LIGHT SCATTERING CHANGES CORRELATED WITH PHOTOSYNTHETIC PHOSPHORYLATION IN CHLOROPLAST FRAGMENTS

Lester Packer

Department of Physiology, University of California
Berkeley 4, California

Received October 8, 1962

It has been established that a close correlation exists between the process of oxidative phosphorylation and the regulation of swelling-shrinkage states of mitochondria. Mitochondrial membrane fragments undergo light scattering changes if they retain the ability to catalyze electron transport with coupled phosphorylation (1). It was postulated that if such changes are a general phenomenon of electron transport coupled phosphorylation reactions, they should be manifest in other systems; in particular, those of chloroplast membranes which contain a phosphorylation system linked to photochemical reactions. The present report describes certain experiments which establish a close relationship between some structural parameter of the chloroplast membrane and coupled cyclic and non-cyclic photophosphorylation.

Methods. Spinach chloroplasts were prepared according to Park and Pon (2) in sucrose (0.50 M), versene (0.010 M, pH 7.4), phosphate (0.03 M, pH 7.4); final chloroplast suspensions were washed with NaCl (0.35 M), Tris-(hydroxymethyl)aminomethane-HCl buffer (0.020 M, pH 7.5) by centrifugation at 600 x g. The chloroplast residue was resuspended in this 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 1000 x g for five minutes. The residue was resuspended in the same medium and saved (1000 x g fraction). The supernatant was recentrifuged at 10,000 x g for 15 minutes, and the resultant residue was suspended in the low salt medium (10,000 x 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. (1,3). 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 approximately 200 lumens. Experiments with cyclic and non-cyclic photophosphorylation and assay of chlorophyll were patterned after procedures described by Whatley and Arnon (4). 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. The increases and decreases in scattered light intensity in response to red light illumination are expressed as percentage changes of the initial scattering level. The concentrations of chlorophyll in the reaction mixture was 5-50 μg per ml, which corresponds to small absorbancy readings, 0.0005-0.0086 at the wavelength used for estimation of chlorophyll concentration (652 mμ).

Experimental. 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, Mg^{++}, 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 (Experiment 2). Experiment 3 proves that in the presence of the complete requirements for non-cyclic phosphorylation, the scattering changes are abolished by 1 μM dichlorophenylmethylurea (DCMU) which blocks the first photochemical reaction. However, the scattering changes induced by red light can be restored under these conditions by reinitiating electron transport with 2,6 dichloro-

*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 are therefore not fluorescence changes. Absorbancy changes were ruled out by showing that the percentage change was independent of the chlorophyll concentration in the range present in the experiments; moreover, the absorbancy of the chlorophyll was too low to effect an apparent scattering measurement.

TABLE I

SCATTERING CHANGES UNDER CONDITIONS OF NON-CYCLIC PHOTOSYNTHETIC PHOSPHORYLATION

The reaction system contained: Tris (0.020 M, pH 7.5), NaCl (0.035 M), and chloroplasts (600 x g fraction, 28 µg chlorophyll/ml). The data are given as percent 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 (5m M) + 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) + DCPIP (30 µM)	10.0
Complete system + DCMU (1 µM) + DCPIP (30 µM) + NH_4Cl (1 mM)	1.0

phenol indophenol (DCPIP) reduced by ascorbate, which bypasses the first light reaction and initiates photophosphorylation (cf 4). Since photophosphorylation occuring 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 scattering changes, these results establish a correlation between photophosphorylation and the scattering.

Washed chloroplast fragments also alter scattering in response to cyclic photophosphorylation. Figure 1 shows that the electron donor, ascorbate-

DCPIP, does not change scattering; but when red light is turned on, a prompt increase in scattering occurs which is complete in one minute. Extinguishing the red light causes a reversal of the scattering change. ADP has little effect on scattering, but in the presence of red light a more rapid and greater scattering change now occurs; reversal of this scattering change is also more rapid. Finally, the most rapid and intense scattering increases were observed when the electron acceptor, ferricyanide, was added. Scattering changes in the cyclic system are abolished by NH_4^+ ions, but are not inhibited by DCMU, as would be expected.

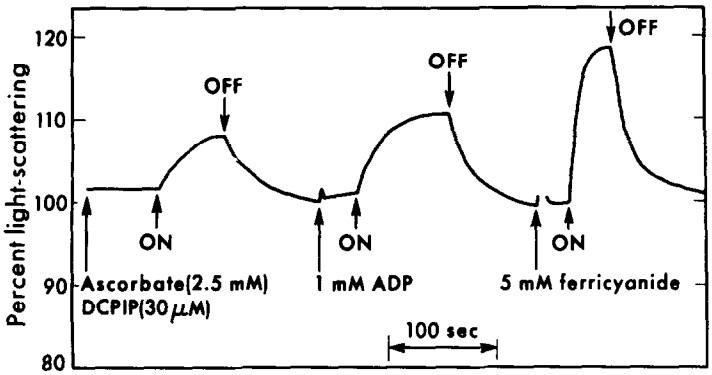

Figure 1. Scattering changes induced by red light in chloroplast fragments under conditions of cyclic photosynthetic phosphorylation. The reaction system contained: Tris (0.02 M, pH 7.5); NaCl (0.035 M); $MgCl_2$ (0.005 M); phosphate (0.004 M, pH 7.5); and chloroplast fragments (10,000 x g fract 40 μg/ml chlorophyll).

In agreement with the lability of the photophosphorylation system in chloroplast fragment systems, it was found that the ability to manifest scattering changes in the presence of the required components decreased after storage of the preparation. However, with fragments stored at 0° C, it was observed that the ability to manifest scattering changes in the presence of the requirements for cyclic photophosphorylation could be partially restored with 3 mM ATP (Table II). Similar effects have also been encountered in

fragmented mitochondrial membranes (1,5) and erythrocytes (6) where shape transformations are brought about by the addition of ATP after aging.

TABLE II

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); DCPIP (30 μM); and chloroplast fragments (10,000 x g fraction, 6.26 μg/ml chlorophyll), aged at 0° for 18 hours. 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

Summary. It was postulated and confirmed here that light scattering changes in chloroplast membranes are manifest during photosynthetic phosphorylation. The correlation of the light scattering with photophosphorylation was demonstrated by both processes having the same requirements (phosphate, ADP, and Mg^{++}) and by their inhibition in the presence of NH_4^+ ions, under conditions where photosynthetic electron transport proceeds in the presence or absence of these factors. The precise interpretation of the nature of the structural changes in chloroplast fragment systems as indicated by scattering changes is still undefined, but it may be of considerable importance that the energy transfer systems both of mitochondria and chloroplasts are capable of bringing about structural changes in their membranes by the action of the phosphorylation reactions in a predictable manner.

References.

1. Packer, L., and Tappel, A.L., Jour. Biol. Chem., 235 : 525 (1960).

2. Park, R.B., and Pon, M.G., Jour. Mol. Biol., 3:1 (1961).

3. Packer, L., Biological Structure and Function, 2, edited by T.M. Goodwin and C. Lindberg, Academic Press, New York, 1961, p. 85.

4. Whatley, F.R., and Arnon, D.L., Methods in Enzymology, 6, edited by S.F. Colowick and N.O. Kaplan, 1962, in press.

5. Packer, L., Fed. Proc., 19:16 (1960).

6. Nakao, M., Nakao, T., and Tatibana, M., Jour. Biochem., 47:694 (1960).