Bacteriochlorophyll and Chlorobium chlorophyll

The absorption spectra of acetone extracts of green sulphur bacteria (*Chlorobium* spp.) suggest the presence of a chlorophyll similar to chlorophyll *a*[1,2,3], but very different from bacteriochlorophyll, which occurs in the purple sulphur and non-sulphur bacteria (Thio- and Athiorhodaceae)[3,4]. LARSEN[3] has suggested that the term "chlorobium chlorophyll" is more appropriate for this pigment than METZNER's original "bacterio-viridin". LARSEN's[3] work also indicated that the phaeophytins of bacteriochlorophyll and chlorobium chlorophyll are spectroscopically different. Recently, however, SEYBOLD AND HIRSCH[5] and PRINGSHEIM[6] have stated that the chlorophyll of the green photosynthetic bacteria, including *C. thiosulphatophilum*, is bacteriochlorophyll.

SEYBOLD AND HIRSCH's claim is based mainly on (a) that bacteriochlorophyll (obtained from purple photosynthetic bacteria and called by them bacteriochlorophyll *a*) is extraordinarily unstable being converted within a few minutes mainly into bacteriochlorophyll *b* which fluoresces bright red in ultraviolet light and exhibits an absorption maximum at 660 mμ as well as at 770 mμ and (b) that as the extracts of the green photosynthetic bacteria also fluoresce bright red and exhibit absorption bands at 660 and 770 mμ, the original pigment was bacteriochlorophyll which had been rapidly changed into bacteriochlorophyll *b* during extraction. This dichotomy of opinion prompted an investigation into the properties of purified "Chlorobium chlorophyll" and a direct comparison of it with bacteriochlorophyll.

C. thiosulphatophilum (kindly provided by Dr. J. LASCELLES, Oxford) was cultured on LARSEN's medium[3] and the pigments extracted with methanol and transferred to ethyl ether. The ether extract was washed free from methanol, dried with anhydrous Na_2SO_4 and the solvent removed *in vacuo*. The residue was dissolved in a trace of ether, diluted with light petroleum and chromatographed on icing (confectioner's) sugar. The carotenoids (mainly γ-carotene[7]) quickly ran through the column and the chlorophyll fraction was developed with light petroleum/ethyl ether (4:1). Two fractions were regularly obtained; the more strongly adsorbed fraction (A) which exists only in trace amounts in extracts of young cultures, fluoresces red in ultraviolet light and exhibits an absorption spectrum with maxima in ethyl ether at 765, 659, 431 and 408 mμ; it occurs in increasing amounts in older cells and it also appears in larger amounts if unpurified solvents are used for extractions. The less strongly adsorbed fraction (B) differs from A only in showing either no band or merely a suggestion of a band at 765 mμ (Fig. 1). The appearance of this band in some preparations is probably due to contamination with traces of A. As B is by far the major component of young cells it is probable that it is the true "chlorobium chlorophyll", whilst A is probably an oxidation product. It appears that the absorption spectrum of chlorobium chlorophyll recorded by SEYBOLD AND HIRSCH[5] and, to a lesser extent, by earlier workers[2,3], represents mixtures of A and B. Apart from the absence of the 765 mμ band in our preparation, the absorption spectrum of chromatographically homogeneous chlorobium chlorophyll differs appreciably from that of KATZ AND WASSINK[2] and of LARSEN[3] only in the region 450–500 mμ; this is because carotenoids were not removed from their extracts.

In spite of their very similar absorption spectra, chlorobium chlorophyll and chlorophyll *a* can easily be differentiated in two ways: (1) by mixed chromatography on either sugar or $CaCO_3$:

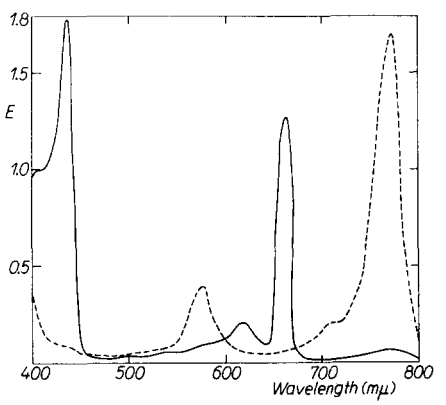

Fig. 1. Absorption spectra in ether of (a) Chlorobium chlorophyll ———; (b) Bacteriochlorophyll - - - - - - -.

