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Nature of the primary electron acceptor in bacterial photosynthesis*

Coupled photooxidation of reaction-center bacteriochlorophyll P890 and photoreduction of ubiquinone-7 in the subchromatophore fragments derived from *Chromatium* has recently been reported[1]. The different responses of the near infrared and ultraviolet absorbance changes under certain redox conditions established that they accompany changes in P890 and ubiquinone, respectively. The rapid onset times for both reactions and their temperature independence from room temperature to 77 °K led to the suggestion that these are probably directly coupled primary photochemical reactions.

Recently the time resolution of the rapid spectrophotometer[2] has been improved by using the Q-switched ruby-laser pulse ($2 \cdot 10^{-8}$ sec) as an excitation source. The time resolution of the detection system has been improved from approx. 10^{-4} to 10^{-6} sec by using a high-speed digitizer in connection with a Fabri-tek model FT-1062 Signal Averager. This note reports some more recent results obtained by reexamining the ubiquinone reaction. The absorbance change at 280 mμ accompanying ubiquinone reduction is presented in the upper record of Fig. 1. At the time resolution available the halftime of this reaction is 1 μsec or less. For purposes of comparison the oxidation of cytochrome-422 in unfractionated *Chromatium* chromatophores was also measured. As seen from the lower record in Fig. 1, cytochrome-422 oxidation has a halftime of 3 μsec, which is in good agreement with that reported by PARSON[3]. It should be noted that the cytochrome-oxidation signal is preceded by a rapid absorbance increase which is presumably a part of the broad 430 mμ absorbance change often observed in bacterial chromatophores[4].

Although the suggestion that ubiquinone is a participant in the primary photochemical process is supported by this extremely rapid onset time measurement, other evidence has been obtained which makes it unlikely that ubiquinone is the primary electron acceptor.

It has been shown earlier[1] that in the presence of reduced 2,6-dichlorophenolindophenol, reduced phenazine methosulfate or *N,N,N',N'*-tetramethyl-*p*-phenylenediamine, the rapid photoreduction of ubiquinone is followed by a secondary electron transfer from these donors, mediated by bacteriochlorophyll. However, if ascorbate is present alone in sufficient amount, the photooxidation of P890 is unaffected, whereas the 275 mμ absorbance change due to ubiquinone reduction completely disappears. In unfractionated *Chromatium* chromatophores, the photooxidation of both P890 and cytochrome-422 are also unaffected by ascorbate.

The chemical reduction of ubiquinone under conditions where the 275 mμ absorbance-decrease signal disappears can be demonstrated by difference spectroscopy using tandem dual-compartment cuvettes. The spectra actually recorded are presented in Fig. 2. Spectrum a is that obtained with a suspension of the photochemically active subchromatophore fragments placed in Compartment 1 of the tandem cuvette, using Tris buffer as the reference solution. Spectrum b was taken with the same tandem cuvette that was used for Spectrum a, except that an additional 1.5 ml of 55 μM ascorbate solution was placed in Compartment 2 of the cuvette. Absorption spectra

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c, d, and e are difference spectra showing the reduction of ubiquinone by ascorbate and other compounds. Spectrum c was obtained using two cuvettes containing solutions identical to those used for Spectrum b, placed in the sample and reference beams. This spectrum represents a baseline with unmixed chromatophore fragments and ascorbate in separate compartments of the tandem cuvettes. Spectrum d was

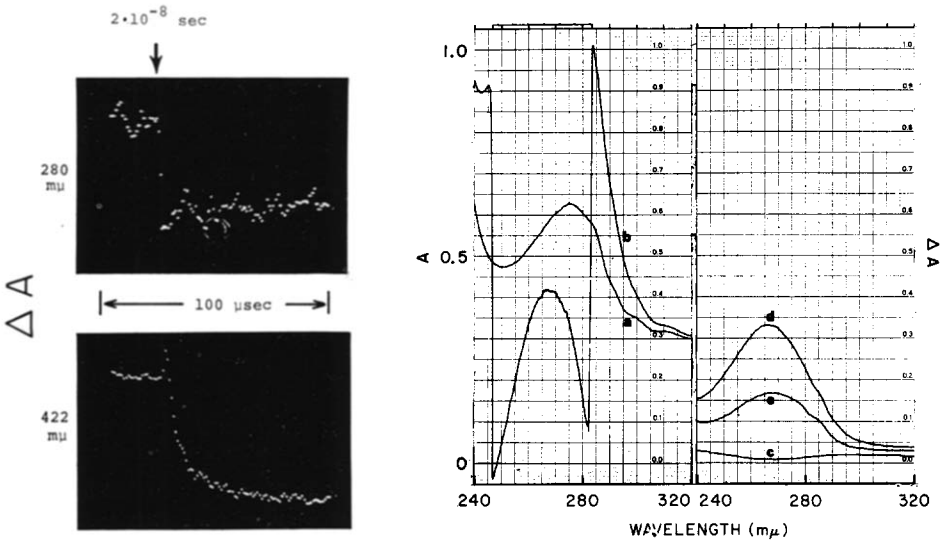

Fig. 1. Absorbance-change transients accompanying ubiquinone reduction in the photochemically active subchromatophore fragments and cytochrome-422 oxidation in unfractionated chromatophores of *Chromatium*. Excitation source: Q-switched ruby-laser pulse with a duration of $2 \cdot 10^{-8}$ sec. The 280-mμ measuring light was a line source from a mercury lamp. The signals were obtained at different sensitivities and with an arbitrary number of flashes to yield a size suitable for presentation. The actual ΔA's are $2.2 \cdot 10^{-3}$ (top) and $3.3 \cdot 10^{-3}$ (bottom), respectively.

Fig. 2. Absorbance (a, b) and difference (c, d, e) spectra of ubiquinone reduction by ascorbate. Absorbance scale applies to all five spectra. Spectrum b is on a folded scale: 0–1–2. Details explained in the text.

measured 15 min after the solutions in the cuvette placed in the reference beam were mixed, *vs.* the unmixed solutions in the sample beam. The difference in absorbance represents losses in absorbance due to the reduction of ubiquinone and oxidation of ascorbate. Spectrum e was obtained by adding excess $NaBH_4$ to rereduce the dehydroascorbate. Thus, the remaining absorbance difference in Spectrum e represents only the loss in absorbance due to the reduction of ubiquinone. Taking the millimolar extinction coefficients of ubiquinone and ascorbate to be 12 and 15, respectively, the magnitudes of the absorbance differences in Spectra d and e are consistent with a quantitative reduction of ubiquinone by an equimolar amount of ascorbate.

The experimental results presented above suggest that some component other than ubiquinone must be accepting the electron directly from photooxidized P890 during the initial charge-separation process, and that the ubiquinone must be part of a tightly organized reaction-center complex to allow its reduction to occur at 77 °K and in less than 1 μsec. This is consistent with the earlier finding that partial extraction of ubiquinone by organic solvent had little effect on its absorbance-change

transient. Following these suggestions, the absorbance-change transient accompanying the reduction of the primary electron acceptor itself would have extremely rapid onset and decay times, which would tax the capability of the present method of detection. Further improvement of instrument time resolution as well as other approaches are planned for a continued investigation of these fundamental aspects of the primary photochemical process in bacterial photosynthesis.

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