BBA 4103

STRUCTURAL CHANGES CORRELATED WITH PHOTOCHEMICAL PHOSPHORYLATION IN CHLOROPLAST MEMBRANES

LESTER PACKER

Department of Physiology, University of California, Berkeley, Calif. (U.S.A.)

(Received January 18th, 1963)

SUMMARY

Mitochondria manifest structural changes in their membranes under conditions of oxidative phosphorylation. It was postulated and confirmed here that similar changes occur under conditions of photophosphorylation in chloroplasts. Spinach chloroplasts isolated in buffered 0.5 M sucrose or 0.35 M NaCl and incubated under conditions of non-cyclic and cyclic phosphorylation at 25 ± 0.1°, on illumination with red light, show a rapid increase in 90° scattering of low-intensity 546-mμ light, which is reversed by turning the red light off. To detect such light-scattering changes, a low chlorophyll concentration is required so that pigments do not mask the scattering response, and also the scattering can be measured at the minimum of the photochemical reaction spectrum. The light-scattering responses are precisely correlated with conditions for non-cyclic and cyclic photophosphorylation, when tested in 0.035 M NaCl media in the presence of phosphate, Mg^{2+}, ADP and appropriate electron acceptors. Sonic treatment caused a decline in the ability to manifest light-scattering responses, and this decline parallels the decline in photophosphorylation. The light-scattering responses are abolished by NH_4^+ and 3-(3,4-dichlorophenyl)-1,1-dimethylurea which inhibit photophosphorylation and photosynthetic electron transport, respectively. The magnitude of the scattering response is dependent on red-light intensity. The angle of dependence of the scattering increase rules out a fluorescence change. Aging of chloroplasts results in a loss of the light-scattering response, but this can be restored by ATP. It is concluded that some structural parameter of the chloroplast membrane changes in response to photophosphorylation. A hypothesis for the coupling of the energy-transducing systems to membrane structure in mitochondria and in chloroplasts is presented.

INTRODUCTION

It has been established that a close correlation exists between the process of oxidative phosphorylation in mitochondria and the regulation of their swelling–shrinkage states[1,2]. Membrane fragments derived from mitochondria undergo structural changes as measured by light-scattering, if they retain the ability to catalyze electron transport

---

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

with coupled phosphorylation[3]. It was postulated that if such structural changes are a general phenomenon of energy-transducing systems in nature, that they should be manifest in other energy-transfer systems, in particular those associated with the chloroplast membrane which catalyze phosphorylation linked to photochemical reactions. The present article describes certain experiments which establish a close relation between some structural parameters of the chloroplast membrane and coupled cyclic and non-cyclic photophosphorylation. A hypothesis is presented for the coupling of energy-transducing systems to membrane structure of mitochondria and chloroplasts.

METHODS

Spinach chloroplasts were prepared according to PARK AND PON[4] in sucrose (0.5 M), Versene (0.010 M, pH 7.4), phosphate (0.03 M, pH 7.4), and finally washed with NaCl (0.35 M), Tris-HCl buffer (0.020 M, pH 7.5) by centrifugation at 600 × *g*. The chloroplast residue was resuspended in this NaCl-Tris medium. In some experiments, this fraction was washed with NaCl (0.035 M)-Tris buffer (0.020 M, pH 7.5) (low-salt medium) by centrifugation at 10 000 × *g* for 15 min, and the resultant residue was suspended in the low-salt medium (10 000 × *g* fraction). The angular scattering changes were measured near the minimum of the photochemical action spectrum (546 mμ) with a Brice-Phoenix light-scattering apparatus modified for recording[5]. Both incident and scattered light were filtered at 546 mμ. Photophosphorylation was activated at 180° from a tungsten source filtered at 660 mμ, and of approx. 200 lumens. Conditions for cyclic and non-cyclic photophosphorylation (*cf.* Fig. 5), assay of chlorophyll and phosphate uptake, were patterned after procedures described by WHATLEY AND ARNON[6,*]. The 90° scattering was adjusted to read 100% on the chart paper by using the minimum intensity of 546-mμ light and the instrument at maximum gain. Increases and decreases in scattered-light intensity in response to red-light illumination are expressed as percentage changes of the initial scattering level. The concentration of chlorophyll in the reaction mixture was usually 5-50 μg/ml, which corresponds to small absorbancy readings, 0.0005-0.0086 at the wavelength used for estimation of chlorophyll concentration (652 mμ). Temperature was controlled during experiments at 25 ± 0.1° with a thermistor-regulated water bath by circulating liquid through a jacketed cell. Thermocouples were employed (inserted into the cuvette) to simultaneously record temperature variations in the system in the absence and presence of red-light illumination in initial experiments[**].

