Light Adaptation of Bacteriochlorophyll-d Producing Bacteria by Enzymic Methylation of their Antenna Pigments

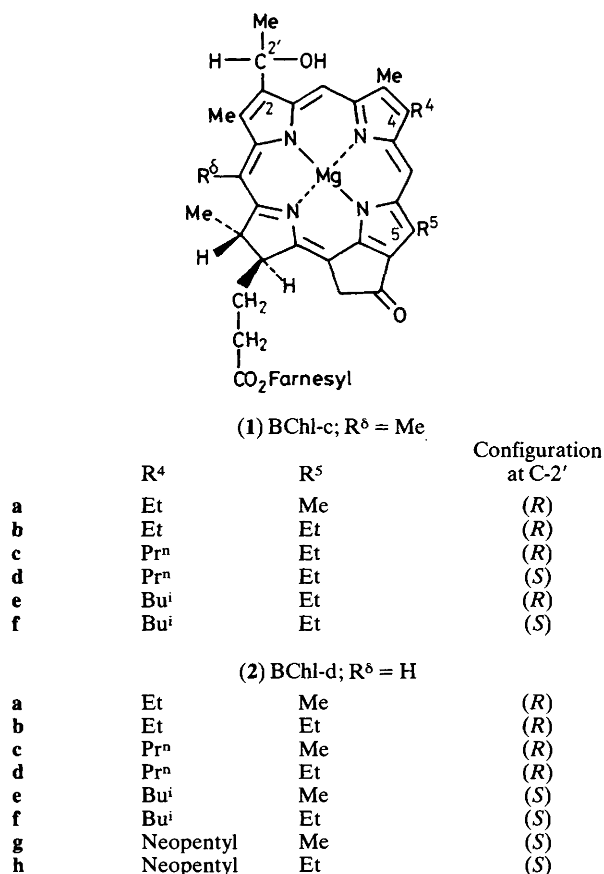
Kevin M. Smith* and Frank W. Bobe

Department of Chemistry, University of California, Davis, California 95616, U.S.A.

The bacteriochlorophyll-d producing strain of bacteria *Chlorobium vibrioforme* forma *thiosulfatophilum* (NCIB Strain 8327) alters its chromophore by modification of its 4- and 5-substituents using methylations with *S*-adenosylmethionine; with time, the strain produces bacteriochlorophyll-c by performing macrocyclic rather than side chain methylation.

Bacteriochlorophylls-c (**1**) and -d (**2**) (BChl-c, -d) are found in strains of green sulphur bacteria. They occur as homologous mixtures (**1a**—**f**; **2a**—**h**), and the structural features in both the BChl-c[1] and BChl-d[2] have been established. Unlike the BChls-d, the BChl-c possess a δ-*meso* methyl substituent which is responsible for a 20 nm red shift of the long wavelength absorption band in living cells (at 750 nm) compared with the BChl-d (at 730 nm).[3] Feeding experiments with ^{13}C-enriched methionine showed[4] the δ-methyl (BChl-c) and extra carbons in the 4- and 5-side chains (BChl-c and -d) to be derived from the S-methyl of methionine.

The presence of minor amounts of BChl-d in BChl-c samples from *Prosthecochloris aestuarii* (Strain C.e.)[1] and other BChl-c strains[5] has been noted. A change in the BChl-d pigment composition of the *Chlorobium vibrioforme* forma *thiosulfatophilum* strain (NCIB 8327) from a roughly 3 : 1 ratio of the 5-ethyl series *vs.* 5-methyl series[2,6] to about 16 : 1 in favour of the 5-ethyl homologues over a period of four years was also observed. Sufficient material had been stored which had been isolated from this strain during subculturing between



