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*TWO LIGHT REACTIONS OF BACTERIOCHLOROPHYLL IN VIVO**

By Roderick K. Clayton

CHARLES F. KETTERING RESEARCH LABORATORY, YELLOW SPRINGS, OHIO

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The chemistry of bacterial photosynthesis begins at a photochemical reaction center[1] that effects a separation of oxidant and reductant at the expense of light energy. Substances that have been implicated in the functioning of the reaction center are one or more cytochromes,[2, 3] a specialized BChl[4] component designated P870,[5–7] and UQ.[8] In cells and chromatophores of photosynthetic bacteria, a light-induced bleaching of P870 is manifested by characteristic absorbancy changes in the region from 700 to 1,250 mμ.[5, 7, 9, 10] This reaction has been identified as a photochemical oxidation, both because chemical oxidation produces the same difference spectrum and because a reducing environment accelerates the reversal, in the dark, of the light reaction.[5, 11] Recently it was shown that this light reaction is accompanied by absorbancy changes corresponding to the reduction of UQ,[8] and by a broad band of increased absorption centered at 435 mμ.[5, 10] A primary photochemical reaction in bacterial photosynthesis was formulated[1] as

$$\text{P870}\cdot\text{UQ} \xrightarrow{h\nu} \text{P870}^*\cdot\text{UQ} \rightarrow \text{P870}^+\cdot\text{UQ}^-, \qquad (1)$$

where P870* represents P870 in an excited state.

Another light reaction in photosynthetic bacteria is the oxidation of Cyt,[2, 3] presumably coupled with the reduction of an electron-acceptor molecule:

$$\text{Cyt}\cdot\text{A} \xrightarrow{h\nu,\ \text{BChl}} \text{Cyt}^+\cdot\text{A}^-. \qquad (2)$$

For reaction (2) to occur reversibly, it is of course necessary that the Cyt become reduced in the dark following its photooxidation. The light reaction of P870 is maximal when the cytochrome reaction is blocked (i.e., under such conditions that the Cyt remains oxidized in the dark), and the P870 reaction becomes truncated when the cytochrome reaction is able to occur vigorously.[3] Both light reactions have been shown to occur with high quantum efficiency at liquid nitrogen temperature, P870 oxidation reversibly and cytochrome oxidation irreversibly.[12, 13]

It has been proposed[1, 3] that reaction (2) is actually a sequel to reaction (1), with oxidized P870 as electron acceptor:

$$\text{Cyt}\cdot\text{P870}^+ \rightarrow \text{Cyt}^+\cdot\text{P870}; \qquad (3)$$

in this formulation the coupled reactions (1) and (3) produce UQ^- and Cyt^+ as

starting points for the electron transfer reactions of photosynthesis. Alternatively, it has been suggested[13, 14] that reaction (2) is the central primary reaction, with BChl (or P870) as electron acceptor:

$$\text{Cyt}\cdot\text{BChl} \xrightarrow{h\nu} \text{Cyt}\cdot\text{BChl}^* \rightarrow \text{Cyt}^+\cdot\text{BChl}^-. \quad (4)$$

In this view, reaction (1) is either an independent event or a nonfunctional light reaction that occurs only when Cyt is in its oxidized state. Development of this point of view has been hampered because until now there has been no evidence for the formation of reduced BChl concomitant to the appearance of oxidized Cyt. The present communication describes a light-induced reaction suggestive of the reduction of BChl in cells and chromatophores of *Rhodopseudomonas spheroides*. This reaction accompanies the oxidation of Cyt, whereas the oxidation of P870 is accompanied by absorbancy changes reflecting reduction of UQ and by an absorption increase at 435 mμ.

Methods.—The cultivation of *R. spheroides* and the preparation of chromatophore fractions has been described in detail elsewhere,[15] as has the technique of observing light-induced absorption spectrum changes and chemically induced difference spectra.[5, 10] In these observations several spectrophotometers were used: the Beckman DK2a, the Perkin-Elmer Model 350, and a single-beam difference spectrophotometer constructed from a Leiss monochromator and an electrical balancing circuit. Results were independent of the instrument used.

Results.—Figure 1 shows light-induced absorption spectrum changes observed in cells of blue-green (carotenoidless) mutant *R. spheroides*, together with an absorption spectrum of the preparation. The cells were harvested toward the end of

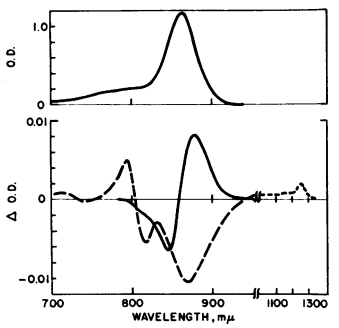

FIG. 1.—Absorption and light-induced difference spectra for cells of blue-green mutant *Rhodopseudomonas spheroides* (strain 2.4.1/CC1/R-26). Cells were suspended in phosphate buffer, 0.01 *M*, pH 7.0, containing 30% albumin to reduce light scattering. *Upper curve*, absorption spectrum. *Lower solid curve*, spectrum of reversible light-induced absorbancy changes in the freshly prepared cell suspension. *Lower dashed curve*, spectrum of light-induced changes in the same cell suspension after 24 hr storage at 3°C.