EXPERIMENTAL

Non-cyclic photophosphorylation

The changes in light-scattering intensity[***] by chloroplast fragments induced with red-light illumination and the conditions for non-cyclic photophosphorylation are shown in Table I. When the requirements for photophosphorylation are present,

---

[*] Chloroplasts prepared by the method of WHATLEY AND ARNON[6] in buffered NaCl manifest the same type of response as when they are prepared by the sucrose method of PARK AND PON[4].

[**] This experiment was carried out by Dr. Y. MUKOHATA.

[***] Collateral experiments showed that these optical changes are dependent on the angle at which the emitted green light was measured, in a manner characteristic of scattering, and that they are therefore not fluorescence changes. A full report of these studies is in preparation.

TABLE I

SCATTERING CHANGES UNDER CONDITIONS OF NON-CYCLIC PHOTOPHOSPHORYLATION

The reaction system contained: Tris (0.020 M, pH 7.5), NaCl (0.035 M), and chloroplasts (600 × *g* fraction, 28 μg chlorophyll/ml). The data are given as per cent increase in scattering intensity following red-light illumination.

	Experiment	*Scattering change (%)*
1	No addition	0.0
	$MgCl_2$ (5 mM)	1.0
	$MgCl_2$ (5 mM) + phosphate (5 mM)	1.5
	$MgCl_2$ (5 mM) + phosphate (5 mM) + ADP (1 mM)	2.0
	$MgCl_2$ (5 mM) + phosphate (5 mM) + ADP (1 mM) + NADP (1 mM)	4.0
	$MgCl_2$ (5 mM) + phosphate (5 mM) + ADP (1 mM) + NADP (2 mM)	8.0
	$MgCl_2$ (5 mM) + phosphate (5 mM) + ADP (1 mM) + NADP (3 mM)	15.0
2	$MgCl_2$ (5 mM) + phosphate (5 mM) + ADP (1 mM)	
	Complete system + NADP (1 mM)	9.0
	Complete system + ferricyanide (2 mM)	9.0
3	$MgCl_2$ (5 mM) + phosphate (5 mM) + ADP (1 mM) + NADP (2 mM)	
	Complete system	14.5
	Complete system + DCMU (1 μM)	0.0
	Complete system + DCMU (1 μM) + ascorbate (2.5 mM) + dichlorophenolindophenol (30 μM)	10.0
	Complete system + DCMU (1 μM) + ascorbate (2.5 mM) + dichlorophenolindophenol (30 μM) + NH_4Cl (1 mM)	1.0

Mg^{2+}, phosphate and ADP, the extent of the scattering change induced by red light is dependent upon the concentration of the electron acceptor, NADP. In agreement with the known requirements of non-cyclic phosphorylation for specific electron acceptors[4], the system manifests scattering changes with either NADP or ferricyanide (Exp. 2). Exp. 3 proves that in the presence of the complete requirements for non-cyclic phosphorylation, the scattering changes are abolished by 1 μM DCMU which blocks the first photochemical reaction. However, the scattering changes induced by red light can be restored under these conditions by re-initiating electron transport with 2,6-dichlorophenolindophenol reduced by ascorbate, which bypasses the first light reaction and initiates photophosphorylation (*cf.* refs. 6 and 7 and Fig. 5). Since photophosphorylation occurring under these conditions is specifically inhibited by NH_4^+ ions, whereas the electron transport is not, and since 1 mM of NH_4Cl largely abolishes the scattering changes, these results establish a correlation between photophosphorylation and the scattering.

The dependence of light-scattering increase induced by red-light illumination upon the concentration of the electron acceptor NADP under conditions of non-cyclic photophosphorylation is shown in Table II.

Washed chloroplast fragments also alter scattering in response to non-cyclic photophosphorylation. Fig. 1 shows that addition of the electron donor, ascorbate–2,6-dichlorophenolindophenol, does not change the scattering level; but when red light is turned on, a prompt increase in scattering occurs which is complete in 1 min. Extinguishing the red light causes a reversal of the scattering change. Addition of ADP has little effect on scattering, but in the presence of red light an accelerated and greater scattering change now occurs; reversal of this scattering change is also

TABLE II

EFFECT OF NADP CONCENTRATION ON SCATTERING CHANGES UNDER CONDITIONS OF NON-CYCLIC PHOTOPHOSPHORYLATION

The complete system contained: Tris (0.02 M, pH 7.5), NaCl (0.035 M), $MgCl_2$ (0.005 M), phosphate (0.004 M), and chloroplasts (49 µg/ml chlorophyll).

