP formation. The second function could be the creation of

optimal conditions for maximal rate and higher efficiency of energy transfer. These optimal conditions could have been attained by the induction of a conformational change necessary for efficient energy transfer. This latter function is not specific and other solutes could replace Mg^{2+} ions. This is further emphasized in the results presented in Table I. Addition of a suboptimal amount of $MgCl_2$ together with $SrCl_2$ was as effective as Mg^{2+} alone at an optimal level.

TABLE I

RELATIONSHIP OF THE OSMOTIC CONCENTRATION TO THE REQUIREMENT FOR Mg^{2+}

Details as described under METHODS, except for the omission of $MgCl_2$ from the reaction mixture.

Expt. No.	*Component added* ($M \times 10^3$)	*Activity*		
		Photoreduction of ferricyanide (*μmoles/mg chlorophyll per h*)	*ATP/2e⁻*	*Photophosphorylation with PMS* (*μmoles/mg chlorophyll per h*)
1	None	—	—	1.7
	$SrCl_2$ (1.5)	—	—	59
	$MgCl_2$ (1.5)	—	—	202
	$MgCl_2$ (1.5) + $SrCl_2$ (1.5)	—	—	417
	$SrCl_2$ (3)	—	—	87
	$MgCl_2$ (3)	—	—	383
2	$MgCl_2$ (1)	82	0.5	—
	$MgCl_2$ (1) + $SrCl_2$ (10)	376	1.3	—
	$MgCl_2$ (11)	378	1.3	—
3	$MgCl_2$ (1)	92	0.5	—
	$MgCl_2$ (1) + NaCl (120)	439	0.9	—
	$MgCl_2$ (11) + NaCl (120)	450	1.0	—
4	$MgCl_2$ (1)	89	0.3	—
	$MgCl_2$ (1) + NaCl (120)	309	0.8	—
	$MgCl_2$ (1) + NaCl (120) + $SrCl_2$ (10)	330	0.8	—
5	$MgCl_2$ (1)	82	0.3	—
	$MgCl_2$ (1) + sucrose (240)	295	1.0	—
	$MgCl_2$ (11) + sucrose (240)	362	1.0	—

Table I also gives values for electron flow and phosphorylation with ferricyanide in the presence of mixtures of several solutes. The effect of added $MgCl_2$ could be reproduced by $SrCl_2$, NaCl or sucrose. No additive effect was observed with these solutes. The increase in the $ATP/2e^-$ ratios observed for saturating amounts of $MgCl_2$, $SrCl_2$, NaCl or sucrose was similar. However, the extent of stimulation of rates of electron transport and phosphorylation attained by sucrose was lower than for sucrose and $MgCl_2$ together.

It was previously shown that the rate of electron flow and phosphorylation depended, among other things, upon the concentrations of inorganic phosphate and magnesium[15]. The apparent K_m determined for these components was between $5 \cdot 10^{-4}$ and 10^{-3} M (refs. 16–18). In view of the above results it was of interest to test whether these relatively high K_m values were, at least partially, due to the osmotic effect

described. As can be seen in Table II, the effect of orthophosphate was strongly diminished by the presence of saturating concentrations of $MgCl_2$. However, even at these concentrations of $MgCl_2$, a stimulatory effect of orthophosphate was obvious. Apparently, the K_m for orthophosphate in the system is indeed high since its effects on the rate of photophosphorylation could not be fully reproduced by $MgCl_2$.

TABLE II

EFFECT OF $MgCl_2$ AND INORGANIC PHOSPHATE ON THE PHOTOPHOSPHORYLATIVE ACTIVITY

Details as described under METHODS, except for the omission of $MgCl_2$ from the reaction mixture.

Components added to reaction mixture (μmoles/ml)	*Photophosphorylation with PMS (μmoles ATP formed/mg chlorophyll per h)*	*Relative activity*
$MgCl_2$ (1) + P_i (1)	42	1.0
$MgCl_2$ (1) + P_i (15)	616	14.7
$MgCl_2$ (3) + P_i (1)	197	1.0
$MgCl_2$ (3) + P_i (15)	820	4.2
$MgCl_2$ (10) + P_i (1)	372	1.0
$MgCl_2$ (10) + P_i (15)	801	2.1
$MgCl_2$ (20) + P_i (1)	383	1.0
$MgCl_2$ (20) + P_i (15)	744	1.9

The stimulation of electron flow by a phosphate-acceptor system has been shown to occur through their effect on dark rather than light reactions of photophosphorylations[15]. Fig. 2 shows the behavior of the PMS-stimulated phosphorylation in the presence and absence of $SrCl_2$. It is clear that the addition of $SrCl_2$ caused both a change in the intercept and in the slope. This, in accordance with the formulation of LUMRY, SPIKES AND EYRING[19], would indicate that $SrCl_2$ affects both the light and dark steps of photophosphorylation.