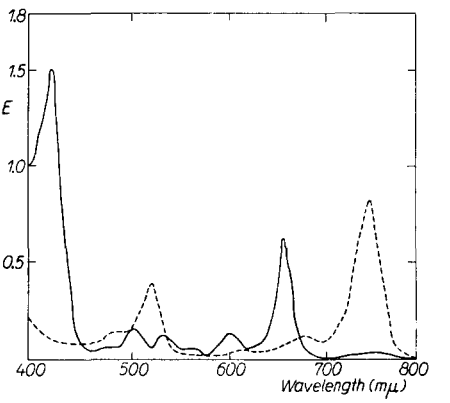

Fig. 2. Absorption spectra in ether of (a) Chlorobium phaeophytin ———; (b) Bacteriophaeophytin - - - - - - -.

chlorophyll *a* is much less strongly adsorbed and can be separated from chlorobium chlorophyll by development with light petroleum/ethyl ether (9:1; (2) by chromatography of their phaeophytins prepared according to the method of VAN NIEL AND ARNOLD[9]. Although the absorption maxima of chlorobium phaeophytin (Fig. 2) (maxima at 660, 604, 549, 515 and 412 mμ in ether) are very similar to those of chlorophyll *a*, the two compounds can again be easily separated on a sugar column.

The positions of these maxima also agree well with those recorded by LARSEN[3] for chlorobium chlorophyll phaeophytin but differ in their relative intensities (possibly because he was dealing with a mixture) and in the fact that he did not observe the 412 mμ band.

The chlorophyll fraction extracted from *Rsp. rubrum*, using the same method as for *Chlorobium* spp, yields on chromatography one major zone, bacteriochlorophyll (maxima at 770, 708, 574 and 396 mμ in ether); adsorbed just below bacteriochlorophyll is a small green band, fluorescing red in ultraviolet light, with main maxima at 438 and 675 mμ in ether. This appears to be SEYBOLD AND HIRSCH'S[5] bacteriochlorophyll *b* and is an oxidative artifact[4], which under our experimental conditions and those of HOLT AND JACOBS[4] is produced only in traces.

It is obvious from the absorption spectra of chlorobium chlorophyll and bacteriochlorophyll and their respective phaeophytins (Figs. 1 and 2) that the two compounds are very different. Their non-identity can further be demonstrated by chromatography on sugar; they possess somewhat similar adsorptive properties but prolonged development with light petroleum containing 30 % ethyl ether (v/v) will separate them; the chlorobium chlorophyll (upper) zone being bluish-green and the bacteriochlorophyll (lower) zone being bluish-grey. The phaeophytins can be similarly separated.

As we agree with HOLT AND JACOBS[1] that bacteriochlorophyll is quite stable and as we cannot reproduce SEYBOLD AND HIRCH'S[5] claim that it is rapidly converted into bacteriochlorophyll *b*, it must be concluded that chlorobium chlorophyll is the naturally occurring chlorophyll in *Chlorobium* spp, and that it is not a degradation product of bacteriochlorophyll produced during experimental procedures. The further claim of SEYBOLD AND HIRSCH[5] that chlorobium chlorophyll and bacteriochlorophyll *b* are identical can easily be disproved although they have similar absorption spectra: (a) they can be separated chromatographically on sugar, chlorobium chlorophyll being more strongly adsorbed, (b) chlorobium chlorophyll is almost completely insoluble in light petroleum whilst bacteriochlorophyll *b* is easily soluble.

The individuality of the various pigments discussed can readily be demonstrated by chromatographing a mixture of them on a sugar column when they will separate in the following order of decreasing adsorptive power: chlorobium chlorophyll; bacteriochlorophyll; bacteriochlorophyll *b*; chlorophyll *a*.

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Galactose 1-phosphate in galactose cataract*

Galactose 1-phosphate (Ga 1-P) accumulates in the red blood cells of galactosaemic infants on a milk diet[1]. At the same time the O_2 uptake of these cells is partially inhibited compared with that of cells taken before galactose feeding[2]. While no causal relationship has as yet been established between these two findings, it seems possible that Ga 1-P acts as, or gives rise to, an inhibitor of glucose metabolism. Since glucose is the main source of energy of the lens and since inhibition of glucose metabolism leads to cataract formation *in vitro*[3], an occurrence of Ga 1-P in the lenses of galactose-fed rats may be of considerable interest.

Experimental: Young male albino rats (initial weight 50–60 g) were fed a diet containing 30 % of galactose; controls were given the same diet without galactose. After varying intervals the animals were killed and the lenses excised. Either whole lenses or the capsules were used,