NADP (mM)	*Scattering increase (%)*
0	2.0
1.1	4.5
2.2	11.0
3.3	15.0

more rapid. Finally, the most rapid and intense scattering increases were observed when the electron acceptor, ferricyanide, was added.

A comparison of the inhibitory effect of ammonia on light-scattering changes produced under conditions of non-cyclic photophosphorylation was made with that of some other inhibitors and is shown in Fig. 2. Light-scattering increases were produced in the presence of ascorbate, dye, and ferricyanide, together with the other basic requirements. After a steady state of light-scattering increase had been reached following red-light illumination, the various inhibitors were added. The upper trace shows that after the light-scattering increase has reached its maximum, 5 mM NH_4^+ abolishes the light-scattering change. Thus ammonia blocks light-scattering changes if added before red-light illumination as shown in Table I, or abolishes the change if added after it has taken place. Similar tests with two other potential uncoupling agents, namely pentachlorophenol and 3-chloro-carbonyl cyanide phenylhydrazone[8,9] (which are potent uncouplers of mitochondrial phosphorylation), are shown in the middle and bottom traces, respectively. It may be seen that both of these substances were capable of abolishing the light-scattering increases produced under conditions of photophosphorylation. In other experiments it was demonstrated that addition of the reagents prior to red-light illumination prevented the structural changes. As pointed out by WHATLEY AND ARNON[10] one cannot be sure whether the

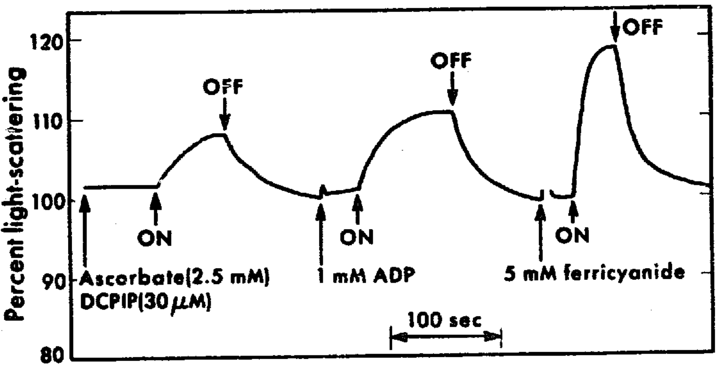

Fig. 1. Requirements for light-scattering change induced by red-light illumination in chloroplast fragments under conditions of non-cyclic photophosphorylation. The reaction system contained Tris (0.02 M, pH 7.5), NaCl (0.035 M), $MgCl_2$ (0.005 M), phosphate (0.004 M, pH 7.5), and chloroplasts (10000 × *g* fraction, 40 µg/ml chlorophyll).

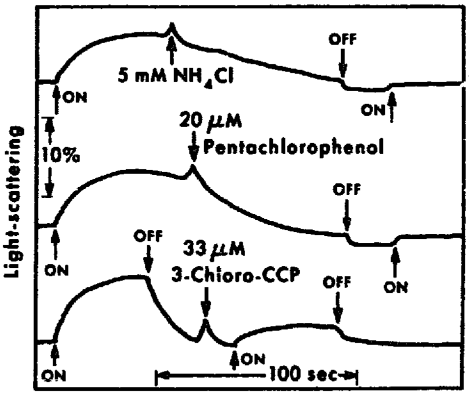

Fig. 2. Inhibitors of light-scattering changes in chloroplast fragments under conditions of non-cyclic photophosphorylation. Test conditions as in Fig. 1.

action of pentachlorophenol and 3-chloro-cyanide phenylhydrazone in this instance is due to an uncoupling of phosphorylation or to an effect on photosynthetic electron transfer, since separate measures of these two activities were not made in this experiment. The results suggest, however, an uncoupling effect of the reagents. NH_4^+ on the other hand at a concentration of 1 mM is known to uncouple phosphorylation under the conditions of this experiment, and to slightly stimulate photosynthetic electron transfer in spinach chloroplasts[10].

