Stereochemistry of the Generation of the Ethyl Group in Bacteriochlorophyll *a* Biosynthesis

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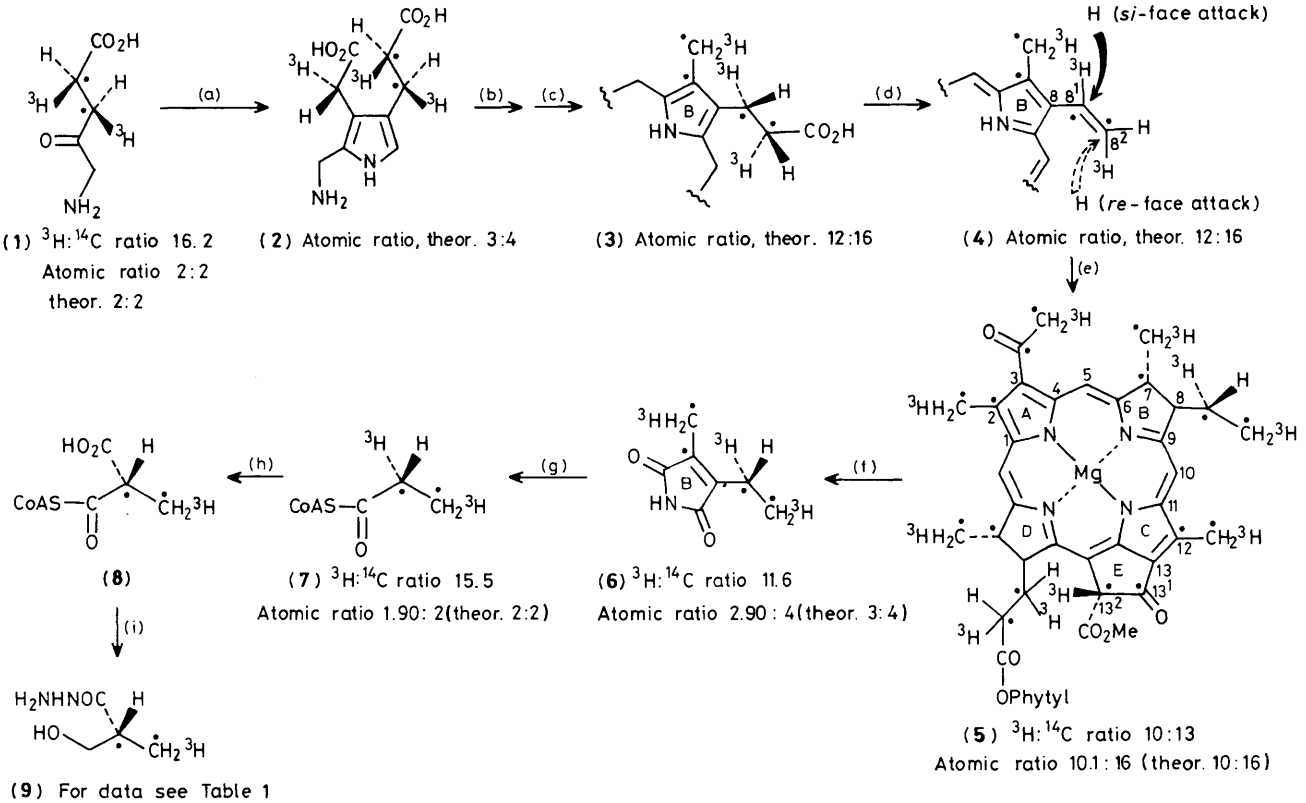
It is shown that the C-8 ethyl group of bacteriochlorophyll *a* (**5**) is formed by the addition of hydrogen to C-8^1 from the *si*-face of the corresponding double bond [**4**; $\Delta(8^1,8^2)$]; this observation, when taken in conjunction with the previous finding regarding the stereochemistry of hydrogen addition to C-8^2, reveals that the overall saturation process occurs *via* the formal *trans*-addition.

