A NEW BACTERIAL CHLOROPHYLL

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Three chlorophyll entities have so far been reported to occur in photosynthetic bacteria, namely bacteriochlorophyll in purple bacteria (van Niel, 1944) and chlorobium chlorophyll-660 (Larsen, 1953) and chlorobium chlorophyll-650 (Stanier & Smith, 1960) in green bacteria. We wish to report on a new chlorophyll from a newly isolated photosynthetic bacterium which is tentatively identified as a Rhodopseudomonas sp. (Aasmundrud & Eimhjellen). The properties of the new chlorophyll have been compared to those of bacteriochlorophyll obtained from Rhps. spheroides.

We propose that the already well established chlorophyll component of the purple bacteria be named bacteriochlorophyll a as suggested earlier by Fischer and Hasenkamp (1935) (Seybold & Hirch, 1954) and, that the new chlorophyll reported in the present communication be named bacteriochlorophyll b.

Experimental.

Rhps. spheroides (strain 2. 4. 9 c obtained from Dr. R. Stanier, Dept. Bact., Univ. California, Berkeley) and the newly isolated photosynthetic bacterium were grown in glass-stoppered bottles completely filled with a medium composed of 0.5 % yeast extract (Difco), 0.05 % $MgSO_4 \cdot 7H_2O$, M/100-phosphate buffer (K_2HPO_4-NaH_2PO_4) and M/100 $(NH_4)_2$-succinate in tap water, pH adjusted to 6.8-7.2. The cultures were incubated in a light cabinet at 25 - 28 C, and soon after maximum growth the cells were collected by centrifugation. The packed cells were extracted twice with methanol at 0 to 5 C. An aliquot of the combined chlorophyll extracts was used for preparation of pheophytins (van Niel and Arnold, 1938) substituting diethyl ether for carbon tetrachloride as the final solvent for the reaction products. The rest of the

methanol extracts was mixed with diethyl ether and the chlorophylls transferred into the diethyl ether layer after adding a 10 % (w/v) aqueous NaCl-solution to the mixture. After drying over anhydrous Na_2SO_4 the solutions of chlorophylls and pheophytins were concentrated in vacuo at room temperature and subjected to circular paper chromatography (Jensen & Aasmundrud, 1962).

The colored zones of the chromatograms were carefully cut out and the pigments eluted with acetone. The absorption spectra of these solutions were immediately determined with the Zeiss PMQ 2 Spectrophotometer.

All solvents for extraction and chromatography were nearly saturated with H_2S-gas (Kaplan & Silberman, 1959) and all procedures were carried out as far as possible in darkness.

Results and discussion.

In Fig. 1 are given the absorption spectra (in acetone) of the new chlorophyll (b. chl. b) and of bacteriochlorophyll a (b.chl. a) after purification of both compounds by paper chromatography. In the red region of the spectrum b.chl. b has an absorption maximum at 794 mμ, whereas b.chl. a has an absorption maximum at 771 mμ. In the Soret region b.chl. b has pronounced peaks at 407 and 368 mμ, whereas b.chl. a has two corresponding peaks at 390 and 358 mμ. The minor peak of b.chl. b at 675 mμ is probably due to a decomposition product (see below). On paper chromatograms the band of b.chl. b had a bluish-green color easily distinguishable from the clear blue color of b.chl. a. In solutions of acetone and diethyl ether the latter chlorophyll exhibited the same color as on paper, whereas a transfer of b.chl. b from paper to these solvents caused a characteristic change in color towards green as judged by the eye.

Fig. 2 gives the spectra of the pheophytins, designated bacteriopheophytin a and bacteriopheophytin b, respectively, after purification by paper chromatography. A difference between the absorption maxima in the red region, similar to that found for the corresponding chlorophylls, is apparent.

Under the experimental procedure followed in these investigations b.chl. a was found to be relatively stable, always giving a single chlorophyll upon rechromatogra-

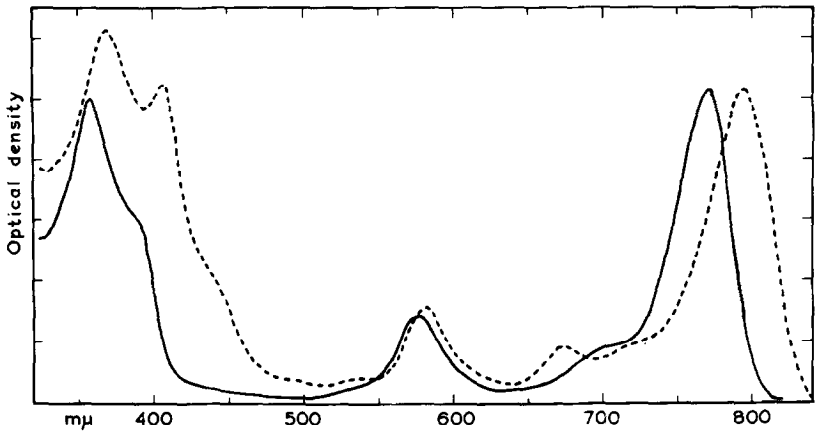

Fig. 1. Qualitative absorption spectra of bacteriochlorophyll a (fully drawn curve) and bacteriochlorophyll b (broken curve) measured in acetone.