An experiment to further illustrate the relationship of scattering changes to non-cyclic photophosphorylation is given in Fig. 3 and Table III. Chloroplasts were prepared and subjected to sonic treatment in a 9-kc sonicator (Raytheon Corporation) for 30, 60 and 120 sec. Following this, the ability of these preparations to manifest

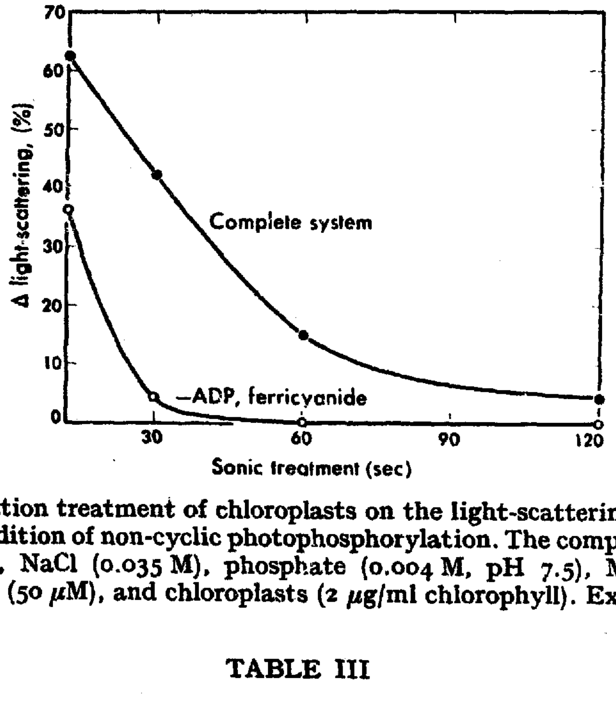

Fig. 3. Effect of sonication treatment of chloroplasts on the light-scattering response induced by red light under the condition of non-cyclic photophosphorylation. The complete system contained: Tris (0.02 M, pH 7.5), NaCl (0.035 M), phosphate (0.004 M, pH 7.5), $MgCl_2$ (0.005 M), ADP (0.001 M), ferricyanide (50 µM), and chloroplasts (2 µg/ml chlorophyll). Explanation in text.

TABLE III

INFLUENCE OF SONIC TREATMENT OF CHLOROPLASTS ON THE RETENTION OF LIGHT-SCATTERING INCREASE PRODUCED UNDER CONDITIONS OF NON-CYCLIC AND CYCLIC PHOTOPHOSPHORYLATION

The basic reaction system contained: Tris (0.02 M, pH 7.5), NaCl (0.035 M), $MgCl_2$ (0.005 M), phosphate (0.004 M, pH 7.5), chloroplast (2 µg/ml chlorophyll), and the additions indicated in the table.

Conditions	*Scattering change (%)*			
	Sonication time (sec)			
	0	*30*	*60*	*120*
Non-cyclic				
No addition	36	4	0	0
ADP (2 mM) + ferricyanide (20 µM)	61	42	15	4.5
ADP (2 mM) + ferricyanide (20 µM) + DCMU (1 µM)	0	0	0	0
Cyclic				
ADP (2 mM), + ferricyanide (20 µM) + DCMU (1 µM) present Ascorbate (2.5 mM) + phenazine methosulfate (20 µM)	57	38	16	3

scattering changes in response to red light with ferricyanide as the electron acceptor was tested. The results are plotted in Fig. 3, where it can be seen that in the presence of the complete system for non-cyclic photophosphorylation, light-scattering changes were produced under red-light illumination, which are related to sonication time, and that without sonication, a light-scattering increase of 61 % occurred. These changes were reversed on turning off the red light and could be reproduced time and again with successive periods of light and dark. With increased sonication time, the extent of the light-scattering response declined. The curve obtained for this exactly parallels the decline in photophosphorylation in the chloroplasts as shown in experiments by PARK[11] under the same conditions. The curve in Fig. 3 labeled "minus ADP, ferricyanide" shows that some light-scattering response is manifested by the preparation which was not subjected to sonic treatment. It is presumed that this response is brought about by endogenous electron acceptors and cofactors present in the unwashed preparation. As expected this response rapidly falls to zero with increasing sonication time. It may be noted here that washed chloroplast preparations never manifested an endogenous light-scattering response. These results provide additional proof for the close parallel between phosphorylation and the ability of chloroplasts to manifest structural changes as judged by light-scattering.

