BBA 46098

# REDOX PROPERTIES OF THE "P-836" PIGMENT COMPLEX OF CHROMATIUM

## G. L. SCHMIDT<sup>1</sup> AND M. D. KAMEN

Department of Biology<sup>1</sup> and Department of Chemistry, University of California, San Diego, La Jolla, Calif. 92 037 (U.S.A.)

(Received November 6th, 1970)

### SUMMARY

The redox potential of the component responsible for the photobleaching at 836 nm in chromatophores of *Chromatium vinosum* strain D was determined to be 320 mV. This result may be of significance for suggestions regarding the existence of a low potential, non-cyclic photosystem in *Chromatium*.

Recently, Thornber<sup>1</sup> isolated a subchromatophore particle from *Chromatium vinosum*, strain D, which exhibited light-induced bleaching at 836 nm. As a light-induced change at approx. 840 nm is seen in *Chromatium* chromatophores<sup>2,3</sup>, we have investigated its redox properties in an effort to determine its possible role as a reaction center.

Classical chromatophores were prepared by the method of Cusanovich and Kamen<sup>4</sup>. Light-induced absorbance changes and redox potentials were determined by the methods of Cusanovich *et al.*<sup>2</sup>. Fig. 1 compares the light-induced changes in the near infrared of anaerobic and aerobic chromatophores. Anaerobic conditions were created by bubbling a suspension of chromatophores in 0.15 M potassium phosphate buffer (pH 7.5) with O<sub>2</sub>-free argon. After recording the difference spectrum presented in Fig. 1a, the suspension was flushed with air and the difference spectrum presented in Fig. 1b was recorded. The magnitude of the photobleaching at 838 nm was found to decrease with time of storage (Fig. 1c).

Quantitation of the 836 nm bleaching is difficult due to baseline shifts and interference of the light-induced changes at 808 and 883 nm. For this reason, the difference between absorbance at 850 nm and 836 nm was measured relative to the redox potential. A mixture of 10<sup>-4</sup> M K<sub>3</sub>Fe(CN)<sub>6</sub> and 10<sup>-4</sup> M disodium 2,6-dibromobenzenoneindo-3'-carboxyphenol was employed as a redox buffer to cover the potential range 200–400 mV.

The effect of redox potential of the 836 nm photobleaching is presented in Fig. 2. The midpoint potential was determined as 320 ( $\pm$ 5) mV. A one-electron titration curve provided the best fit for the experimental points. As a control, the light-induced change at 883 nm was also titrated, using 10<sup>-3</sup> M K<sub>3</sub>Fe(CN)<sub>6</sub> as a redox buffer to cover the potential range 520–350 mV. As shown in Fig. 3, the midpoint potential was found to be approx. 495 mV, which agrees with a previous report<sup>2</sup>.

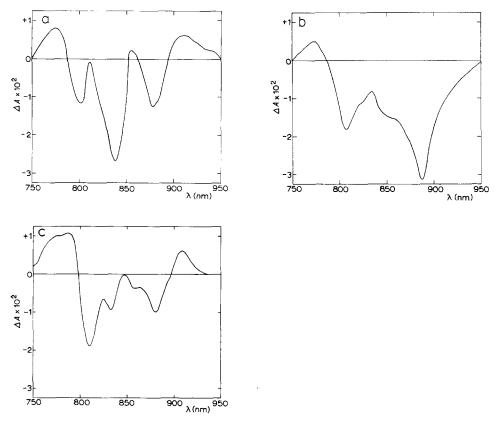


Fig. 1. Light-induced absorbance changes in the near infrared spectrum of Chromatium chromatophores. Chromatophores were suspended in 0.15 M potassium phosphate buffer (pH 7.5) at a bacteriochlorophyll concentration of 10  $\mu$ M. Actinic light intensity was 1·10<sup>4</sup> ergs·cm<sup>-2</sup>·sec<sup>-1</sup> after passing through a filter with a 500–650 nm band pass. (a) Anaerobic chromatophores. (b) Aerobic chromatophores. (c) Anaerobic chromatophores after 17 days storage under argon at 4°.

