CYTOCHROMES IN CHLOROPHYLL-CONTAINING PARTICLES OF CHROMATIUM AND CHLOROBIUM THIOSULFATOPHILUM *

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Since the photosynthetic bacteria appear to contain the simplest structural units capable of photophosphorylation (Anderson and Fuller, 1958), the occurrence and nature of cytochromes in purified particles from photosynthetic bacteria can provide information for investigation of their function. This paper reports observations on the cytochrome content of purified chlorophyll-containing particles from the purple sulfur photoanaerobe, Chromatium strain D, and the green sulfur photoanaerobe, Chlorobium thiosulfatophilum. Information is presented about the relationship of these cytochromes to cytochrome f and to those isolated from Chromatium by Newton and Kamen (1956) and Bartsch and Kamen (1960).

MATERIALS AND METHODS

Chromatium was grown autotrophically according to Hendley (1955), and C. thiosulfatophilum was grown in the manner described by Larsen (1953). Chromatophores of Chromatium were isolated by the procedure of Anderson and Fuller (1958). The green particles of C. thiosulfatophilum

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were obtained by crushing 10 g of packed frozen cells in a Hughes press after which 10 μg of DNA'ase was added and the mixture suspended in 25 ml of 0.25 M Tris** buffer at pH 7.5 containing 0.001 M EDTA.** This suspension was centrifuged at 32,000 X G for 25 minutes and the residue resuspended, homogenized and centrifuged again. The supernatant fractions were combined and spun in a Spinco Model E ultracentrifuge for 30 minutes at 41,000 X G. The supernatant suspension was placed in tubes containing a small amount of washed glass wool, centrifuged at 144,000 X G for 120 minutes and the sediment resuspended in 0.1 M Tris buffer at pH 7.5 containing 0.001 M EDTA. After repeating this operation the particles were subjected to low speed centrifugation to remove aggregates.

Chromatophores of Chromatium yielded a single major peak when subjected to ultracentrifugal analysis. Preparations from Chlorobium tended to aggregate; yet, they were free of slower moving components when subjected to ultracentrifugal analysis. Further analytical data on this preparation of particles from C. thiosulfatophilum has been presented by Bergeron and Fuller (1960). Attempts to analyze the preparations by moving boundary electrophoresis were unsuccessful because of the low specific gravity of the particles.

Bacteriochlorophyll and carotenoid were determined in the Chromatium chromatophores by measuring absorbance at 800 and 490 mμ using millimolar extinction coefficients of 93.4 and 150, respectively. Chlorophyll from particles of C. thiosulfatophilum was estimated after extraction with acetone (-10°C) and was calculated from the specific extinction coefficient of 98.0 l/g/cm at 650 mμ (Stanier and Smith, 1960). Conti and Vishniac

** Abbreviations: Tris for tris(hydroxymethyl)aminomethane; EDTA for ethylenediamine tetraacetate.

(1960) reported that the chlorophyll in this strain is a "650" type with an estimated molecular weight of 880.

The particles were extracted with acetone (-10°C), acetone containing 10% methanol, and finally with 50% $(NH_4)_2SO_4$ solution at pH 8.0 to remove organic solvents. The extracted particles were homogenized in water containing 1% methyl cellulose to prevent the settling of particles. A Cary recording spectrophotometer with a full-scale 0.1 OD slide wire was used for these studies. It was assumed that cytochromes with peaks at 552 and 555 mμ have the same extinction coefficient as that given by Bartsch and Kamen (1960) for cytochrome c of Chromatium (ϵ mM=44.6, reduced minus oxidized, difference spectrum). The cytochromes with alpha bands at 630 -640 and 612 mμ were estimated from the extinction coefficient given by the same authors for the cytochrome termed RHP (ϵ mM=7.2, oxidized form). Total cytochrome was estimated also by absorbance at 550mμ assuming ϵ mM=28.38 (Drabkin, 1941).

