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A new chlorophyll from green bacteria

The green photosynthetic bacteria are characterized by the presence of either chlorobium chlorophyll (bacterioviridin)-650 or chlorobium chlorophyll-660 (numerical designation based on the position of the red absorption peak in ether)[1]. Most strains of Chlorobium contain chlorophyll-660 which has its major red peak between 740 and 750 mμ in vivo. KATZ AND WASSINK[2] first reported an additional minor absorption peak at 810 mμ for Chlorobium limicola in vivo and a corresponding minor peak at 780 mμ in ethanol extracts. LARSEN (Fig. 6-11 in reference[3]) also observed this 810 mμ absorption band in another Chlorobium strain and a corresponding peak at 770 mμ in acetone extracts. In strain L of C. thiosulfatophilum the minor red peak appears at 800 mμ, clearly resolved from the main peak of chlorophyll-650 at about 725 mμ[1,4]. We have observed the minor red peak once again in Chloropseudomonas ethylicum, strain 2K[5,6], as a shoulder at about 810 mμ on the side of the 750 mμ peak of chlorophyll-660[7]. The evidence presented in this paper indicates that the absorption peak appearing between 800 and 810 mμ in vivo does not belong to chlorophyll-650 (or -660), but instead belongs to another chlorophyll species very similar to bacteriochlorophyll.

By the application of gradient differential centrifugation to a crude extract of C. thiosulfatophilum L, BERGERON AND FULLER[4] obtained a "yellow" zone in which the ratio of the 800 mμ peak to the 725 mμ peak was 1:4 compared to 1:16 for the original extract. We have obtained from C. thiosulfatophilum L and Cpg. ethylicum 2K a fraction with the major red absorption peak at 805 or 810 mμ (Fig. 1) by following the general procedure of GIBSON[8] for extracting cytochromes from green bacteria. Washed bacteria

in borate buffer were frozen and thawed three times. The crude extract was centrifuged 90 min at 140,000 g. The pellet (fraction 1) contained the bulk of the chlorophyll-650 or -660. To the supernatant was added 20% (w/v) $(NH_4)_2SO_4$. The precipitate (fraction 2) contained the pigment with the absorption band at 805 or 810 mμ. The supernatant (fraction 3) contained essentially all of the cytochrome material.

Fraction 2 was extracted first with 80% methanol at 4° and subsequently with 80% methanol at 40°. The cold methanol extract contained most of the chlorophyll-650 or -660 which had been present in fraction 2. The warm methanol extracts showed major peaks at 339±1, 364±2, 606±4, and 770±1 mμ (Fig. 1). The pigments were transferred to ether by mixing an equal volume of ether to the methanol extract and slowly adding 10% (w/v) NaCl until two phases separated. The wet ether extract showed major peaks at 358±1, 391±1, 578±2, and 770±1 mμ (Fig. 1). (In the case of C. thiosulfatophilum L, additional peaks due to chlorophyll-650 were observed at about 427 and 658 mμ in the warm methanol extracts and at about 427 and 650 mμ in ether (Fig. 1).)

Spectroscopically the main pigment from fraction 2 closely resembles bacteriochlorophyll (see Table I). (SMITH AND BENITEZ[9] previously pointed out that the 780 and 770 mμ bands in the extracts of KATZ AND WASSINK and LARSEN correspond to bacteriochlorophyll.) However, we shall use the designation "chlorophyll-770" based on the position of the red absorption maximum in ether, until further experiments are carried out to test whether or not this new chlorophyll from green bacteria is identical to bacteriochlorophyll from purple bacteria.

The overlap of the 780 mμ fluorescence band of chlorophyll-660[10] and the 810 mμ absorption band of chlorophyll-770 *in vivo* makes it highly probable that excitation energy is transferred from the former to the latter. Light absorbed by either chlorophyll-650 (or -660) or chlorophyll-770 *in vivo* causes the oxidation of c-type cytochrome(s) in both *C. thiosulfatophilum* and *Cps. ethylicum*[11]. We suggest that chlorophyll-650 (or -660) may serve as an accessory pigment for collection of light in green bacteria, while chlorophyll-770 serves as the terminal excitation energy acceptor which couples light absorption to photosynthetic electron transfer.