Cyclic photophosphorylation

It seemed of interest to examine the ability of chloroplasts to manifest structural change under the influence of cyclic as compared with non-cyclic photophosphorylation. The same experimental condition employed above was used to demonstrate this relationship. The experiment was performed by blocking non-cyclic photophosphorylation by the addition of DCMU. Table III shows that 1 μM DCMU completely blocks scattering changes under all four conditions of sonication time. 2.5 mM ascorbate and 20 μM phenazine methosulfate were then added to the reaction system. The amount of ascorbate added was much in excess of the 20 μM ferricyanide added earlier, and all the ferricyanide would be expected to be chemically reduced under this condition. Phenazine methosulfate provided the necessary electron acceptor for cyclic phosphorylation (*cf.* ref. 6 and Fig. 5). It can be seen (Table III) that a virtually complete restoration of light-scattering response is brought about under the conditions for cyclic phosphorylation. Separate estimations were carried out under these conditions of the uptake of inorganic phosphate at 30°. It was found that 10.6 μM of phosphate disappeared per mg chlorophyll per hour at 30°*. The magnitude of the phosphorylation cannot be directly equated with light-scattering increases for two reasons: (a) The amount chlorophyll per ml employed in the phosphate determination was higher, and (b) the light intensity employed was different. But the experiment clearly shows active cyclic photophosphorylation. Under similar conditions it has been demonstrated that flavin mononucleotide (*cf.* ref. 6 and Fig. 5) also supports cyclic photophosphorylations, and light-scattering responses are obtained under these conditions.

Additional requirements for scattering response under conditions of photophosphorylation

Light intensity: Assuming that the correlation between the light-scattering response and photophosphorylation is rigorous, it was predicted that the magnitude

---

* These estimations were carried out by R. H. MARCHANT.

of the light-scattering response should be controlled by the light intensity. This important experiment is shown in Fig. 4. It can be seen that when the red-light intensity was varied between 233 lumens (corresponding to 140 setting on the rheostat) and 116 lumens (corresponding to rheostat setting of 80) the magnitude of the scattering response diminished as the red-light intensity diminished. A control was performed at the end of the experiment by returning the level of red-light illumination to that for the first light-scattering cycle. It may be seen that the larger scattering response returns, although within the time interval of the experiment some interaction has occurred. The maximum reversible light-scattering changes obtained at the highest light intensity over many experiments were 62 and 98 % for non-cyclic and cyclic photophosphorylation respectively.

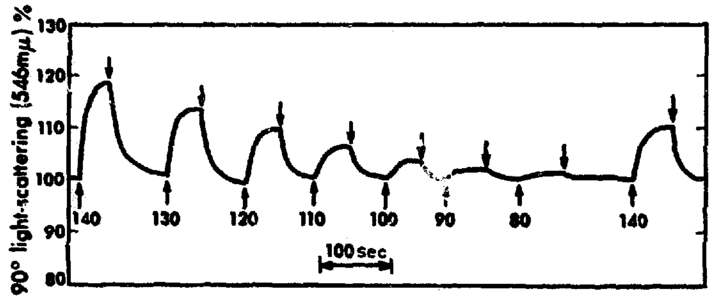

Fig. 4. Influence of red-light intensity upon the light-scattering response produced under the condition of non-cyclic photophosphorylation. Conditions as in Fig. 3. The numbers below the trace refer to rheostat settings (see text for explanation).

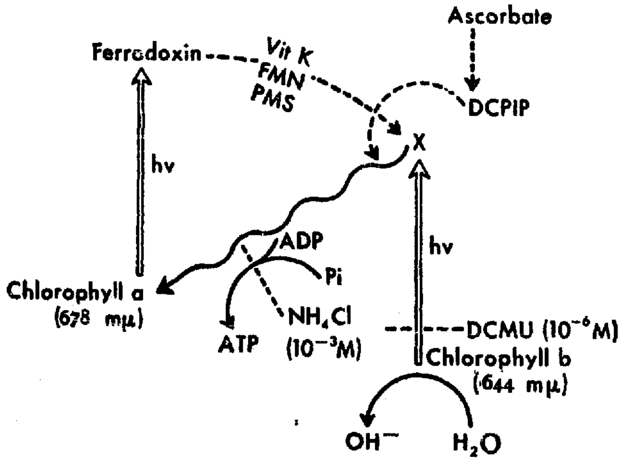

Fig. 5. Scheme for photosynthetic electron transport and non-cyclic phosphorylation in spinach chloroplasts, after ARNON[7].

