BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

Zn-COPROPORPHYRIN CATALYZED PHOTOREDUCTION OF SPINACH FERREDOXIN*

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The photoreduction of spinach ferredoxin (Fd) by chloroplasts has been described by various workers (Horio and Yamashita, 1962; Whatley, Tagawa and Arnon, 1963; Chance and San Pietro, 1963). Furthermore, the reduction of ferredoxin by chloroplasts in the light with its subsequent oxidation in a dark reaction leading to the formation of reduced pyridine nucleotide has also been demonstrated (Whatley et al., 1963). Chance and San Pietro (1963) and Chance et al. (1965) have studied the kinetics of Fd reduction by illuminated chloroplasts and shown that the rate of reduction by isolated chloroplasts was consistent with its possible role as an intermediate electron-transferring agent during NADP reduction.

The NADP-reducing system of spinach chloroplasts is composed of two components, ferredoxin and a flavoprotein enzyme referred to as ferredoxin-NADP reductase. Evidence has been presented that both components are required for the photoreduction of pyridine nucleotides by chloroplasts, with Fd being reduced initially by the chloroplast (Tagawa and Arnon, 1962; Keister et al., 1960 and 1962; Shin et al., 1963; Davenport and Hill, 1960; Davenport, 1963).

Ferredoxin has been noted as the earliest chemically isolatable reductant formed during conversion of light energy into chemical energy in photosynthesis (Arnon, 1965). Recently, the photoreduction of Fd in the presence of

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coproporphyrin and an electron donor has been reported (Kassner and Kamen, 1967). This reaction - characteristic of the chlorophyll catalyzed photoreduction of pyridine nucleotides first described by Krasnovsky and Brin (1948) - raises the possibility that Fd can function as a primary electron acceptor during photosynthesis.

The present report describes the Zn-coproporphyrin catalyzed photoreduction of Fd. This metalloporphyrin appears to be a much more potent photocatalyst than the free base porphyrin. Furthermore, this reaction represents an example of light-induced electron transport catalyzed by a metalloporphyrin other than chlorophyll.

Preparations and Methods - Zn-coproporphyrin was prepared by mixing aliquots of aqueous stock solutions of zinc sulfate (15% excess) and coproporphyrin at room temperature (30 minutes prior to its use in the reaction solution). Ferredoxin was prepared as previously described (Kassner and Kamen, 1967). A Fd-NADP reductase was prepared by repeated chromatography on DEAE-cellulose of the acetone-extract of spinach leaves.

The reactions were carried out at room temperature in Thunberg type cuvettes which contained the Fd, metalloporphyrin, and electron donor in the optical compartment; and, where indicated, NADP plus Fd-NADP reductase in the sidearm. The solutions were deaerated by repeated exchange of the air with prepurified argon after which the system was evacuated. Spectra were measured with a Cary 14R spectrophotometer following illumination as previously described (Kassner and Kamen, 1967).

Results and Discussion - Zn-coproporphyrin, like the free base porphyrin, exhibits fluorescence in polar solvents such as water and also phosphorescense. Such properties are characteristic of pigments such as chlorophyll which can photosensitize chemical reactions. It was therefore anticipated that Zn-coproporphyrin would be photoactive in the reduction of Fd in the presence of a suitable electron donor. Fig. 1 illustrates the progressive bleaching of one solution of Fd following increasing periods of illumination of an an-

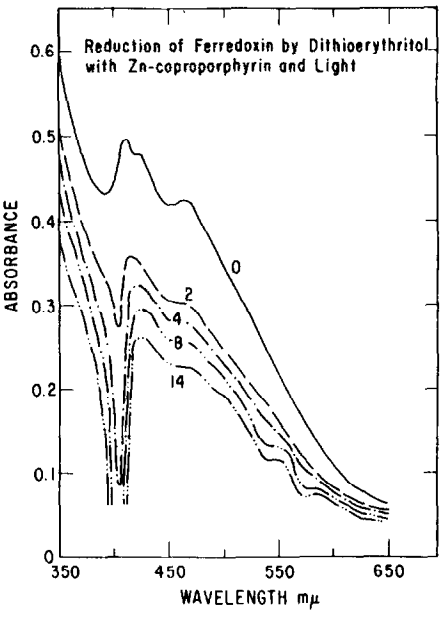

Fig. 1. Photoreduction of ferredoxin in the presence of Zn-coproporphyrin and dithioerythritol. Spectral changes in oxidized Fd on illumination were recorded at indicated intervals as a difference spectrum between a sample cuvette containing 0.150 μmoles Fd, 50 μmoles Tris-HCl buffer pH 7.95, 0.015 μmoles Zn-coproporphyrin, 15 μmoles DTE in 3.2 ml and a reference cuvette containing Zn-coproporphyrin, DTE and Tris buffer at the same concentration. The extinction coefficient used for Fd is ϵ_{420}=10.0 cm^{-1} mM^{-1}. Illumination at 2.0 x 10^6 ergs/cm^2-sec.

