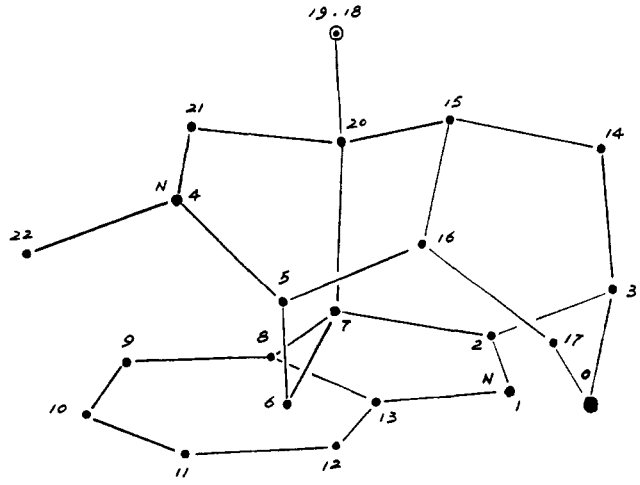

Figure 1. Three-dimensional projection of koumine hydrobromide from X-ray crystallographic analysis.

The relative configuration of koumine was also deduced from an analysis of the ^1H NMR spectrum of the alkaloid and its derivatives. Thus, the coupling constant between H3 and H15 of 1.0–3.2 Hz indicated a planar W-type coupling[5] and, hence, a cis relationship of these two hydrogens. The coupling constant between H5 and H16 (2.0–4.1 Hz) suggests that these protons are trans,[6] whereas H15 and H16, which have a coupling constant of 10.0–12.4 Hz corresponding to a dihedral angle of ca. 0°, must be cis. The methyl protons of the ethyl group in **5** appear at an unusually high field (δ 0.48), and a Dreiding model (see also Figure 1) indicates that they lie within the shielding zone of the aromatic ring.[7] This conformation also places the *N*-methyl group near the benzene ring of **5**, and, in agreement with this, it was found that irradiation of the NCH_3 proton signal caused a 5.3% nuclear Overhauser enhancement of H9. The interatomic separation of these groups is ca. 3.1 Å based on the configuration shown for **1**.

The stereostructure **1** (relative configuration) was finally confirmed by an X-ray analysis of koumine hydrobromide.[8] A three-dimensional projection is shown in Figure 1. The novel skeleton of koumine places it biogenetically in the strychnos family of alkaloids,[9] which includes the related oxindole gelsemine (**9**).[10] However, the C_9 mevalonoid segment of **1**, presumably derived from secologanin, is attached to the tryptamine unit in a unique fashion.[11,12]

Supplementary Material Available: ^1H and ^{13}C NMR data as well as proton coupling constants of koumine and its derivatives (4 pages). Ordering information is given on any current masthead page.

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(8) Crystals of koumine hydrobromide are orthorhombic and belong to the space group D_2^4-$P2_12_12_1$, D_2^4-$P2_12_12_1$, a = 14.307, b = 12.053, and c = 9.862 Å. Intensities were collected on a PW-1100 four-circle diffractometer by using Mo Kα radiation; 1431 independent reflections with values 2.9–26.5° were measured. The position of the bromine atom was obtained from a three-dimensional Patterson synthesis and all nonhydrogen atoms were located in a one-cycle Fourier synthesis. After a second cycle with full matrix least-squares refinement, the R value was 0.13 (Yao, Z. H.; Wan, Z. L.; Liang, D. C., private communication).
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In Vivo Liquid Solution ENDOR and TRIPLE Resonance of Bacterial Photosynthetic Reaction Centers of *Rhodopseudomonas sphaeroides* R-26

