EVIDENCE FOR THE PHOTOCHEMICAL REDUCTION ON COENZYME Q IN CHROMATOPHORES OF PHOTOSYNTHETIC BACTERIA

Roderick K. Clayton

Biology Division, Oak Ridge National Laboratory,* Oak Ridge, Tennessee

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Chromatophores of photosynthetic bacteria exhibit several classes of light-induced absorption spectrum changes (Arnold and Clayton, 1960; Clayton, 1962a-d), including the following: (1) Light changes the absorption spectrum of bacteriochlorophyll (BChl). The main effect is a bleaching of the long wave band at 870-890 mμ. Chemical oxidation, but not reduction, causes similar changes (cf Duysens, 1952; Goedheer, 1960). It has been concluded provisionally that the light-altered form of BChl is oxidized BChl. (2) Light generates new absorption bands at 420-450, 715, and 1000-1250 mμ. Oxidation, but not reduction, produces similar bands. Each of these bands represents a transition whose energy is about 0.4 ev less than that of a corresponding BChl band (the absorption bands of BChl in vivo are at 375, 590, and 800-900 mμ). These new bands, therefore, may belong to the light-altered (oxidized) BChl. (3) Light-induced spectral changes in the region 240-350 mμ suggest the reduction of Coenzyme Q (CoQ). These changes are the main subject of this communication.

Absorbancy changes have been recorded with a Beckman DK-2a Spectrophotometer. Absorbancy at a chosen wave length was recorded vs time while the sample was exposed intermittently to a beam of exciting light. Filters placed in the paths of the exciting and measuring beams prevented the detector from responding directly to the exciting light.

* Operated by Union Carbide Corporation for the United States Atomic Energy Commission.

The changes listed above as (1)-(3) are reversible, with decay times of a few seconds, in dried films of chromatophores as well as in aqueous chromatophore suspensions. They all show the same kinetics. They are illustrated, at four wave lengths, in Fig. 1.

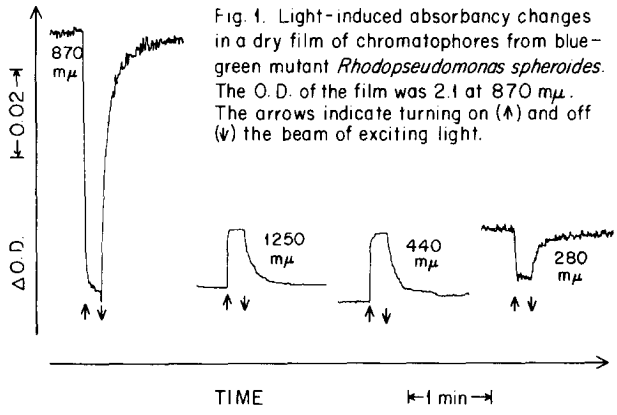

Fig. 1. Light-induced absorbancy changes in a dry film of chromatophores from blue-green mutant *Rhodopseudomonas spheroides*. The O.D. of the film was 2.1 at 870 mμ. The arrows indicate turning on (↑) and off (↓) the beam of exciting light.

Chromatium chromatophores (donated by Dr. R. C. Fuller) yielded the difference spectra shown in Fig. 2. Curve A was obtained by preparing an ethanol solution of material extracted from the chromatophores with iso-octane. The absorption spectrum of this solution was recorded before and after reduction with borohydride, and the difference plotted. This spectrum reflects the reduction of CoQ_7 and of a lesser amount of vitamin K (Fuller et al., 1961). Curve B is a corresponding "reduced-minus-oxidized" spectrum for chromatophores suspended in dilute aqueous Na_2CO_3 and exposed to borohydride. Chemical oxidation of Chromatium chromatophores, using 10^{-3} M $K_2S_2O_8$; had little effect on their absorption spectrum at 240-350 mμ. Curve B differs from Curve A in showing a greater +ΔO.D. around 315 mμ; this probably reflects the δ-band of reduced cytochrome.