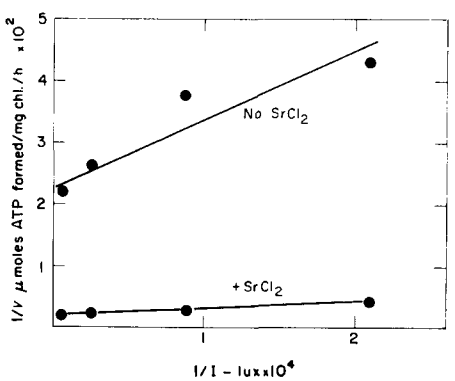


Fig. 2. The effect of $SrCl_2$ on light and dark stages of photophosphorylation. Details as described in METHODS. PMS was used as a cofactor.

Formation of a high-energy intermediate

The stimulation of electron flow and phosphorylation and the higher efficiency of energy transfer attained by increasing the solute concentration suggested that a

similar relationship might be observed for the intermediate stages of ATP formation[20]. The formation of an intermediate in ATP synthesis has been previously correlated with a photoinduced shrinkage of chloroplasts[10,21,22,11,23]. As shown in Fig. 3, the formation of the non-phosphorylated intermediate was markedly increased when the concentration of solutes in the light-stage reaction mixture was raised. The addition of these same solutes to the dark-stage reaction mixture had no effect. As in the case of the one-stage phosphorylation reaction, the stimulation by $MgCl_2$ and $SrCl_2$ was similar to and more effective than that of NaCl or sucrose. The effect of $CaCl_2$ can also be seen to resemble its effect on photophosphorylation. It stimulated at concentrations below 5 mM and was inhibitory above this concentration. Some inhibition was also observed when $CaCl_2$ was present only in the dark stage.

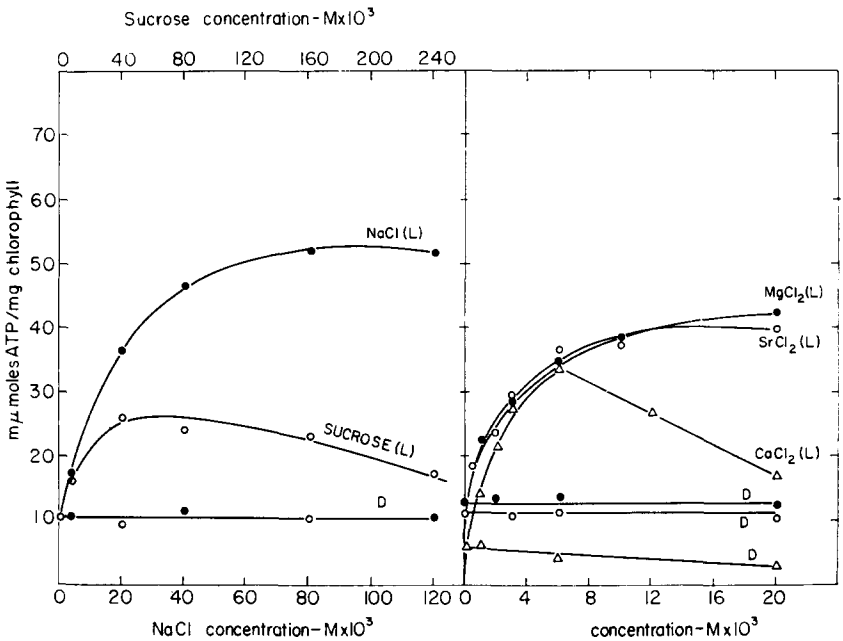


Fig. 3. The effect of the concentration of solutes on the formation of a high-energy intermediate (X_E). Details as described in METHODS. L designates the addition of solute to the light-reaction mixture. D designates that the solutes were added to the dark-stage reaction mixture.