Chlorophyll concentration: It seemed worthwhile to attempt to illustrate the presence of the scattering phenomenon in chloroplasts over a range of chlorophyll concentration. This problem seemed particularly acute in chloroplasts because of their enormous pigment concentration. Experience with mitochondria and cells[1,12] has shown that the demonstration of light-scattering responses is most feasible under conditions when the particle–particle interactions are reduced to a minimum, *i.e.*, at low concentration. Table IV shows that the ability of chloroplasts under conditions of non-cyclic photophosphorylation to manifest light-scattering changes, is markedly dependent on the chlorophyll concentration. High concentrations of chlorophyll

TABLE IV

INFLUENCE OF CHLOROPHYLL CONCENTRATION ON SCATTERING CHANGES UNDER CONDITIONS OF NON-CYCLIC PHOTOPHOSPHORYLATION

The complete system contained: Tris (0.02 M, pH 7.5), NaCl (0.035 M), $MgCl_2$ (5 mM), phosphate 4 mM), ADP (1 mM), NADP (2.2 mM), and spinach chloroplasts. Other conditions as in text.

Chlorophyll (μg/ml)	*Scattering change (%)*
16.3	14.0
49.0	13.0
163.0	4.5
815.0	0.0

completely masked the light-scattering response. Attempts were always made to maintain the concentration of chlorophyll in the reaction system below an amount giving a 37 % transmittance change when added to the medium at 546 mμ, the wavelength used for light-scattering measurements. Pigment concentration in excess of 37 % transmittance change would be expected to interfere with angular scattering measurement, although many workers in photosynthesis (see review by French[13]) have taken note of light-scattering effects in chloroplast systems in their attempts to resolve absorption spectra. The presence of light-scattering changes linked to functional changes in chloroplasts has not been previously observed, possibly for this reason.

Role of ATP: Since the product of photophosphorylation is ATP, it seemed important to examine its direct effect on chloroplast structure as judged by light-scattering changes. It was found that fresh chloroplasts with high phosphorylating activity do not show an effect of ATP on the light-scattering response produced by red-light illumination under conditions of photophosphorylation, nor does ATP manifest a light-scattering effect on chloroplasts in the dark. Upon aging of the chloroplast, however, an ATP effect develops. Upon storage of chloroplasts at 0°, the ability to manifest light-scattering changes under conditions of photophosphorylation declines. The addition of ATP has been shown to restore the ability of such systems to manifest scattering changes linked to photophosphorylation. Such a restoration effect for chloroplasts aged for 18 h is illustrated by Table V. It can be seen that 3 mM ATP restores the scattering response, and that this scattering response

TABLE V

RESTORATION OF SCATTERING CHANGES IN AGED CHLOROPLASTS BY ATP

The reaction system contained: Tris (0.02 M, pH 7.5), NaCl (0.035 M), $MgCl_2$ (0.005 M), phosphate (0.004 M), ferricyanide (2 mM), ADP (1 mM), ascorbate (2.5 mM), dichlorophenolindophenol (30 μM), and chloroplast fragments (10 000 × *g* fraction, 6.26 μg/ml chlorophyll), aged at 0° for 18 h. Data are presented as in Table I.

	Scattering change (%)
No addition	0.5
ATP (1 mM)	1.5
ATP (2 mM)	6.5
ATP (3 mM)	8.5
ATP (3 mM) + NH_4Cl (2 mM)	1.0

is still sensitive to the inhibitory action of NH_4^+. In other experiments with damaged chloroplasts, ATP has also been observed to induce light-scattering increases in the dark[14]. These experiments suggest that ATP may have an effect on the restoration of photophosphorylation in damaged systems (see DISCUSSION).