	R^4	R^5	Configuration at C-2′
	(**1**) BChl-c; R^δ = Me		
a	Et	Me	(*R*)
b	Et	Et	(*R*)
c	Pr^n	Et	(*R*)
d	Pr^n	Et	(*S*)
e	Bu^i	Et	(*R*)
f	Bu^i	Et	(*S*)
	(**2**) BChl-d; R^δ = H		
a	Et	Me	(*R*)
b	Et	Et	(*R*)
c	Pr^n	Me	(*R*)
d	Pr^n	Et	(*R*)
e	Bu^i	Me	(*S*)
f	Bu^i	Et	(*S*)
g	Neopentyl	Me	(*S*)
h	Neopentyl	Et	(*S*)

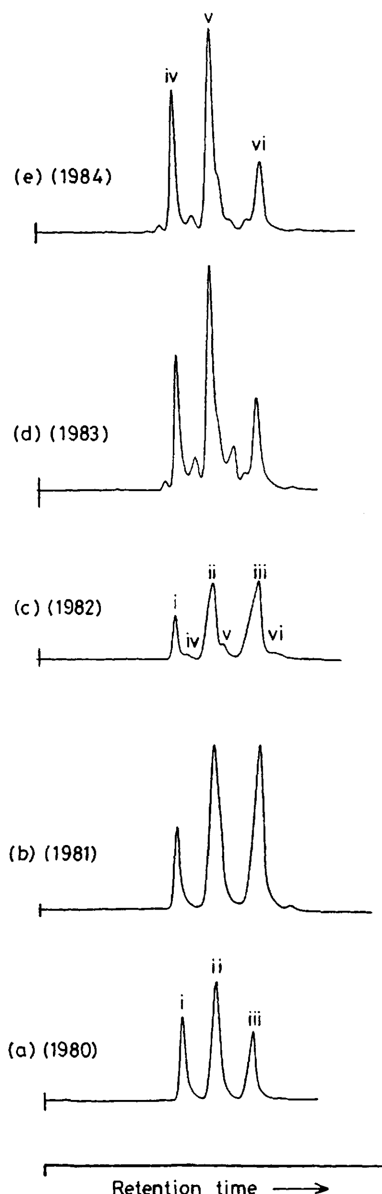


Figure 1. H.p.l.c. chromatograms [Conditions:[2] Waters Associates C-18 μBondapak reversed-phase column, Waters Z-Module, 2.6 ml min^{-1} of 85 : 15 methanol–water. Variable wavelength detector (Perkin–Elmer LC55B) set at 650 nm] of the natural mixture of methyl 5-ethylbacteriopheophorbides (Ebph) isolated from *C. vibrioforme* forma *thiosulfatophilum* between 1980 and 1984. Peak identities were established by comparison with authentic samples:[1,2] (i) 4-Et-Ebph-d, (ii) 4-Pr^n-Ebph-d, (iii) 4-Bu^i-Ebph-d, (iv) 4-Et-Ebph-c, (v) 4-Pr^n-Ebph-c, (vi) 4-Bu^i-Ebph-c. 2′(*R*) and -(*S*) absolute stereochemistries of these pigments will be detailed in a full paper.

1980 and 1984 so that reversed phase h.p.l.c. measurement of this time-dependent change was possible. The traces for the 5-ethyl series are shown in Figure 1. A trend to increased methylation in the *Chlorobium* strain is observable (Figures 1 a–c). Two years after receiving the strain additional peaks also appeared (Figure 1c) in the h.p.l.c. tracings of the methyl bacteriopheophorbides.† In 1983 (Figure 1d) and 1984 (Figure 1e) the pigments experienced a nearly complete switch to a class of methyl bacteriopheophorbides with increased retention time due to the presence of one additional δ-meso methyl group (*cf.* the BChl-c) for each homologue. Likely explanations for the switch to BChl-c production by the BChl-d bacterial strain are: (i) contamination of the BChl-d strain with a BChl-c bacterium, (ii) presence of a minor BChl-c producing colony in the original strain which eventually took over, and (iii) a mutation, adaptation, or selection by the BChl-d strain with caused production of the BChl-c as a result of external environmental pressures.