Chloroplast volume

The study of a possible correlation between the solute effect on electron flow and photophosphorylation and chloroplast shrinkage was thought desirable. We measured, therefore, by the "chlorocrit" technique, the change in the packed volume of the chloroplasts in the presence and absence of added solutes. As shown in Table III, the addition of sucrose, NaCl, $MgCl_2$ or $CaCl_2$ to a chloroplast suspension markedly decreased their packed volume.

Light scattering

Several reports have appeared[9,19,11,24] of light-dependent scatter changes exhibited by spinach chloroplast suspensions which seem to reflect a conformational

TABLE III

THE EFFECT OF INCREASING SOLUTE CONCENTRATION ON THE PACKED VOLUME OF SWOLLEN CHLOROPLASTS

Details as described in METHODS. A volume of 0.25 ml of chloroplast suspension in 0.01 M Tris–HCl (pH 7.8) was mixed with 0.05 ml of solutions of each of the solutes added. Chlorophyll concentration: 1.2 mg/ml.

Solutes added	*Concentration* ($M \times 10^3$)	*Relative packed volume*
None	—	1.00
Sucrose	40	0.78
	80	0.64
NaCl	20	0.80
	40	0.70
$MgCl_2$	3	0.51
	9	0.43
$CaCl_2$	3	0.47
	9	0.43

change in the chloroplast structure. This conformational change was related to the formation of a high-energy intermediate of photophosphorylation. A difference spectrum similar to that induced by light[10] was observed for chloroplasts suspended in a medium containing increasing concentrations of solutes with respect to chloroplasts suspended in a medium not containing those solutes. This is illustrated in Fig. 4 where the observed changes in scattering were obtained by the addition of NaCl. The difference spectrum from 300 mμ to 750 mμ shows a general increase in scattering.

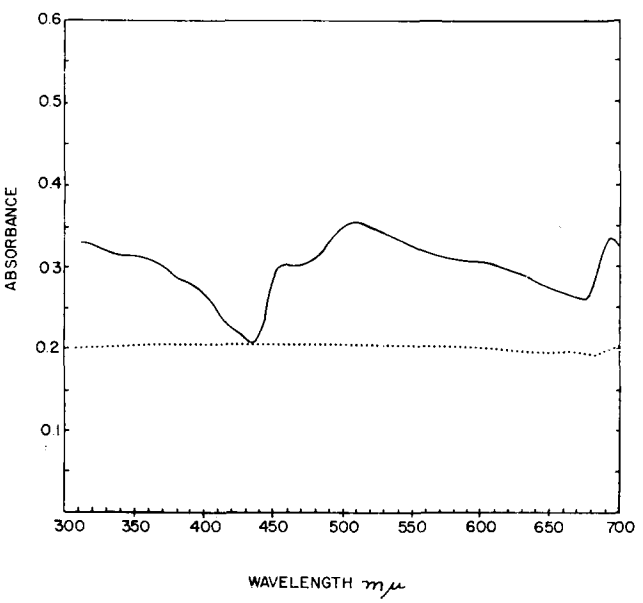
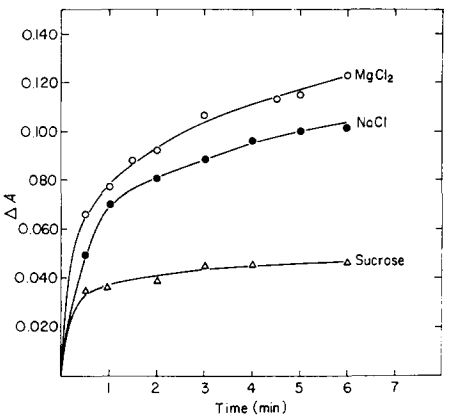


Fig. 4. Difference spectrum of NaCl-treated *vs.* water-treated chloroplasts. Details as described in METHODS. ·····, base line prior to the addition of NaCl; ———, scan after addition of NaCl (138 μmoles/ml) to the sample cuvette and an equivalent volume of water to the reference cuvette.

The magnitude of this increased scattering was smaller in the regions 670–685 mμ and 410–450 mμ.

A time course for the change in scattering induced by addition of NaCl, $MgCl_2$ or sucrose is shown in Fig. 5. As illustrated, the induced change in scatter is most rapid during the first minute. The concentration dependence of the scatter change is given in Fig. 6. Half-maximal increase in rate was observed around 3 mM, 30 mM and 40–50 mM for $MgCl_2$ or $SrCl_2$, for NaCl and for sucrose, respectively. The extent of scatter change elicited by sucrose was smaller than that reached by addition of salts, in agreement with the data on electron transport, phosphorylation and the formation of a high-energy intermediate (Figs. 1, 3).