The B-ring ethyl group is a common feature of the plant chlorophylls, bacteriochlorophyll *a* (**5**, ^{3}H = H) and bacteriochlorophylls *c* and *d* (*Chlorobium* chlorophylls) and arises *via* the formal addition of hydrogen to a precursor vinyl group at a stage between magnesium protoporphyrin-IX monomethyl ester and chlorophyllide *a*.[1] An important



Scheme 1. The fate of ^{3}H in the incorporation of (2*R*,3*R*)-[2-^{3}H,3-^{3}H;2,3-$^{14}C_2$]ALA (**1**, • = ^{14}C) into bacteriochlorophyll *a* (**5**, • = ^{14}C) and thence into propionate. (a) In this ALA-dehydratase catalysed reaction a ^{3}H from one of the units of ALA is lost. (b, c) The labelled atoms are unaffected in these reactions. (d) The protons in the C-3^1 and C-8^1 H_{si} positions are removed in the formation of the two vinyl groups in rings A and B. (e) This multistep transformation is attended by the loss of one ^{3}H in the formation of the C-4 acetyl group and another in the creation of the ring-E carbonyl group at C-13^1. The status of the hydrogen atom in the formation of the position 13^2 is not known, but the ^{3}H:^{14}C data indicate the retention of ^{3}H at this position. (f) As expected, oxidative degradation proceeds without loss of labelled atoms from ring B. (g) O_3 gave propionate which was either converted into the 4-bromophenacyl derivative for the determination of the ^{3}H:^{14}C ratio or propionyl CoA for enzymic carboxylation. (h) (**8**) was converted into (**9**) by successive treatment with Ni/H_2; CH_2N_2; and NH_2NH_2.

Table 1. The ^3H:^{14}C ratios of 3-hydroxyisobutyric acid hydrazide (**9**) obtained following carboxylation of propionate derived from ALA stereospecifically (two independent incorporation experiments) or randomly tritiated at C-2 and C-3 or for several control incubations consisting of propionate randomly tritiated at C-2 only. The ^3H:^{14}C ratios for each experimental sample run should be examined vertically.

	^3H:^{14}C ratio of the degradation product of bacteriochlorophyll *a* derived from: (2*R*,3*R*)-[2-^{3}H,3-^{3}H; 2,3-$^{14}C_2$]ALA				(2*RS*,3*RS*)-[2-$^3H_2$,3-$^3H_2$; 2,3-$^{14}C_2$]ALA		^3H:^{14}C ratio of control propionate and its carboxylation product [2-$^3H_2$;2-^{14}C]propionate			
(a) Propionate	8.25	8.25	15.50	14.10	13.11	13.11[a]	4.0	4.0	5.1	5.1
(b) 3-Hydroxyisobutyric acid hydrazide	4.05	3.71	6.80	6.76	8.62	8.44	2.0	1.8	2.6	2.61
% Decrease in ^3H:^{14}C ratio in the conversion (a) → (b)	51	55	56.2	52.1	34.3	35.6	50	55	51	51.2

[a] Propionate obtained from this type of experiment will contain one stereospecifically located ^3H at C-2, and two ^3H atoms at C-3.

mechanistic aspect of the generation of the ethyl group is the stereochemistry of hydrogen addition at each centre of the B-ring vinyl group precursor and thus the overall steric course of the saturation process. Battersby and co-workers[2] have shown that hydrogen addition at C-8^2 of the C-8 vinyl group [$\Delta(8^1,8^2)$] occurs from the bottom face of the double bond, as illustrated in structure (**4**).

We envisaged that the experimental protocols developed during our previous investigations on porphyrin and bacteriochlorophyll *a* biosynthesis[3,4] would enable the selective and facile probing of the stereochemistry of hydrogen addition to C-8^1 of the vinyl group and hence describe the overall steric course of hydrogenation of $\Delta(8^1,8^2)$ during bacteriochlorophyll *a* biosynthesis in *Rhodopseudomonas spheroides*.

(2*R*,3*R*)-[2-^{3}H,3-^{3}H;2,3-$^{14}C_2$]-5-aminolaevulinic acid[3] (**1**, ALA) was incorporated into bacteriochlorophyll *a* (**5**) over a 12 h period by cultures of *R. spheroides* growing semi-anaerobically in the light (incorporation *ca.* 20%). After purification of the photosynthetic pigment, magnesium and the phytyl side chain were removed by methanolysis and the B-ring aromatised[5] to produce a chlorin which, upon chromic acid oxidation, yielded the B-ring as ethyl(methyl)maleimide (**6**). Conditions for the efficient ozonolytic degradation of ethyl(methyl)maleimide without tritium labilisation have previously been established in our laboratory,[4] and using this method ethyl(methyl)maleimide was degraded and the resulting acetic acid and propionic acid were characterised as their 4-bromophenacyl esters.