aerobic solution containing Zn-coproporphyrin and dithioerythritol (DTE). The reference solution contained Zn-coproporphyrin at approximately the same concentration as the reaction solution. The initial bleaching following illumination for two minutes is characteristic of the reduction of Fd as previously observed in the prophyrin-ascorbate system (Kassner and Kamen, 1967). Further illumination causes, in addition to the reduction of Fd, a bleaching of Zn-coproporphyrin as evidenced by the appearance of minima at 404.5, 437, and 573 mμ, wavelengths characteristic of the absorption maxima of Zn-coproporphyrin. It, therefore, appears that as the concentration of oxidized Fd decreases to about 50% of its original concentration in the reaction solution, photobleaching of Zn-coproporphyrin proceeds. Subsequently, the rate of bleaching of Zn-coproporphyrin increases as the concentration of reduced Fd approaches a maximum.

Whatley et al. (1963) and Fry et al. (1963) reported that Fd reduced by chloroplasts in the light was stable and could be largely reoxidized by NADP even after a considerable dark period. Likewise, if Fd was reduced by dithionite, it could be reversibly oxidized to a large extent with oxygen

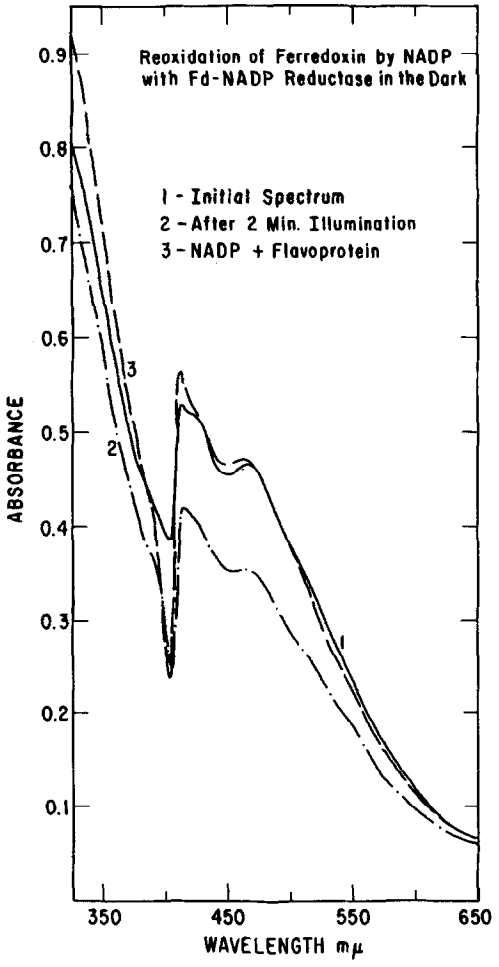

Fig. 2. Reoxidation of ferredoxin by NADP in the presence of Fd-NADP reductase in the dark following partial photoreduction. Shown are spectral changes in an anaerobic solution of Fd as a difference spectrum. The sample cuvette contained 0.15 μmoles Fd, 50 μmoles Tris-HCl buffer pH 7.95, 0.015 μmoles Zn-coproporphyrin, 15 μmoles DTE in 3 ml. The reference cuvette contained Zn-coproporphyrin, DTE, and Tris buffer at approximately the same concentration as the sample. Spectra were recorded before and after illumination; and then after addition of 3 μmoles NADP, 6 mμ moles Fd-NADP reductase, and 100 μmoles Tris-HCl buffer pH 7.95 (in 0.19 ml). The extinction coefficient used for Fd-NADP reductase is ϵ_{456}=11.0 $cm^{-1}mM^{-1}$. Illumination at 2.0 x 10^6 ergs/cm^2-sec.

(Fry et al., 1963). It was also shown that Fd could be reoxidized with oxygen following partial photoreduction in the porphyrin-ascorbate system (Kassner and Kamen, 1967). Similarly, bleaching of Fd following illumination in the above system is largely reversible after addition of NADP and Fd-NADP reductase. Fig. 2 illustrates the reoxidation of one solution of Fd by NADP in the dark following partial photoreduction in the presence of Zn-coproporphyrin and DTE.

There are two significant features of the present finding. One is that it has been shown in a model system that a metalloporphyrin other than chlorophyll can be used as a photocatalyst to promote electron transfer from a donor to an acceptor other than oxygen in aqueous solution. Secondly, the rate of reduction of Fd in the above system appears to be about 10-fold faster than that for the free base porphyrin at equal concentrations of porphyrin and Zn-coproporphyrin under the conditions of the experiment, based on the extent of bleaching following illumination for 2 minutes. Mg-coproporphyrin also appears to catalyze the photoreduction of Fd in the above system but at a rate which is somewhat slower than that of Zn-coproporphyrin. The above results lead to the proposal that a Zn-pigment like that described above could be photoactive in electron transfer in photosynthesis.

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