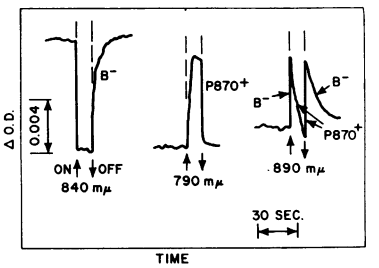

FIG. 2.—Kinetics of light-induced absorbancy changes in wild-type *R. spheroides* cells (strain 2.4.1) suspended in 30% aqueous albumin, pH 7.0. Portions of the traces labeled B^- pertain to one light reaction; those labeled $P870^+$ pertain to another (see text). Arrows show when the exciting light was turned on and off. Wavelengths pertain to the measuring light. For the traces at 890 and 790 mμ, the exciting light intensity was 1,100 arbitrary units; for that at 840 mμ, the intensity was 460 units. These units can be assessed by reference to Fig. 3.

exponential growth and suspended in 30 per cent aqueous albumin to reduce their turbidity. The lower solid curve pertains to the freshly harvested and resuspended cell suspension ("fresh cells"); the dashed curve describes the behavior of the same cells after 24 hr storage at 3°C ("stored cells"). The stored cells showed the light-induced oxidation of P870 and also the absorption increase at 435 mμ. They did not show a change corresponding to cytochrome oxidation. In contrast the fresh cells showed, in the visible, only a change representing light-induced cytochrome oxidation. The P870 oxidation and the 435-mμ absorption increase were entirely lacking in these fresh cells. Instead they showed in the infrared the light-induced change shown in Figure 1 (lower solid curve). This change appears to be a bathychromic shift of a band centered at the absorption maximum of BChl.

Because of the wavelengths involved, both types of light-induced changes shown in Figure 1 appear related to BChl. Evidence already exists that P870 is BChl in a specialized environment.[7] For convenience, the two infrared reactions will be termed B^- (lower solid curve, fresh cells) and $P870^+$ (dashed curve, stored cells). The term B^- is chosen because of a tentative identification of this reaction with the reduction of BChl, or of P870 (see below). $P870^+$ encompasses the bleaching of P870 and related effects near 800 and 1,250 mμ.

Reactions B^- and $P870^+$ could often be observed simultaneously in cells or in freshly prepared chromatophores. The two reactions could then be distinguished kinetically, as shown in Figure 2 for cells of wild-type *R. spheroides*. At 890 mμ, using strong exciting light, B^- (absorption increase at this wavelength) showed a rapid onset and a slow decay, whereas $P870^+$ (absorption decrease) rose slowly and decayed rapidly. The kinetics of $P870^+$ are seen in isolation at 790 mμ, where B^- is nil. B^- could be isolated by using weaker exciting light (see the 840-mμ trace in Fig. 2). In fact, $P870^+$ became appreciable only at exciting light intensities sufficient to saturate B^- (see Fig. 3). Correspondingly, in the visible part of the spectrum an oxidation of cytochrome was effected by weak exciting light, but the 435-mμ absorption increase appeared only under stronger excitation.

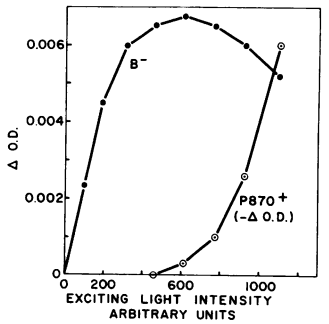

FIG. 3.—Light - saturation curves for the two kinds of light-induced change shown in Fig. 2, using the same preparation of wild-type *R. spheroides* cells, measured at 890 mμ.

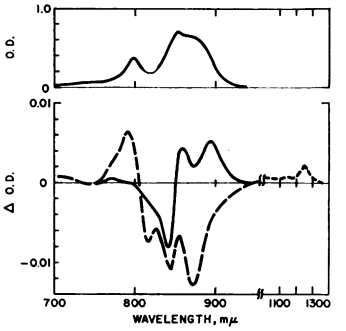

FIG. 4.—Spectra of the two kinds of light-induced change shown in Fig. 2, using the same preparation. The two effects were discriminated kinetically as indicated in Fig. 2. *Upper curve*, absorption spectrum. *Lower solid curve*, spectrum of B^- reaction. *Lower dashed curve*, spectrum of $P870^+$ reaction.