Evidence for two photosystems in photosynthetic bacteria has been reviewed by HIND AND OLSON<sup>5</sup>. In the case of *Chromatium*, this evidence is based on the presence of a low potential cytochrome (C-552) and a high potential cytochrome (C-555)<sup>6,7</sup>, the cytochrome action spectra of Morita<sup>8</sup>, and the redox potential correlations of Cusanovich and Kamen<sup>2</sup>. On the other hand, Seibert and DeVault<sup>9</sup> and Parson and Case<sup>10</sup> have presented evidence which is interpreted to indicate that both C-552 and C-555 are photooxidized by the cyclic system of which the P-883 reaction center chlorophyll is a component. The present report agrees with that of Cusanovich and Kamen<sup>2</sup>, who measured the midpoint potential of P-883 at 489 mV. According to Ross and Calvin<sup>11</sup>, the maximum free energy which can be trapped by *Chromatium* bacteriochlorophyll is 0.79 eV per quantum. Therefore, while light absorption either by the P-883 or the P-836 pigment complex could possibly result in NAD<sup>+</sup> photoreduction, one may suggest that, by virtue of its lower potential, the P-836 pigment complex could function as a component in the reaction center for the non-cyclic low potential photosystem postulated to occur in *Chromatium*.

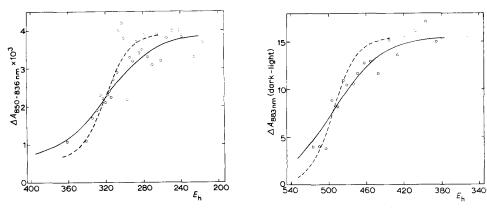


Fig. 2. Redox titration of 838 nm light reaction. The redox buffer was 10-4 M K<sub>3</sub>Fe(CN)<sub>6</sub> and 10-4 disodium 2,6-dibromobenzenoneindo-3'-carboxyphenol. Other conditions were as described –, calculated 1 electron change with  $E_{
m m}$ , = 320 mV; ----, calculated 2 electron change with E, = 320 mV.

Fig. 3. Redox titration of 883 nm light reaction. The redox buffer was 10-3 M K<sub>3</sub>Fe(CN)<sub>6</sub>. Other conditions were as described in Fig. 1. ——, calculated 1 electron change with  $E_{\rm m}$ , = 495 mV; ——, calculated 2 electron change with  $E_{\rm m}$ . = 495 mV.

### ACKNOWLEDGEMENTS

This work was supported by grants-in-aid from the National Institutes of Health (HD-01262) and the National Science Foundation (GB-7033X) to one of us (M.D.K.). The senior author was supported by a predoctoral fellowship from the National Institutes of Health (I-FoI-GM-41, 206-02).

### REFERENCES

- I J. P. THORNBER, Biochemistry, 9 (1970) 2688.
- 2 M. A. Cusanovich, R. G. Bartsch and M. D. Kamen, Biochim. Biophys. Acta, 153 (1968) 397.
- 3 B. KE, L. P. VERNON, A. GARCIA AND E. NGO, Biochemistry, 7 (1968) 311.
- 4 M. A. Cusanovich and M. D. Kamen, Biochim. Biophys. Acta, 153 (1968) 376.
- 5 G. HIND AND J. M. OLSON, Ann. Rev. Plant Physiol., 19 (1968) 249.
- 6 J. M. Olson and B. Change, Arch. Biochem. Biophys., 88 (1960) 26.
- 7 J. M. Olson and B. Change, Arch. Biochem. Biophys., 88 (1960) 40. 8 S. Morita, Biochim. Biophys. Acta, 153 (1968) 241.

- 9 M. SEIBERT AND D. DEVAULT, Biochim. Biophys. Acta, 205 (1970) 220.
- 10 W. W. PARSON AND G. D. CASE, Biochim. Biophys. Acta, 205 (1970) 232.
- 11 R. T. Ross and M. Calvin, Biophys. J., 7 (1967) 595.

Biochim. Biophys. Acta, 234 (1971) 70-72