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In the last few years ENDOR has proved a powerful tool for the investigation of primary reactants in bacterial photosynthesis.[1-8] It has been concluded that a "special pair" of bacteriochlorophyll (BChl) upon excitation donates an electron to an electron-transport chain, leaving behind a cation radical accessible to ESR techniques.[1,2,7,8] Together with primary (bacteriopheophytin = BPh) and secondary acceptors (ubiquinones), the special pair BChl is situated in the "reaction center" protein complex (RC), which can be isolated from several bacteria.[7,9] The steric arrangement of the pigment molecules in the complex is strongly related to their photochemical function. Details of this arrangement are still unknown.[7,10]

By liquid-solution ENDOR highest spectral resolution could be achieved with the isolated pigments, thereby yielding up to 10 proton and 4 nitrogen isotropic hfs couplings and a detailed picture of the spin density distribution of the monomeric radical ions of BChl *a* and BPh *a* in vitro.[3-6] In vivo ENDOR studies of whole cells,[1] chromatophores,[2] or RC's[11,12] have been carried out so far only at low temperatures, i.e., in solid solution. In these cases dipolar broadening masks all those interactions which do not belong to nuclei with relatively small hfs anisotropy, e.g., rotating methyl groups. Consequently, only a few broad ENDOR lines could be detected. Nevertheless, these investigations served as a strong support of the special pair model for the primary donor,[1,2,7,8] which had originally been proposed from ESR data (see ref 8). This communication presents the first successful ENDOR experiments of RC's in aqueous solution at room temperature and demonstrates the possibility of studying biological samples at their correct physiological temperatures.

Reaction centers were isolated from *Rhodopseudomonas sphaeroides* R-26 by a modification of the method of Clayton and Wang.[13] The crude reaction centers obtained after ammonium sulfate fractionation were purified on DEAE-cellulose (Whatman DE 52, 2.5-cm i.d. × 20 cm for 1 μmol of reaction centers) and

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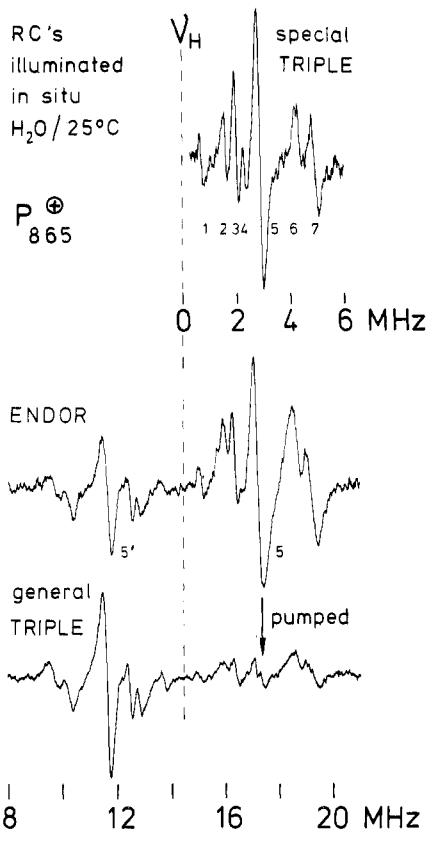

Figure 1. ENDOR/TRIPLE spectra of P_{865}^+ (cation of primary electron donor) in RC's of *Rps. spaeroides* R-26 (3.06×10^{-4} M in 0.01 M Tris buffer, pH 7.5, containing 0.1% lauryldimethylamine oxide (LDAO). Time constant: 1 s; rf 100 W pumped and scanned power; special TRIPLE, single scan; other spectra, nine scans.

reconstituted with ubiquinone 10 (Sigma). They were then concentrated by membrane filtration (BM 100, Berghof) to 3.06×10^{-4} M. Samples (0.2–0.4 mL) were taken neat or diluted with ethylene glycol (1:1, v/v). They were flushed with high-purity nitrogen, repeatedly degassed and regassed with nitrogen, sealed off at a slightly reduced pressure, and transferred to the Pyrex capillary ENDOR tube (1.0-mm i.d.) attached to the degassing vial. The samples were in situ illuminated in the spectral range 470–900 nm (Xe high-pressure lamp, 500 W). Solid $K_3[Fe(CN)_6]$ could be added from a side arm to the solution. The ESR, ENDOR, and TRIPLE (electron-nuclear-nuclear triple resonance) spectra were obtained with a spectrometer rather similar to that described previously.[14]

In g factor [2.0025 (1)] and line width (9.5 G) the light-induced ESR signal compared well with the signal observed for the cation radical of the primary donor.[8] From the light saturation behavior of this signal, we estimated a lifetime of some 10 ms, which is typical for reaction centers at ambient temperatures.[15-17] The light-induced signal disappeared upon thermal denaturation of the RC's.

