LIGHT-INDUCED CONFORMATIONAL CHANGES OF CHLOROPLASTS PRODUCED BY HIGH ENERGY INTERMEDIATES OF PHOTOPHOSPHORYLATION†

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Several laboratories have reported that chloroplast suspensions show light-induced, reversible light-scattering and absorption changes. (Packer, 1963; Itoh et al, 1963: Jagendorf and Hind, 1963). It has been shown that inhibitors of electron transport (DCMU, o-phenanthroline, etc.) and uncouplers of photophosphorylation (NH_4Cl, Cl-CCP[1]) abolish the changes, suggesting that the light-scattering changes are a manifestation of chloroplast structural changes related to energy conservation mechanisms. This is in analogy to a considerable body of work on mitochondrial structural changes which are considered to be related to oxidative phosphorylation (Lehninger, 1962).

While attempting to show NADP photoreduction by $TMQH_2$ with spinach chloroplasts, it was noticed that a

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[1] Abbreviations used: Cl-CCP, m-chloro carbonyl cyanide phenylhydrazone; DCMU, dichlorophenylmethyl urea; TMQ, trimethyl-1,4-benzoquinone; AA, ascorbic acid; DPIP, 2,6-dichlorophenolindophenol.

control consisting of chloroplasts, buffer, and $TMQH_2$ gave a ΔA 340 mμ of about 0.5 absorbancy unit per min, which was shown not to be due to endogenous nucleotide. Further work revealed that the light-induced absorption change is non-specific and has characteristics similar to light-scattering changes. The experiments outlined below relate the light-induced conformational changes to electron transfer and photophosphorylation and lead us to a somewhat different interpretation than that offered by Packer (1963).

Chloroplasts were prepared according to the method of Black et al (1963). Absorption measurements were made either on a Beckman Model DB, Bausch and Lomb Model 505, or a Cary Model 14R recording spectrophotometer (all were equipped for simultaneous illumination and recording). Illumination was provided by incandescent lamp with a 5 cm water filter and a Corning 2403 red filter. ATP formation was assayed by the ^{32}P method of Avron (1960). Light-scattering changes were measured with a Brice-Phoenix Model 1000 light-scattering photometer, in a manner similar to that reported by Packer (1963). The actinic light source was the same as the measuring beam. It was found that either 90° light-scattering changes or absorption changes are satisfactory for measuring the light-induced conformational changes. We routinely used the absorption technique for the work reported here.

The light-induced conformational changes of fresh chloroplasts may be elicited by a number of redox compounds such as quinones, flavins, dyes, etc. (Table 1). The total absorption increase measured at 520 mμ amounts to 0.3-0.6

Table 1. Activation of light-induced absorption changes of spinach chloroplast suspensions measured at 520 mμ. Reaction mixtures consisted of 20-25 μg chlorophyll equivalent chloroplasts per ml, 0.066 M Tris pH 6.0. The data are given as the ratio ΔA 520 mμ per min (initial rate) with a compound to ΔA 520 mμ per min with no additions.

Compound Added	Initial Rate	
	Fresh Chloroplasts	Aged Chloroplasts
None	1	1
0.7 mM TMQ	30	2
0.17 mM Ferricyanide	10	2
0.7 mM $TMQH_2$	30	30
0.013 mM PMS	27	30
0.07 mM FMN	20	2

units when the suspension has 20-25 μg chlorophyll per ml. Ninety-degree light-scattering changes were found to agree kinetically with the absorption changes. Allowing the chloroplasts to age at 0-4°C until they are no longer capable of reducing Hill reagents such as ferricyanide or DPIP causes a loss of the light-induced absorption change with an oxidized cofactor. However, if a reduced cofactor such as $TMQH_2$ or the AA-DPIP couple is used, the absorption change reaction occurs upon illumination. The light-minus-dark difference spectrum from 250 mμ to 720 mμ is the same for both fresh and aged particles. It is characterized by a general increase in absorbancy as the wavelength decreases,

with negative peaks at 680-690 mμ and 430-440 mμ. The negative peaks are near absorption peaks as predicted from the theory of large-particle light-scattering (Latimer, 1963).