DISCUSSION

It has been postulated and confirmed that structural changes measured by light-scattering occur in the chloroplast membrane under conditions of photosynthetic phosphorylation. Correlation of the light-scattering response with photophosphorylation was demonstrated by the similarity in the requirements for both processes (these requirements are: phosphate, ADP and Mg^{2+}, the need for an electron acceptor such as NADP and ferricyanide for non-cyclic photophosphorylation) and by the inhibition of the light-scattering response by substances such as NH_4^+ and DCMU which block photophosphorylation and photosynthetic electron transport respectively. Light-scattering responses were also found with cyclic phosphorylation using phenazine methosulfate or FMN as the electron mediators. Further support for the correlation of structural changes with the photophosphorylation process was indicated by the parallel decline in light-scattering response and photophosphorylation, but not Hill-reaction activity[11], after sonic treatment of chloroplasts, by the requirement for red-light illumination, and by the restoration effect of ATP on aged or damaged chloroplast preparations. These results indicate a very precise correlation between photophosphorylation and some structural parameter of the chloroplast membrane. A similar relationship has been found to exist between oxidative phosphorylation in mitochondria and mitochondrial membrane structure[1-3]. Under conditions of oxidative phosphorylation in intact, tightly coupled mitochondria, a light-scattering change (increase) is also produced upon initiation of the phosphorylation process, which is reversed when oxidative phosphorylation stops in a manner exactly analogous to the chloroplast results. Damage of the mitochondrial membrane either by aging or by the preparation of fragmented membranes under appropriate conditions leads to a requirement for ATP in addition to the conditions for phosphorylation in order that light-scattering increases can occur. It may also be noted that an ATP requirement for shape transformation in aged erythrocytes has been reported[15], just as in the case of mitochondria and chloroplasts.

Because of the close correlation of photophosphorylation with light-scattering, it seems possible that the light-scattering technique, requiring only a few seconds, may become a powerful tool for future studies on the interdependence of chloroplast structure and energy-transfer reactions. For example, it would seem that the controlled destruction of chloroplasts into subunits still retaining the capacity for photophosphorylation might be possible by assay of the light-scattering response. The light-scattering response may also provide a mechanism for determining an action spectrum for photophosphorylation, and these experiments are currently in progress. The effect of ATP on the restoration of structural changes linked to photophosphorylation in aged chloroplasts also suggests means for studying further the requirement for integrity of chloroplast structure in photophosphorylation. Many questions remain unanswered at this time; foremost amongst these would seem to be the absence of a precise interpretation of the nature of the changes in the chloroplast of which light-scattering is a measure. Experiments are currently underway in this laboratory to further define the nature of this physical change.

As recently reviewed by FRENCH[13,16], light-scattering studies have in the past been employed only to correct absorbancy spectra of pigment in chloroplast systems. The presence of a high concentration of pigments in a membrane structure which manifests light-scattering has prevented earlier workers from obtaining precise absorption spectra of photosynthetic pigments. Elimination of this background either by correction in intact chloroplast materials, or by the elimination of the background scattering by extracting the pigments, or by fractionating the chloroplasts into subunits, *i.e.* quantosomes[17], has enabled certain investigators to eliminate the scattering contribution in the absorbancy of photosynthetic pigments. The reverse situation, namely the action of the photosynthetic pigment reactions on the light-scattering properties has not been previously observed. This appears to be due to the interference of photosynthetic pigments with the measurement of light-scattering changes as shown in Table IV. High concentrations of pigments mask the ability to observe changes in light-scattering geared to photophosphorylation.

The physiological significance of structural changes in the chloroplast membrane in response to photophosphorylation is still unknown. In mitochondria where similar reactions exist, model experiments with muscle-mitochondrial models[18] and certain experiments on the Crabtree effect in ascites tumour cells[12] suggest that changes in mitochondrial membrane structure, driven by phosphorylation, may lead to altered ATP availability for extramitochondrial processes. It may be that similar effects on the permeability of the chloroplast membrane may be brought about by metabolically driven changes in membrane structure. It is hypothesized, as illustrated in Fig. 6, that in chloroplasts and mitochondria, a coupling exists between the energy-transducing systems and their membrane structure. In the intact membranes of chloroplasts and mitochondria a low light-scattering and low-shrinkage state exists during electron transport in the absence of phosphorylation. Under conditions of oxidative phosphorylation, energy-linked intermediates are synthesized in increasing concentration. The fate of these intermediates are then two-fold, their energy can be conserved to make ATP or alternatively utilized for coupled mechano-chemical work[1,2], or the translocation of ions[19]. In damaged membranes, however, high concentrations of an energy-linked intermediate generated by the phosphorylation