Figure 1 shows the liquid-solution[18] ENDOR and TRIPLE spectra obtained by desaturating the ESR signal described above. The line widths in the ENDOR spectrum could be reduced to less than 200 kHz. Even higher resolution and better S/N ratio could be achieved in the special TRIPLE mode;[14,19] seven hfs couplings have been clearly resolved (see Figure 1, top). An eighth coupling detected in 50% ethylene glycol resulted in a splitting of the largest line near 2.8 MHz in the special TRIPLE spectrum. The relative signs of hfsc's were determined in the general TRIPLE mode.[14] Figure 1 (bottom) shows a typical spectrum: additional pumping on line 5 produced enhancement of all low-frequency ENDOR lines, whereas all high-frequency lines are deenhanced. Pumping on line 5′ yields just the opposite effect. From this we conclude that all seven (eight) hfs couplings have the same sign; positive signs are much more likely since the majority of protons in the pigments are of β and γ type. The hfsc's have the following values: +1.44 (2), +3.50 (2), +4.00 (3), +4.45 (5), +5.60 (5), +8.70 (5), and +9.50 (5) MHz. The number in brackets is the error in the last decimal place. To be certain that we have studied the cation radical of the primary donor and that no additional impurities were present, the RC's have been oxidized chemically by $K_3[Fe(CN)_6]$. The visible, ESR, and ENDOR spectra were identical with those obtained from the light-induced radicals.

Comparing our hfsc's with the in vitro data of the monomeric BChl *a* cation radical in solution[3] shows that the hfsc's in the primary donor cation in the RC are all reduced in magnitude. They are, however, not scaled down by a constant factor of about 2 as expected for an essentially symmetric dimer[20] sharing the unpaired electron and neglecting environmental effects.[22] The finding of a factor of approximately 2 for *all* observable coupling constants in low-temperature frozen-solution ENDOR is to date the strongest evidence for this special pair model.[23] Our results imply a more complex situation for the pigments involved in the primary donor of bacterial reaction centers at room temperature. They emphasize the potential of the increased resolution inherent in liquid-state ENDOR in refining the first-order model. The many factors which may contribute[25] can only be sorted out by detailed future work, including selectively deuterated systems and RC's isolated from other bacteria.

Frozen-solution ENDOR spectra (in 50:50 H_2O–ethylene glycol, vol %) have been followed down to 140 K. Spectra obtained by in situ illumination and chemical oxidation were again identical. The hfs data[29] turned out to be rather strongly dependent on

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(19) Under certain conditions (favorable relaxation rates, strongly saturating radio frequency fields) the line intensities in the special TRIPLE mode are roughly proportional to the number of protons involved.[14] However, we have not used these intensities for assignment purposes, since we are not sure that these favorable conditions hold in our case. Studies on selectively deuterated samples, which are in progress, should give a much more rigorous assignment.
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temperature and the freezing procedure. A completely quantitative agreement of our results with those from RC's,[12] chromatophores,[2] (80 K, 50% glycerol), or whole cells[1] (100 K, 50% glycerol) was not observed. This would not be expected on account of the different experimental conditions (RC solutions in different solvent mixture, temperature, in situ illumination). It should be noted that the kinetics of $BChl^+$ re-reduction also decreases strongly above 150 K.[15-17] Further experiments at lower temperatures and conditions comparable with those in the previous studies cited are in progress.