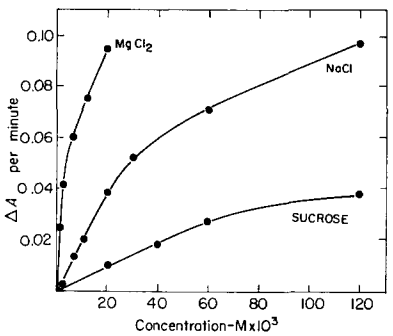


Fig. 5. Time course of solute-induced scatter changes at 510 mμ. Details as described in METHODS. $MgCl_2$, 10 μmoles/ml; NaCl, 60 μmoles/ml; sucrose, 120 μmoles/ml.

Fig. 6. Concentration dependence of the solute-induced change in scatter at 510 mμ. Details as described in METHODS.

It was observed that the total scatter of a chloroplast suspension at pH 7.8 was lower than at pH 6. Also, the light-induced change of scatter was shown to be maximal at pH 6 (ref. 10). Therefore, the extent of scatter change induced by several solutes was determined at different pH values. As shown in Table IV, the changes elicited by NaCl and sucrose were found to be pH dependent. However, they were

TABLE IV

EFFECT OF pH ON THE SCATTER CHANGES

Details as described in METHODS; the pH of the buffer solution (Tris–maleate) was adjusted before addition of the chloroplast suspension. pH was checked before and after addition of solutes. Chlorophyll concentration: 25 μg/ml.

Components added ($M \times 10^3$)	*Change of scatter*					
	pH 5.7	*5.9*	*6.6*	*6.8*	*7.2*	*7.8*
	ΔA/min at 510 mμ					
Sucrose (120)	—	0.016	—	0.028	0.040	0.054
NaCl (60)	0.069	0.073	—	0.092	0.102	0.117
$MgCl_2$ (10)	0.114	—	0.104	—	0.101	0.108
$SrCl_2$ (10)	0.118	—	0.102	—	0.121	0.120

maximal at the more basic pH. In the case of $MgCl_2$ or $SrCl_2$ no significant change was observed in this range of pH values.

The light-induced scatter changes studied by DILLEY AND VERNON[10] were found to be diminished by the presence of a phosphate-acceptor system. However, the solute-induced scatter change measured herewith was not diminished by the presence of a complete phosphate-acceptor system.

Fragments of chloroplasts were also found to be highly dependent on the solute concentration of the medium. Table V shows that the photophosphorylation activity of these broken chloroplasts could be stimulated by addition of a solute, but only to about 70 % of the extent reached by "swollen" chloroplast preparations. The scatter change elicited by addition of $MgCl_2$ to these fragments was also reduced to the same degree.

TABLE V

COMPARISON OF CHLOROPLASTS AND CHLOROPLAST FRAGMENTS

Details as described under METHODS. PMS served as the cofactor in the photophosphorylation experiments.

$MgCl_2$ added ($M \times 10^3$)	*Photophosphorylation*		*Scatter*	
	Chloroplasts	*Fragments*	*Chloroplasts*	*Fragments*
	(*μmoles ATP/mg chlorophyll per h*)		(*ΔA/min*)	
None	113	163	—	—
1	271	214	0.017	0.010
3	543	344	0.031	0.022
6	653	457	0.053	0.042

DISCUSSION

The results reported indicate the existence of a positive relationship between a conformational change or shrinkage induced by addition of several solutes to a chloroplast suspension, and the increased rates of electron flow, formation of a high-energy non-phosphorylated intermediate and ATP formation. The solute-induced conformational change could be measured either by changes in scatter (small-angle scattered light), or determination of the packed volume of the chloroplasts. This change in scatter was independent of the absence or presence of the phosphorylating components ADP, P_i and Mg^{2+}, and of a cofactor for photophosphorylation. It has recently been reported that photooxidation of internal cytochrome *f* by photosystem I of swollen chloroplasts did not occur unless sucrose was added[26]. Therefore, we believe the conformational change induced by solute to represent a condition or structural change necessary for allowing electron transfer to proceed. The marked change in the $P/2e^-$ ratio induced by added solute also indicates a function in producing a tighter coupling between ATP formation and electron transfer. The enhanced rate of formation of a high-energy, non-phosphorylated intermediate (X_E), under these conditions, could be the result of either or both of these effects.