Curve C of Fig. 2 shows the spectrum of light-induced changes in Chromatium chromatophores (note that the ordinate for Curve C is exaggerated ten-fold). Essentially the same spectrum is displayed by aqueous suspensions and dried films of chromatophores from Rhodopseudomonas spheroides and Rhodospirillum rubrum. Curve C is similar to curves A and B, and to "reduced-minus-oxidized" spectra of CoQ (cf Crane, 1961; Fuller et al.,

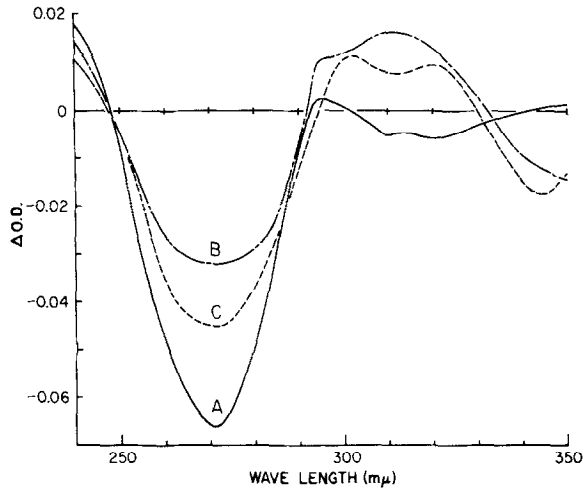

Figure 2. Difference spectra of Chromatium chromatophore suspensions and extracts. A, Ethanol solution of iso-octane extract (reduced - oxidized); B, suspension in aqueous solution of 0.025 M Na_2CO_3 (reduced - oxidized); C, suspension in H_2O (light - dark). The curves are normalized to a density of 1 mg of dry chromatophores/ml and 1-cm optical path. The ordinate of curve C is exaggerated ten times.

1961). Vitamin K probably does not contribute to the light-dark spectra of chromatophores since R. rubrum contains CoQ_9 but no vitamin K (Lester and Crane, 1959).

At 280 mμ the $\Delta\varepsilon$ (oxidized - reduced) of vitamin K is zero, whereas that of CoQ is 10.0 $mM^{-1}cm^{-1}$ (Crane, 1961). On this basis the CoQ_7 content of Chromatium chromatophores, determined from Curve A, Fig. 2, is 4.9 μmole per g of dry mass. Fuller et al. (1961) reported a value of 5.6 μmole/g. If the light reaction at 280 mμ represents CoQ reduction with $\Delta\varepsilon$ = 10.0 $mM^{-1}cm^{-1}$, the amount of CoQ that participates in reversible photoreduction (Curve C) is 0.37 μmole/g.

The BChl content of these Chromatium chromatophores was found to be 24.5 μmole/g [cf 26.5 μmole/g reported by Fuller et al. (1961)]. In their light reaction, the altered BChl molecules have lost from 50 to 100% of their absorbance at the long wave maximum, 890 mμ in Chromatium (Clayton, 1962b, c). The $\Delta\varepsilon$ for the light reaction of BChl thus is uncertain by a factor of 2. Allowing for this uncertainty, one finds that 0.2 to 0.4 μmole/g of BChl participates in the reversible light reaction in Chromatium chromatophores.

Another light reaction in chromatophores is the oxidation of one or more cytochromes (Duysens, 1954; Smith and Ramirez, 1959; Clayton, 1962d; Olson, 1962). This reaction occurs in dried films and in aqueous suspensions of Chromatium chromatophores, but its reversal (in the dark) is slower than that of the other reactions described here. In light-adapted preparations the cytochrome remains fully oxidized while the reactions of types (1) - (3) above proceed reversibly (Clayton, 1962d). When fully dark-adapted, the chromatophore suspension of Fig. 2 showed light-induced spectral changes corresponding to the oxidation of 0.41 μmole/g of cytochrome. This figure is based on Olson's (1962) value of 62 $mM^{-1}cm^{-1}$ for the $\Delta\varepsilon$ of the cytochrome reaction at 423 mμ.

The data of the last three paragraphs are shown in Table 1.

Table 1

Light-reacting Components of Chromatium Chromatophores

Material	Total μmole/g	Light-induced change, μmole/g
Bacteriochlorophyll	24.5	0.2 - 0.4 (oxidized)
Coenzyme Q	4.9	0.37 (reduced)
Cytochrome		0.41 (oxidized)

These findings implicate Coenzyme Q as a primary electron acceptor, and bacteriochlorophyll as a primary electron donor, in bacterial photosynthesis.

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