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TABLE I

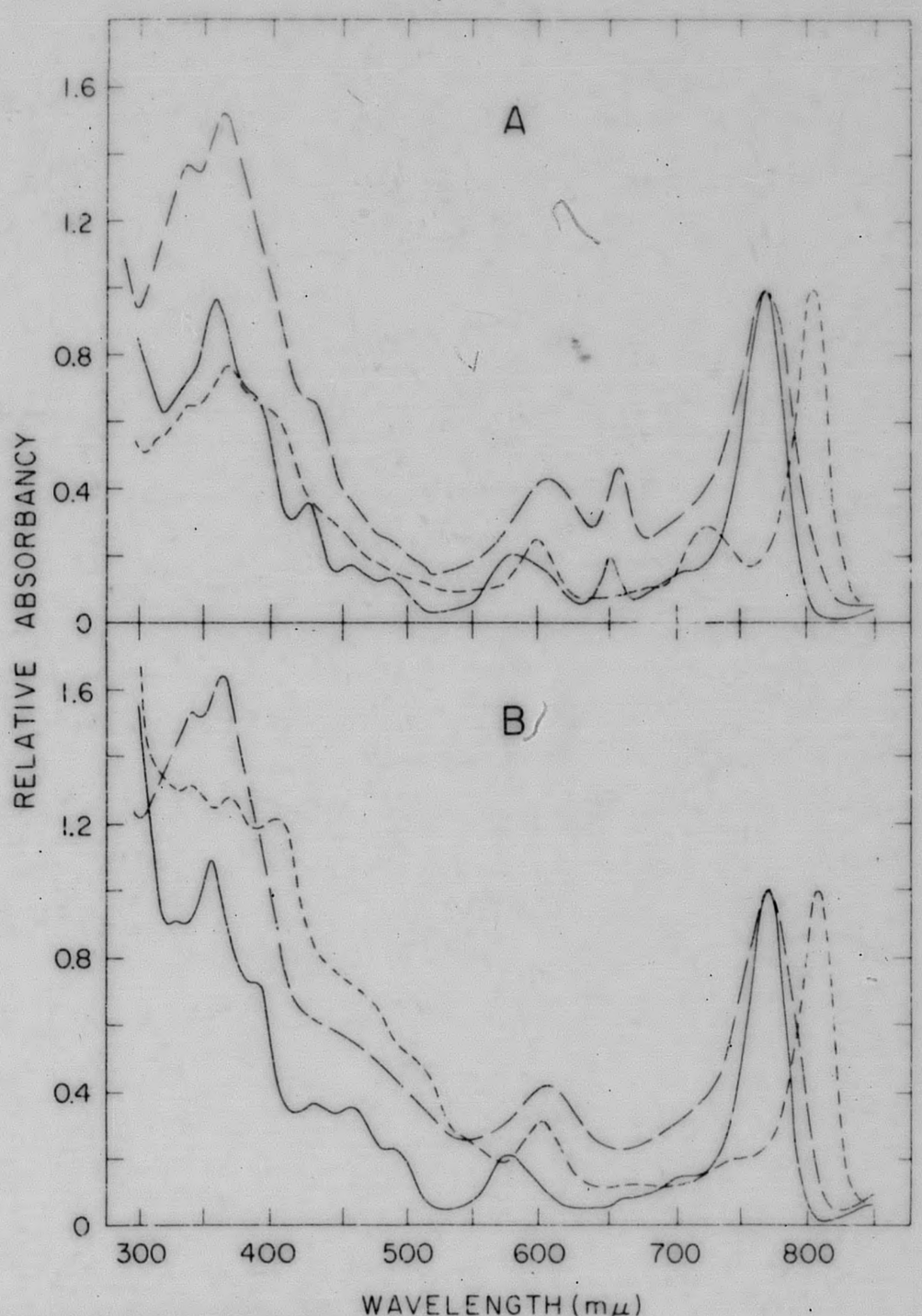
ABSORPTION MAXIMA OF CHLOROPHYLL-770 AND BACTERIOCHLOROPHYLL

Solvent	Chlorophyll-770* (C. thiosulf. L)	(Cps. ethylicum 2K)	Bacteriochlorophyll (R. rubrum[9])	(Chromatium[12])
	mμ	mμ	mμ	mμ
Methanol	770	770±1	772	772
	606±4	604±3	608	609
	364±2	364±2	365	366
	338	339±1	-	340
Ether	770	772	773	773
	~580	577	577	575
	~390	392	391.5	391
	~360	357	358.5	359

* in 80% methanol and wet ether

FIGURE LEGEND

Fig. 1. Absorption spectra of fraction 2 extracts from (A) C. thiosulfatophilum L and (B) Cpa. ethylicum 2K in (1) .005 M TRIS buffer pH 7.5 ·····, (2) 80% methanol (warm extraction) - - - -, and (3) wet ether ————. The height of the red absorption maximum is arbitrarily set equal to 1.0 for each curve.



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ANL Neg. No. 4-108-62 Fig 1