The relationship between the light-induced conformational change studied by

other groups of investigators[7–10] and the solute-induced conformational change is not readily apparent. However, a competition between the effect of sucrose on chloroplast volume in the dark and the shrinkage induced by illumination was reported by ITOH[4]. Most workers interpret the light-induced increase in scattering as due to the formation of high-energy intermediates. The changes in scattering observed herewith do not appear to be a consequence of a high-energy condition, being rather a prerequisite for the formation of high-energy intermediates. Inhibitors and uncouplers of photophosphorylation did not affect the absorption changes induced by NaCl (unpublished data).

The enhanced rate of photophosphorylation observed in the presence of solutes which by themselves do not support ATP formation, and the non-additivity of their effect, indicate that their mode of action is compatible with the postulated induction of the necessary conformational change for the effective functioning of the photosynthetic apparatus. Since it is possible to replace a large fraction of the requirement for Mg^{2+} by other solutes, a dual role of Mg^{2+} in photophosphorylation is evident: a general one, replaceable by other solutes, and a specific one, localized at the terminal steps of ATP formation.

Broken chloroplasts were shown to respond in a manner almost identical to whole chloroplasts (Table V). The membrane affected by these changes must therefore be an internal lamellar one, and not the chloroplast membrane.

REFERENCES

1 M. AVRON AND N. SHAVIT, *Natl. Acad. Sci. – Natl. Res. Council Publ.*, 1145 (1963) 611.
2 K. NISHIDA, *Plant Cell Physiol. Tokyo*, 4 (1963) 247.
3 T. OHNISHI, *J. Biochem. Tokyo*, 55 (1964) 494.
4 M. ITOH, *Plant and Cell Physiol. Tokyo*, 6 (1965) 221.
5 E. GROSS AND L. PACKER, *Biochem. Biophys. Res. Commun.*, 20 (1965) 715.
6 A. B. TOLBERG AND R. I. MACERY, *Biochim. Biophys. Acta*, 109 (1965) 424.
7 M. ITOH, S. IZAWA AND K. SHIBATA, *Biochim. Biophys. Acta*, 66 (1963) 319.
8 L. PACKER, *Biochim. Biophys. Acta*, 75 (1963) 12.
9 A. T. JAGENDORF AND G. HIND, *Natl. Acad. Sci. – Natl. Res. Council Publ.*, 1145 (1963) 599.
10 R. A. DILLEY AND L. P. VERNON, *Biochemistry*, 3 (1964) 817.
11 G. HIND AND A. T. JAGENDORF, *J. Biol. Chem.*, 240 (1965) 3202.
12 Z. GROMET-ELHANAN AND M. AVRON, *Plant Physiol.*, 40 (1965) 1053.
13 M. AVRON, *Anal. Biochem.*, 2 (1961) 535.
14 A. T. JAGENDORF AND M. AVRON, *Arch. Biochem. Biophys.*, 80 (1959) 246.
15 D. W. KROGMANN AND A. T. JAGENDORF, *Biochim. Biophys. Acta*, 30 (1958) 144.
16 M. AVRON AND A. T. JAGENDORF, *J. Biol. Chem.*, 234 (1959) 967.
17 M. AVRON, A. T. JAGENDORF AND M. EVANS, *Biochim. Biophys. Acta*, 26 (1957) 262.
18 M. AVRON, *Biochim. Biophys. Acta*, 40 (1960) 257.
19 R. LUMRY, J. D. SPIKES AND H. EYRING, *Ann. Rev. Plant Physiol.*, 5 (1954) 298.
20 G. HIND AND A. T. JAGENDORF, *Proc. Natl. Acad. Sci. U.S.*, 49 (1963) 715.
21 L. PACKER AND R. H. MARCHANT, *J. Biol. Chem.*, 239 (1964) 2061.
22 G. HIND AND A. T. JAGENDORF, *J. Biol. Chem.*, 240 (1965) 3195.
23 R. A. DILLEY AND L. P. VERNON, *Arch. Biochem. Biophys.*, 111 (1965) 365.
24 S. IZAWA, *Biochim. Biophys. Acta*, 102 (1965) 373.
25 M. AVRON AND N. SHAVIT, *Anal. Biochem.*, 6 (1963) 549.
26 M. AVRON AND B. CHANCE, in *Energy Conversion by the Photosynthetic Apparatus*, Brookhaven Symposium No. 19, 1966, p. 149.