The spectra of B^- and $P870^+$ in the foregoing suspension of wild-type *R. spheroides* cells are shown in Figure 4. Incomplete kinetic separation of the two reactions is suggested by the appearance of hybridization: each curve partakes a little of the shape of the other. When B^- is eliminated, for example by adding sodium azide or (in chromatophores) deoxycholate, the spectrum of $P870^+$ assumes the simple form shown in Figure 1.

The B^- and $P870^+$ reactions respond in opposite ways to redox agents. Aeration, sufficient to oxidize endogenous cytochromes, reversibly abolishes B^- and enhances $P870^+$. Mild reductants (potential about 100 mv) enhance B^- and also enhance the photooxidation of Cyt, while diminishing the $P870^+$ reaction.

The $P870^+$ light reaction is mimicked by chemical oxidation (potential 470 mv).[5] Reduction of chromatophores with hydrosulfite produces difference spectra that resemble crudely a superposition of B^- and of $P870^+$ with its sign reversed. These spectra were variable, as though the relative contributions of B^- and "reversed $P870^+$" differed in different preparations.

Discussion.—The reaction designated B^- accompanies the photochemical oxidation of Cyt and involves absorbancy changes near the principal absorption maximum of BChl. It is natural, therefore, to regard B^- as a manifestation of BChl reduction accompanying the oxidation of Cyt. This interpretation is strengthened by the fact that both B^- and the Cyt oxidation are abolished by aeration and enhanced in a reducing environment. Finally, there is some evidence that chemical reduction produces changes similar to those of B^-.

The two sets of reactions, [Cyt^+, B^-] and [$P870^+$, UQ^-], interact in the sense that the second is maximal when the first is inoperative. Interaction of two light reactions is also implicit in the kinetics of the $P870^+$ reaction. When the B^- reaction is absent, $P870^+$ shows kinetics appropriate to a single light reaction: the decay in the dark is somewhat slower than the rise at the onset of illumination. But when B^- occurs, as in Figure 2, $P870^+$ shows the peculiar kinetic pattern of a decay that is much faster than the rise. This can come about only if the decay of $P870^+$ is influenced by the product of another light reaction. A relationship between [Cyt^+, B^-] and [$P870^+$, UQ^-] is suggested further by the fact that P870 and light-reacting Cyt are present, in chromatophores, at equal concentrations,[8] and both survive treatments that destroy all of the light-harvesting BChl.[7]

The occurrence of Cyt oxidation in fresh cells with high quantum efficiency in dim light[16] encourages the belief that [Cyt^+, B^-] reflects a primary event in photosynthesis. It remains to be seen whether the second primary light reaction [$P870^+$, UQ^-] plays an essential role in photosynthesis, or whether it serves a secondary function or no function at all.

Summary.—Photosynthetic bacteria exhibit two kinds of reversible light-induced absorption spectrum changes near the infrared absorption maximum of bacteriochlorophyll. One of these reactions reflects the oxidation of P870, a specialized bacteriochlorophyll component. This reaction is accompanied by spectral changes that correspond to reduction of ubiquinone. The other infrared light reaction accompanies cytochrome oxidation and may reflect the reduction of bacteriochlorophyll.

* Contribution 123 from the Charles F. Kettering Research Laboratory.

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INBRED GENETIC LOADS AND THE DETERMINATION OF POPULATION STRUCTURE

BY HOWARD LEVENE*

DEPARTMENTS OF MATHEMATICAL STATISTICS AND ZOOLOGY, COLUMBIA UNIVERSITY

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Morton, Crow, and Muller, in a pioneering paper,[1] gave a method for determining from inbred and outbred individuals whether the genetic load in a population was due mainly to deleterious genes maintained by mutation pressure (mutational load) or to genes maintained because the heterozygote was superior to the homozygotes (the segregational load of Crow[2] or the balanced load of Dobzhansky[3]). Morton, Crow, and Muller applied their method to some human data from France and the United States, and concluded that the load due to inbreeding was mainly mutational. The method was subsequently applied by many authors. Perhaps the best and most extensive human data is that of Neel and Schull[4] from Japan, while the best nonhuman data so far published is that of Dobzhansky, Spassky, and Tidwell[5] on Drosophila. These two studies did not rule out a major role for overdominant loci. Recently there has been some controversy over the validity of the approach of Morton, Crow, and Muller, and it has been criticized on mathematical and theoretical grounds by Li[6, 7] and Sanghvi[8] and defended by Crow.[9] The present paper is devoted exclusively to continuing this discussion and to an examination of whether or not population structure can be determined by this method in practice. The question of whether, in Dobzhansky's terminology, the classical or the balanced theory is in fact more nearly correct can be attacked in many other ways, and will not be considered here.

Consider a single locus with two alleles. Under the assumption of random mating, zygotic frequencies will be p^2 AA:$2pq$ Aa:q^2 aa. Let the expected number of offspring one generation later of each of the three genotypes be w_1, w_2, w_3, respectively. The expected number of offspring of a random individual one genera-