RESULTS

Cytochrome content of the pigmented particles from Chromatium and C. thiosulfatophilum is presented in table 1. Our values indicate a lower amount of cytochrome per mole of chlorophyll in Chromatium than reported by Newton and Newton (1957).

The reduced minus oxidized difference spectra of chromatophores of Chromatium and green particles of Chlorobium are shown in figures 1 and 2. In Chromatium particles absorption bands occurred at 552, 523 and 422 mμ, without a band at 406 mμ as reported for cytochrome c of Chromatium by Bartsch and Kamen (1960). In addition, a wide band in the region 630 to 640 mμ may suggest the presence of an RHP or an a type cytochrome. The asymmetry of the 552 band and a slight shoulder seen at

Table 1

Estimation of Cytochromes in Chlorophyll-containing Particles of Chromatium and Chlorobium

Constituent	Chromatium	C. thiosulfatophilum
	m moles/1.0 ml suspension	
Cytochromes:		
type 555, 552	0.91×10^{-5}	0.85×10^{-5}
type 612	none	1.66×10^{-5}
type 630-640	0.78×10^{-5}	none
Total Cytochrome (ext. coef.)	1.69×10^{-5}	2.51×10^{-5}
Total Cytochrome (pyridine hemochromogen)	3.18×10^{-5}	1.59×10^{-5}
Chlorophyll	7.10×10^{-4}	2.34×10^{-3}
Carotenoids	4.00×10^{-4}	4.48×10^{-4}
Protein[1]	4.56 mg/ml	24.0 mg/ml
Molar Ratios, Chlorophyll:		
Carotenoid: Cytochrome[2]	40: 20: 1	100: 20: 1
Pyridine hemochromogen	20: 10: 1	150: 30: 1

[1] Protein determined by the spectrophotometric method of Warburg and Christian (1941).

[2] Calculated from total cytochrome determined by extinction coefficients.

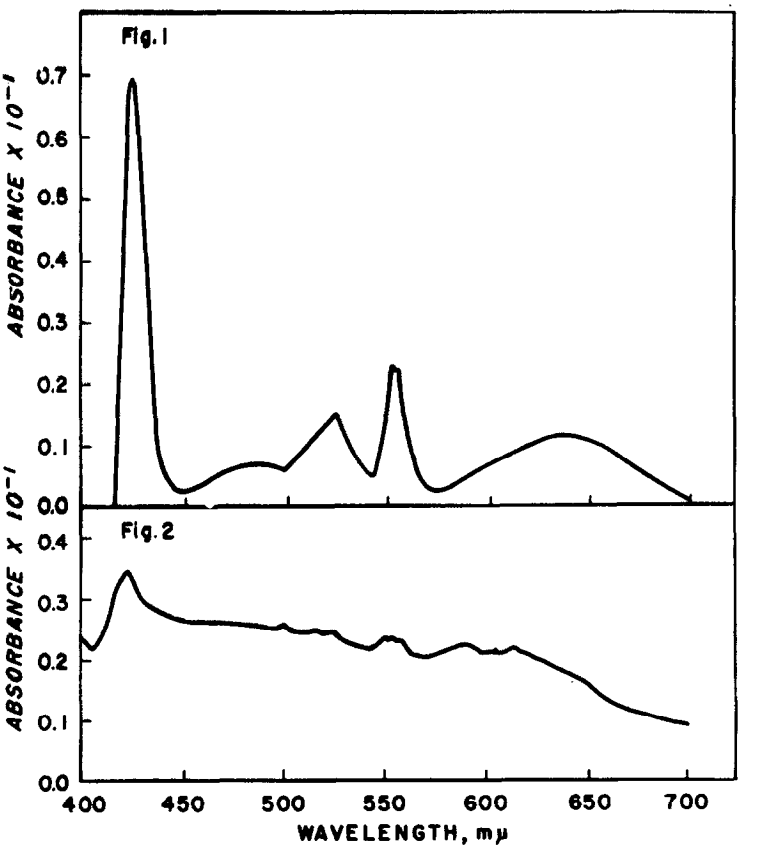

Fig. 1. Difference spectrum (reduced minus oxidized) of Chromatium chromatophores after extraction of pigments.