phy. In dilute solutions, b.chl. b, however, was very unstable. Chromatography of such solutions usually gave two bands, one grass-green and the other greyish-green, in addition to the main bluish-green band; in these cases the former compounds amounted to approximately 30 - 50 % of the total. The decomposition seemed in part to be due to the unavoidable exposure to light during the chromatographic procedure. In the spectrophotometer the decomposition of b.chl. b revealed itself by a disappearance of the peak at 794 mμ and a concomitant increase of an absorption peak at 675 mμ. In the absorption curve given for b.chl. b (Fig. 1) a minor absorption at the latter wavelength can be noted. The spectrum of the colored compound in the grass-green band of the chromatogram had a prominent peak at the same wavelength, the only one in the red region. Acetone extracts of the bacterium containing b.chl. b had no absorption peak at 675 mμ. The chlorophyll in such extracts seemed stable for many hours when stored at room temperature in the total absence of light, since no change in the absorption spectrum took place. By successive exposure of the acetone extract to diffuse daylight for short periods of time the absorption at 794 mμ was gradually lowered and completely gone after 40 minutes of total light exposure. Concomitantly with the lowering and the disappearance of the absorption peak at 794 mμ an absorption at 675 mμ appeared and increased to a maximum value. In addition, paper chromatography of concentrated acetone extracts prepared in absolute darkness

gave only one clear-cut chlorophyll band. Our results thus indicate that only one chlorophyll is present in the intact cells.

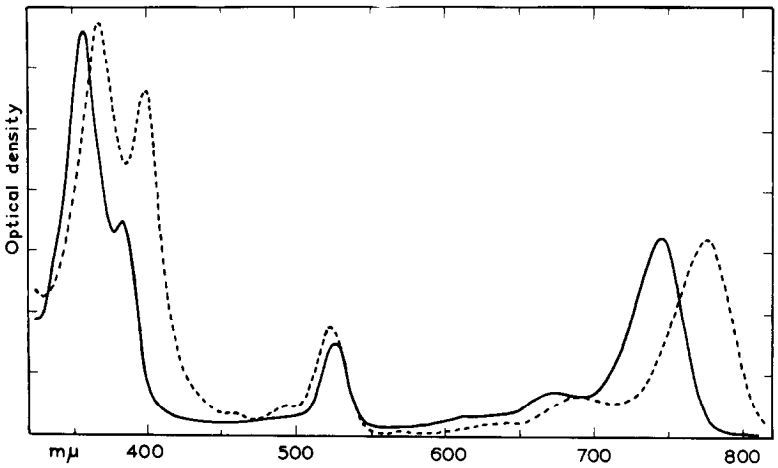

Fig. 2. Qualitative absorption spectra of bacteriopheophytin a (fully drawn curve) and bacteriopheophytin b (broken curve) measured in acetone.

Bacteriopheophytin b decomposed more easily than bacteriopheophytin a.

A comparison of the chromatographic behavior of the two bacteriochlorophylls and their pheophytins are summarized in Table 1.

Table 1.

(R-values are R_f x 100)

	R 1[a)]	R 2[b)]	R 3[c)]	Color of zone
Bacteriochlorophyll a	46	58	-	blue
Bacteriochlorophyll b	48	40	-	bluish-green
Bacteriopheophytin a	62	79	55	red-violet
Bacteriopheophytin b	58	66	48	reddish brown

a) Sucrose impregnated Whatman No. 1 (Sporer et al. 1954)
Solvent system: 0.5 % n-butanol in petroleum ether (b.r. 60 - 80 C).
b) Schleicher & Schüll No. 996 paper (with $CaCO_3$).
Solvent system: 5 % acetone + 1 % n-butanol in petroleum ether.
c) Schleicher & Schüll No. 667 paper (with Al_2O_3).
Solvent system: 10 % acetone in petroleum ether.

Cochromatography of the two pheophytins on the alumina containing paper gave two well separated zones, the identification of which was considerably simplified by their

distinct difference in color. Due to the great instability of b.chl. b no cochromatography with b.chl. a was attempted.

Also b.chl. a gave decomposition products by photobleaching, one of which is chromatographically very similar to the grass-green break-down compound from b.chl. b mentioned above. The absorption spectra of these 2 compounds are virtually identical. This observation and the striking conformity of the spectra of the two pheophytins, suggests a close relationship between bacteriochlorophyll b and bacteriochlorophyll a.

Olson and Romano (1962) have recently reported on a new chlorophyll from green sulfur bacteria. The absorption spectrum given by these authors is similar if not identical to that of b.chl. a. We have confirmed Olson and Romano's finding; Chl. limicola and Chl. thiosulfatophilum, strain L and NCIB 8346, do contain a chlorophyll different from chlorobium chlorophyll-650 and -660. The new chlorophyll component and its pheophytin have absorption spectra identical to those of b.chl. a and bacteriopheophytin a, respectively, (from Rhps. spheroides) and furthermore, they have chromatographic behavior indistinguishable from the latter compounds (unpublished results).

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