Possibilities (i) and (ii) were eliminated by preparation of pure subcolonies (Professor Pfennig) absorbing at 752 nm (BChl-c) and 732 nm (BChl-d). Both of these types of colony were morphologically identical with each other and quite unlike our other source of the BChl-c [*Prosthecochloris aestuarii* (Strain C.e.)]. The homologue ratios of all known BChl-c producing strains are consistent, the 4-ethyl-5-ethyl homologue usually being present to about 70% of the total pigment composition.[7] The homologue composition of the altered strain (Figure 1e) more closely resembles *C. vibrioforme* forma *thiosulfatophilum* (Figure 1a) than known BChl-c producers.

Most of the pigments in green sulphur bacteria act as 'antenna' molecules. On the basis of spectrophotometry[8] and e.s.r. spectroscopy[9] the antenna array has been proposed to consist of an aggregate[8] of about 13 BChl molecules.[3a] The magnitude of red shiift from monomer (*e.g.* 662 nm) to aggregate (748 nm) can be shown to be related to the size of the aggregate in organic solents.[8,10] Likewise, the absorption properties in living cells [714 nm; 4-ethyl (strain B1-20);[2] 728 nm; all 4-ethyl, propyl, isobutyl, neopentyl homologues] can be related to the 4- and 5-substituents. Thus, a modification of absorption properties in the BChl-d series occurs first by alkylation of the 4-side chain (714 to 728 nm) and then, finally, the δ-*meso* position (728 to 752 nm). This drive to absorption maxima at longer wavelength is in response to reduced availability of light.[11]‡

These data point to a light-adaptation process in which the lipophilicity (*i.e.* number of methyl units attached to the 4-, 5-, and δ-positions) of the macrocycle periphery determines the extent of aggregation in the antenna pigment. This may be due to hydrophobic interactions, or because the additional methyl units attached to the 4-position shift the absolute configuration at position 2 from pure (*R*) in case of 4-ethyl to pure (*S*) in case of 4-isobutyl or 4-neopentyl,[1,2] which in turn might determine the size of the *in vivo* aggregates.

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† The methyl bacteriopheophorbides were obtained using standard methodology.[2]

‡ Even pure cultures of bacteria producing the BChl-d, when placed in dim light for six months, are altered such that they produce approximately equal amounts of the BChl-c and BChl-d.[11]

References

1 K. M. Smith, G. W. Craig, L. A. Kehres, and N. Pfennig, *J. Chromatogr.*, 1983, **281**, 209.
2 K. M. Smith and D. A. Goff, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1099.
3 (a) J. M. Olson, *Biochim. Biophys. Acta*, 1982, **594**, 33; (b) A. Gloe, N. Pfennig, H. Brockmann, Jr., and W. Trowitzsch, *Arch. Microbiol.*, 1975, **102**, 103.
4 G. W. Kenner, J. Rimmer, K. M. Smith, and J. F. Unsworth, *J. Chem. Soc., Perkin Trans. 1*, 1978, 845.
5 W. R. Richards and H. Rapoport, *Biochemistry*, 1967, **6**, 3830.
6 D. A. Goff, Ph.D. Dissertation, University of California, Davis, 1984.
7 K. M. Smith, M. J. Bushell, J. Rimmer, and J. F. Unsworth, *J. Am. Chem. Soc.*, 1980, **102**, 2437.
8 K. M. Smith, L. A. Kehres, and J. Fajer, *J. Am. Chem. Soc.*, 1983, **105**, 1387.
9 J. A. Betti, R. E. Blankenship, L. V. Natarajan, L. C. Dickinson, and R. C. Fuller, *Biochim. Biophys. Acta*, 1982, **680**, 194.
10 R. J. Abraham, K. M. Smith, D. A. Goff, and F. W. Bobe, *J. Am. Chem. Soc.*, 1985, **107**, 1085; K. M. Smith, F. W. Bobe, D. A. Goff, and R. J. Abraham, *ibid.*, 1986, **108**, 1111.
11 F. W. Bobe, Ph.D. Dissertation, University of California, Davis, 1985.