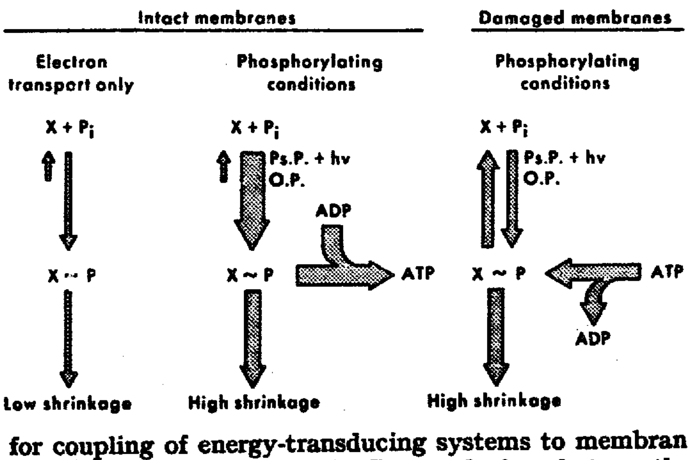

Fig. 6. Hypothesis for coupling of energy-transducing systems to membrane structure of mitochondria and chloroplasts. The abbreviation Ps.P. stands for photosynthetic phosphorylation and O.P. for oxidative phosphorylation.

process are difficult to maintain because of its hydrolysis under these conditions in both mitochondria and chloroplasts, and it appears that ATP is required to maintain sufficient levels of the intermediate to drive structural changes.

Whatever the final physiological significance of the physical change, and the precise mechanism of the process may be, it would seem important that the primary energy-transducing systems of the mitochondrion and of the chloroplast are both capable of bringing about structural changes in their membranes in a predictable manner.

NOTE ADDED IN PROOF

Recently ITOH, IZAWA AND SHIBATA[20] have observed that spinach chloroplasts undergo shrinkage and a change in axial ratio (from 1.96 to 2.34) on illumination. Since these changes are reversed in the dark, they may be regarded as a possible indication of the nature of structural changes related to the phosphorylation process in chloroplast membranes.

Received June 21st, 1963.

ACKNOWLEDGEMENT

This research was supported by the National Science Foundation. The author would like to thank Dr. David O. Hall for stimulating discussions on photosynthetic phosphorylation.

REFERENCES

[1] L. PACKER, *J. Biol. Chem.*, 235 (1960) 242.
[2] L. PACKER, in T. W. GOODWIN AND O. LINDBERG, *Biological Structure and Function*, Vol. II, Academic Press, London, 1961, p. 85.
[3] L. PACKER AND A. L. TAPPEL, *J. Biol. Chem.*, 235 (1960) 525.
[4] R. B. PARK AND M. G. PON, *J. Mol. Biol.*, 3 (1961) 1.
[5] L. PACKER, *J. Biol. Chem.*, 237 (1962) 1327.
[6] F. R. WHATLEY AND D. I. ARNON, in S. F. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. VI, in the press.
[7] D. I. ARNON, *Federation Proc.*, 20 (1961) 1012.
[8] P. G. HEYTLER AND W. W. PRICHARD, *Biochem. Biophys. Res. Commun.*, 7 (1962) 272.
[9] G. R. SCHONBAUM, L. PACKER AND C. R. BOVELL, *Arch. Biochem. Biophys.*, in the press.
[10] M. LOSADA AND D. I. ARNON, in R. M. HOCHSTER AND J. H. QUASTEL, *Metabolic Inhibitors*, Academic Press, New York, in the press.
[11] R. B. PARK, *J. Mol. Biol.*, in the press.
[12] L. PACKER, *J. Biol. Chem.*, 235 (1960) 1234.
[13] C. S. FRENCH, in *Photoperiodism and Related Phenomena in Plants and Animals*, American Association for the Advancement of Science, Washington, D.C., 1959, p. 15.
[14] L. PACKER, R. H. MARCHANT AND Y. MUKOHATA, *Biochem. Biophys. Res. Commun.*, in the press.
[15] M. NAKAO, T. NAKAO AND M. TATIBANA, *J. Biochem.*, 47 (1960) 694.
[16] C. S. FRENCH, in W. RUHLAND, *Handbuch der Pflanzenphysiologie*, Vol. II, Springer-Verlag, Berlin, 1960, p. 252.
[17] K. SAUER AND M. CALVIN, *Biochim. Biophys. Acta*, 64 (1962) 2324.
[18] S. WATANABE AND L. PACKER, *J. Biol. Chem.*, 236 (1961) 1201.
[19] G. P. BRIERLEY, E. BACHMAN AND D. E. GREEN, *Proc. Natl. Acad. Sci. U.S.*, 48 (1962) 1928.
[20] M. ITOH, S. IZAWA AND K. SHIBATA, *Biochim. Biophys. Acta*, 66 (1963) 319.