Action spectra were measured for fresh and aged chloroplasts between 630 and 720 mμ. With fresh chloroplasts there is a broad peak of maximal reaction from about 660 mμ to 680 mμ when either TMQ or $TMQH_2$ is the cofactor. The reaction with aged particles has a single, sharper maximum in the region of 680 mμ when either $TMQH_2$ or the AA-DPIP couple is used. This is indicative that the long-wavelength pigment system (system I of the Duysens and Amesz, 1962) is capable of mediating the events leading to the conformational change(s).

Experiments in which both ATP formation and the conformational change reaction (indicated by absorption change) were measured showed that formation of ATP resulted in a diminished conformational change. The presence of quinacrine, an uncoupler of photophosphorylation (Baltscheffsky, 1960), stimulates the absorption reaction and inhibits ATP formation. Other experiments showed that quinacrine exerts this effect independent of the presence of a phosphate acceptor system. The uncoupler antimycin A also stimulates the light-induced absorption change at 520 mμ (when the pH is below 7). Fig. 1 shows a typical reaction with a quinacrine-treated chloroplast suspension. These uncouplers stimulate the initial forward and back reaction rates and also cause the reaction to plateau quickly, whereas the control reaction is somewhat slower and is biphasic. The uncouplers NH_4^+ (9×10^{-3} M) and Cl-CCP (3.2×10^{-5} M) inhibit the conformational change

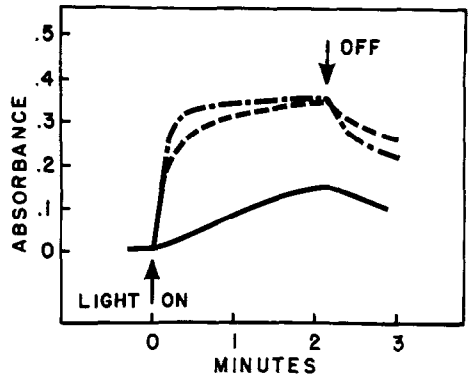

Fig. 1 Effect of quinacrine on the kinetics of the TMQ-stimulated light-induced absorption change reaction at 520 mμ.
Reaction mixtures contained 25 μg chlorophyll equivalent chloroplasts per ml, 0.066 M Tris pH 6.0, plus additions as follows:
———— control, no additions; --- 0.11 mM TMQ; -.-.-.- 0.11 mM TMQ plus 2 x 10-5M quinacrine.

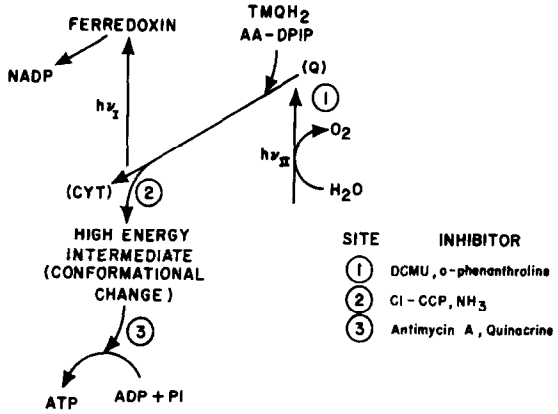

Fig. 2 Relationship between photosynthetic electron transfer and the high energy intermediate(s) responsible for the observed conformational changes. The accompanying table lists the inhibitors which act at the three sites indicated.

80 and 96 per cent respectively, while inhibiting photophosphorylation greater than 95 per cent (Krogmann and Jagendorf, 1959) (Heytler, 1963). It thus appears that

the two groups of uncouplers act at different sites in the process of photophosphorylation. NH_4^+ and Cl-CCP may be visualized as inhibiting the formation of an early high energy intermediate(s), whereas quinacrine and antimycin A (which allow these intermediate(s) to form) inhibit the coupling of the high energy intermediate(s) to ATP formation. The scheme shown in Fig. 2 indicates the relationship between electron transfer, the conformational change, and ATP synthesis as deduced from these experiments.

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