INTERACTIONS BETWEEN FERREDOXIN AND CHLOROPHYLL IN A MODEL MEMBRANE SYSTEM

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ABSTRACT Mixing between ferredoxin, Fd, and chlorophyll, Chl, occurs at an air-water interface. A complex is formed between the two in the mole ratio 2 Fd:1 Chl. The complex bleaches in light with a quantum yield of 0.4.

In the electron transport processes of photosynthetic bacteria and green plants, ferredoxin has been identified as an electron acceptor (1, 2). In the present work it is shown that Fd is surface active. Thus it is possible to study not only the properties of Fd at an air-water interface, but also reactions between Fd and Chl.

The techniques used in this work have been described previously by Aghion et al. (3) and by Brody (4). The spectrophotometer used to measure absorption spectra of films at an air-water interface is described in a paper by Brody.[1]

Reproducible isotherms for Fd are obtained if the ionic strength in the subphase is 0.3 or greater. With the aqueous phase buffered at pH 7.7, the area per molecule of Fd, A_{10}, is 167 A^2 at a surface tension π of 10 dynes/cm. The surface potential at $\pi = 10$ dynes/cm is $\Delta V_{10} = 170 \pm 20$ mv.

The properties of the Fd film are not modified by irradiation either in air or nitrogen atmosphere. The presence of ascorbate (10^{-3} M) in the subphase has no effect on the stability of the film.

Both A_{10} and ΔV_{10} vary with the pH of the aqueous phase. Between pH 7.0 and 7.4 a maximum value is obtained for A_{10} equal to 216 A^2; at pH 7.4 a minimum value is obtained for ΔV_{10} equal to 140 mv. At pH 8.2 a minimum value is obtained for A_{10} mv of 157 A^2 and a maximum value for ΔV_{10} of 210 mv.

In order to study interactions between Chl and Fd it is necessary to work at pH 7.7 (or higher) where Fd is in the nonexpanded state. At lower pH's Chl degrades to pheophytin rapidly; only at alkaline pH's is Chl stable (5). Consequently, the interaction studies are carried out at pH 7.7 where the area of Fd on the surface is minimum.

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[1] Brody, S. S. 1971. *Z. Naturforsch. Teil B.* **26**:922.

That mixing between Chl and Fd takes place on the surface is demonstrated spectrophotometrically. It is found that the optical density of a Chl film is decreased quantitatively ($\pm 2\%$) by the amount of Fd added to the surface. The relationship used is $OD_{Chl} \times A_{Chl} = OD_{Chl+Fd} \times A_{Chl+Fd}$ where OD and A refer to optical densities and total areas of the pure and mixed films, respectively. The red absorption maximum for the Chl film is 672 nm at $\pi = 8$ dynes/cm; the extinction coefficient is 6.6×10^4 cm^2/mole; the ratio of the blue (437 nm) to red absorption bands is 1.25. After Fd is mixed with Chl on the surface the extinction coefficient for Chl is 6.1×10^4 cm^2/mole and the blue to red absorption ratio is 1.47.

In all mixed films of Chl and Fd the experimentally measured isotherm, EXPER, is greater than the theoretically calculated isotherm, THEOR. (The latter is determined by adding together the isotherms of Fd and Chl which are obtained in separate experiments.) The observation that EXPER $>$ THEOR is a sufficient condition to demonstrate an interaction or complex formation between Chl and Fd. The increase in size of the film upon complex formation could be indicative of a conformation change of the protein or a change in orientation of Fd or Chl on the surface.

The difference between the experimental and theoretical isotherms, (EXPER − THEOR), measured at 10 dynes/cm, is a maximum when $(Fd)/(Chl) \simeq 2$. Thus it appears that there may be a complex of the form Fd-Chl-Fd. Using this stoichiometry for the complex, the area calculated for the complex A_{10} is 520 A^2.

The Chl-Fd complex is sensitive to light in a nitrogen atmosphere. One indication of a reaction is that the slope of the isotherm always increases after irradiation. In some experiments the slope increases 30%. Associated with this change in slope is a decrease in the area of the complex. These effects are in contrast to the absence of any changes upon irradiating a film of Chl in nitrogen (3). The surface potential of the Chl-Fd film, in nitrogen, increases about 20 mv after irradiation; this value varies considerably between experiments.

A bleaching of Chl in the Chl-Fd complex is observed after irradiating the film. A decrease is also observed in the ratio of the absorption bands A 672/A 436, A 672/A 499, A 672/A 631. The photoreaction is most likely a reduction of Fd coupled to an oxidation of Chl. Absorption bands characteristic of oxidized Chl in methanolic solution, at 595 and 750 nm (6), are not observed in the Chl film. Furthermore there is no spectral evidence of pheophytinization. A conservative estimate for the quantum yield of bleaching of Chl is 0.4. Complete data and details of this work are published elsewhere.[1]

I would like to thank Marie-Noelle Jaumain for her assistance in carrying out the experimental portion of this work.

This work was supported by research grants from the National Institutes of Health (RO-1-EY 00173) and the National Science Foundation (GB 18354).

The author was supported, in part, by a U.S. Public Health Service Research Career Program Award (K3-GM 17918).

Received for publication 7 May 1971.

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