Fig. 2. Difference spectrum of green particles of Chlorobium thiosulfatophilum after extraction of pigments. A 0.1 O.D. full-scale slide wire was used.

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556 mμ adds substance to the postulate that at least two cytochromes occur in these particles. A difference spectrum of untreated particles minus oxidized particles showed that a reduced cytochrome was present with bands at 555, 522 and 421 mμ, not unlike those of cytochrome f; bands were not seen in the 630 mμ region. The reduced maxima of Chromatium cytochrome c were 416, 523 and 552 mμ (Bartsch and Kamen, 1960). Our

data would imply that chromatophores of Chromatium contain at least two cytochromes, one with bands at 555, 522 and 421 mμ (reduced form) and another with bands at 630-640, 552, with the gamma band not definitely known (408 mμ).

Figure 2 shows the reduced minus oxidized difference spectrum of the extracted particles from C. thiosulfatophilum. Absorption maxima were observed at 422, 548-557, 589 and 612 mμ. We postulate the presence of two cytochromes in these particles, one with maxima at 555, 522, and 422 mμ and another with an alpha band at 612 mμ. Our assumptions are supported by the finding of a wide band at 548 to 557 mμ and another at 612 mμ. Values in table 1 have been calculated using the same extinction coefficients used for cytochromes of Chromatium.

DISCUSSION

Our observation that bacterial photosynthetic units appear to contain at least two cytochromes, an f type and an a type, observed in chloroplasts by Davenport (1952), lends support to the ideas of Kamen (1957), Duysens (1957) and Arnon (1959) that cytochromes participate in the electron transport chain of the photosynthetic apparatus. Evidence for a cytochrome f in chromatophores of photosynthetic bacteria minimizes the possibility that it could function specifically in the process of oxygen evolution. It could be inferred that the absence of a cytochrome b_6 in chromatophores may indicate its possible involvement in non-cyclic electron transport of green plants as visualized by Arnon (1959). The amount of cytochromes in clean chromatophores discounts the possibility of contamination with soluble cytochromes. Thus, it is believed that these cytochromes are structural components of the chromatophore.

References

Anderson, I. C. and Fuller, R. C., Arch. Biochem. Biophys. 76, 168 (1958).

Arnon, D. I., Nature 184, 10, (1959).

Bartsch, R. C. and Kamen, M. D., J. Biol. Chem. 235, 825, (1960).

Bergeron, J. A. and Fuller, R. C., Biological Structure and Function. The IUB/IUBS Joint Symposium, Copenhagen, 1960.

Duysens, L. N. M., in "Research in Photosynthesis". Proc. Gatlinburg Conference, 1955, 164, Interscience Publ. Inc., New York (1957).

Conti, S. F. and Vishniac, W., Nature (in press).

Drabkin, D. L., J. Biol. Chem., 146, 387 (1941).

Hendley, D. D., J. Bact., 70, 625 (1955).

Kamen, M. D., in "Research in Photosynthesis", Proc. Gatlinburg Conference, 1955, 149, Interscience Publ. Inc. (1957).

Larsen, H. Det. Kgl. Videnskabers Selskabs Skrifter, Trondheim (1953).

Newton, J. W. and Newton, G. A., Arch. Biochem. Biophys., 71, 250 (1957).

Newton, J. W. and Kamen, M. D., Biochim. Biophys. Acta, 21, 71 (1956).

Stanier, R. Y. and Smith, J. H. C., Biochim. Biophys. Acta, 41, 478 (1960).

Warburg, O. and Christian, W., Biochem. Z., 310, 384 (1941).

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