EPR, ENDOR, and TRIPLE Resonance Studies of Modified Bacteriochlorophyll Cation Radicals

H. Käss,† J. Rautter,† W. Zweygart,† A. Struck,‡ H. Scheer,‡ and W. Lubitz*,†

Max-Volmer-Institut für Biophysikalische und Physikalische Chemie, Technische Universität Berlin, Strasse d. 17. Juni 135, D-10623 Berlin, FRG, and Botanisches Institut der Ludwig-Maximilian-Universität München, Menzinger Strasse 67, D-80638 München, FRG

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A series of substituted bacteriochlorophyll molecules, all used in reconstitution experiments of reaction centers of *Rhodobacter sphaeroides* (Struck et al. *Biochim. Biophys. Acta* **1991**, *1060*, 262-270), were characterized by EPR, electron-nuclear double (ENDOR), and electron-nuclear-nuclear triple (TRIPLE) resonance spectroscopy in their monomeric radical cation states. Effects of different substituents at position 3 in the porphyrin macrocycle were considered, especially for two "crosslinks" between plant and bacterial chlorophylls. These are 3-vinylbacteriochlorophyll where the "bacteria" acetyl group at position 3 was substituted by vinyl and 3-acetylchlorophyll where the "plant" vinyl group was substituted by acetyl. In addition, effects of substitutions at position 13[2] were studied. All major hyperfine coupling constants of proton and nitrogen nuclei were elucidated from the spectra and assigned to molecular positions by comparison with the parent radicals. The data were compared with those calculated by an INDO-type program, showing that INDO essentially models the effect of the different substituents correctly.

Introduction

Chlorophyll (Chl) and bacteriochlorophyll (BChl) molecules play a multifarious role in plant (oxygenic) and bacterial photosynthesis, respectively. In the light harvesting "antenna" complexes (LHC), they are involved in the absorption of light and the transfer of the excitation energy; in the reaction centers (RCs), they take part in the light-induced charge separation and the subsequent electron transport processes.[1] In addition, (B)Chls have recently become prime candidates in photodynamic therapy because they are excellent singlet oxygen sensitizers, have strong absorptions in the red and near-infrared optical ranges, and are rapidly degraded.[2]

For a profound understanding of the structure-function relationship of photosynthetic complexes, a knowledge of both the spatial and electronic structure is required. Here, the crystallization and subsequent X-ray structure analysis of the RCs from two purple bacteria, *Rhodopseudomonas (R.) viridis*[3] and *Rhodobacter (R.) sphaeroides*,[4] have been of key importance. The latter contains three protein subunits (L, M, and H), a BChl *a* dimer (D), two monomeric BChl *a*, two bacteriopheophytins (BPhe) *a*, two ubiquinones Q_A and Q_B, and a nonheme Fe^{2+}. The cofactors are arranged in two branches which are related to each other by an approximate C_2-symmetry axis running through the dimer and the iron. In spite of the high symmetry, electron transfer (ET) proceeds only via one pigment branch.[4] The first ET steps are very fast, highly directional, and require no activation energy; the quantum yield of the process is very close to unity. Apart from the protein, holding the prosthetic groups in the right juxtaposition for optimum ET, the *choice* of the cofactors is also an important factor in this highly optimized process: Different bacteria may contain different metals, quinones, and pigments in their RCs.[5] For example, three major classes of BChl's are found: BChl *a* (e.g. in *R. sphaeroides*), BChl *b* (in *R. viridis*), and BChl *g* (in *Heliobacterium chlorum*).[6] The major structural difference between these species lies in the substitution pattern of the bacteriochlorin macrocycle, i.e. in the substitution at positions C-3 and C-8 in rings A and B (Figure 1a). In contrast to the bacterial species, the plant chlorophylls have a dehydrogenated ring B (Figure 1c).

During the last few years, site-directed mutagenesis has been widely used to alter the amino acid surrounding of the cofactors with the aim of elucidating the role of the protein in the light-induced charge-separation process.[7] Another supplementary approach to determine the structure-function relationship of the RC is the specific chemical alteration of the cofactors themselves. Removal and replacement has been reported for the two quinones[8] and the metal[9] in the RC of *R. sphaeroides*. Recently, Scheer et al. reported the first successful exchange of the (monomeric) BChl's and BPhe's in RCs of the same species.[10] This paved the way for replacing the native pigments with a variety of other species. Thereby the specific properties of the protein-binding pocket can be probed. Furthermore, pigments with altered spectroscopic and redox properties can be introduced which change the RC function.

A first step in this endeavor is the synthesis and characterization of suitable pigment molecules with modified subsitutents. In the past, the chemical and physical properties of chlorophylls and many chlorophyll derivatives have been studied in great detail, whereas those of the bacteriochlorophylls are less well known.[1] To understand the structure-function relationships of BChl's and Chl's in more detail, it is important to obtain structural links between the different naturally occurring species. One such link is [3-acetyl]-Chl *a*, bearing the 3-acetyl group characteristic for BChl *a* and *b* but the unsaturated ring B of the plant chlorophylls (Figure 1c). The complementary link is [3-vinyl]-BChl *a*, which differs from Chl *a* by the hydrogenated double bond in ring B and from BChl *a* by the presence of the vinyl group instead of the acetyl group at position 3 (Figure 1a). The vinyl and acetyl groups are both conjugated to the π-macrocycle; therefore, a species with a nonconjugated alkyl group will complete the picture: [3-α-hydroxyethyl]-BChl *a*. Another interesting region of the BChl macrocycle is the sterically crowded region between rings D and E. We have, therefore, prepared a species with an opened ring E (Figure 1b) and various compounds with different substituents at position C-13[2] (Figure 1a). The latter are also interesting in view of the possible enolization and epimerization at this position and the putative involvement of such species in the primary processes of photosynthesis.[11] Futhermore, it should be pointed out that the position of the closest approach between

* To whom correspondence should be addressed.
† Technische Universität Berlin.
‡ Universität München.
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