The merit of the degradative scheme utilised (*vide supra*) is that the propionic acid so derived is directly comparable to the C-8 ethyl group of bacteriochlorophyll *a* (see Scheme 1); thus the stereochemical processes that have occurred during the evolution of the ethyl group will be reflected in the distribution of tritium within C-2 and C-3 of the propionic acid. The steric orientation of tritium at C-2 of the latter was determined by utilising propionyl CoA carboxylase[6] which is known stereospecifically to replace the 2-H$_{re}$ atom of propionyl CoA with a carboxy group to yield (2*S*)-methyl malonyl CoA[7] which may then be isolated as 3-hydroxyisobutyric acid hydrazide (**9**).

A summary of the ^3H:^{14}C atomic ratios obtained for ALA, bacteriochlorophyll *a*, ethyl(methyl)maleimide, propionic acid, and methyl malonyl CoA (as hydroxyisobutyric acid hydrazide) along with those theoretically expected from our previous work, are shown in Scheme 1 and Table 1. A comparison of the ^3H:^{14}C ratios of propionic acid samples with 3-hydroxyisobutyric acid hydrazide (Table 1) illustrates that propionic acid samples derived from bacteriochlorophyll *a* synthesised from (2*R*,3*R*)-[2-^{3}H,3-^{3}H;2,3-$^{14}C_2$]ALA lost between 50 and 57% of their associated tritium upon incubation with propionyl CoA carboxylase. This result is consistent with the tritium in these samples of propionic acid being resident in the 2-H$_{re}$ position. As expected from this result, propionic acid derived from bacteriochlorophyll *a* synthesised from (2*RS*,3*RS*)-[2-$^3H_2$,3-$^3H_2$;2,3-$^{14}C_2$]ALA lost between 34 and 36% of its associated tritium upon enzymic carboxylation (Table 1).

On the basis of this localisation, the following regressive analysis can be formulated (Scheme 1). Since the 2-H$_{re}$ atom of propionyl CoA is equivalent to the C-8^1-H$_{re}$ atom of bacteriochlorophyll *a* (**5**) it follows that the addition of a hydrogen equivalent to C-8^1 of the vinyl group of (**4**) must have occurred from the *si*-face (top face as shown) forcing the tritium resident at C-8^1 of (**4**) into the H$_{re}$ position in the reduction product (**5**). This result, coupled with the demonstration by Battersby and co-workers[2] that hydrogen addition to C-8^2 of (**4**) occurs from the *re*-face of the double bond[2]† allows the overall steric course of the saturation at $\Delta(8^1,8^2)$ of (**4**) to be designated *trans*.

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References

1 For a review see: M. Akhtar and P. M. Jordan, in 'Comprehensive Organic Chemistry,' ed. W. D. Ollis, Pergamon Press, London, 1978, p. 1121.
2 A. R. Battersby, A. L. Gutman, C. J. R. Fookes, M. Gunther, and H. Simon, *J. Chem. Soc., Chem. Commun.*, 1981, 645.
3 J. S. Seehra, P. M. Jordan, and M. Akhtar, *Biochem. J.*, 1983, **209**, 709.
4 G. F. Barnard and M. Akhtar, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2354; M. Akhtar and C. Jones, *Methods Enzymol.*, Vol. 123, in the press.
5 J. M. Golden, R. P. Linstead, and G. H. Whitham, *J. Chem. Soc.*, 1958, 1725.
6 P. Diziol, M. Haas, S. W. Graves, and B. M. Babior, *Eur. J. Biochem.*, 1980, **106**, 211.
7 J. Retey and F. Lynen, *Biochem. Z.*, 1965, **342**, 256.

† It should be noted that, because of the pattern of isotopic labelling used in ref. 2, the *si*-face at C-8^2 in the latter work is equivalent to the *re*-face in structure (**4**) (magic of the priority rule!).