We believe this is the first report on liquid-solution ENDOR of large protein complexes under physiological conditions (H_2O, 25 °C). The high spectral resolution achievable by this method is expected to eventually contribute to a better understanding of the nature of the primary donor in bacterial photosynthesis and biological problems involving unpaired electrons in general.

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(29) The ENDOR spectrum of chemically oxidized reaction centers at 140 K for instance shows lines corresponding to splittings of 0.8, 5.2, and 6.8 MHz and a broad feature corresponding to 12.5 MHz. Furthermore, shoulders are observed which correspond to splittings of about 1.9, 3.9, and 7.5 MHz. Previous values are 2.0, 4.2, and 8.0 MHz for chromatophores[2] (80 K) and 0.8, 2.2, 4.7, and 7.0 MHz for whole cells[1] (100 K). Some of the features in our spectra may, however, arise from residual anisotropic motion of the cation radical inside the protein. Such a process might also be responsible for the observed temperature dependence of our data and their deviation from the results of previous studies.

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Stereospecific Hydrogen–Deuterium Exchange via an Enolate Ion

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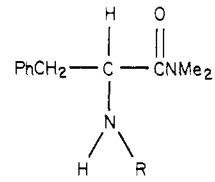
Although enolates are the most commonly encountered and most synthetically useful type of carbanion, there are no examples of stereospecific[1] reactions at the α carbons of simple enolates. Stereoselective reactions are common,[2] particularly in cyclic systems, but there is no evidence of any inherent dissymmetry for the defining three-atom system even under conditions where ion pairing produces well-documented facial dissymmetry in other types of carbanions.[3] Specifically, in *tert*-butyl alcohol-*O*-*d*, potassium *tert*-butoxide catalyzed hydrogen–deuterium exchange at the asymmetric α cation of appropriate amides and esters occurs with a rate constant, k_e, which is equal to the rate constant for loss of optical activity k_α.[4]

We were thus unprepared for the accidential discovery that *N*-pivalylphenylalanine dimethylamide (**1b**) undergoes exchange with a small but reproducible excess of exchange with retention of configuration, $k_e/k_\alpha > 1$. For reactions carried out in *t*-BuOD with [KO-*t*-Bu] = 0.3–0.5 N at 30 °C, the second-order $k_e = (2.2 \pm 0.1)10^{-3}$ M^{-1} s^{-1} (six separate runs, 2–6 points each, deuterium content determined mass spectrometrically). The second-order rate constant for loss of optical activity is $k_\alpha = (0.93 \pm 0.07)10^{-3}$ M^{-1} s^{-1} (three separate runs carried out simultaneously with the exchange runs in a thermostated polarimeter cell). Because the polarimetric method is subject to error if small amounts of highly optically active impurities are present, k_α was also measured by using the chiral NMR shift reagent, tris[3-[(trifluoromethyl)-hydroxymethylene]-*d*-camphorato]europium(III). With this technique, $k_\alpha = 0.92 \times 10^{-3}$ M^{-1} s^{-1} at 30 °C (five points using three different sets of NMR peaks). Thus a reliable value of $k_e/k_\alpha = 2.4$ was determined from separate kinetic runs. This value was confirmed by measuring deuterium content and optical rotation of the same sample, isolated from a single run.



1a, R = H
b, R = $COCMe_3$
c, R = COPh
d, R = $COCD_3$
e, R = $COOCMe_3$
f, R = $CHPh_2$
g, R = CPh_3

For a determination of the structural requirements for this unique behavior, a series of differently substituted compounds were run under similar conditions. The N-unsubstituted parent compound **1a** underwent exchange with complete racemization; $k_e/k_\alpha = 1.0$ at 50 °C. A small but significant excess of retention could be observed with the benzoyl derivative **1c** $k_e/k_\alpha = 1.4$, but with the trideuterioacetyl compound **1d**, $k_e/k_\alpha = 1.0$ (both at 30 °C). Simply displacing the *tert*-butyl group one atom farther from the reaction site by using the *tert*-BuOC derivative **1e** also eliminated the stereospecificity; $k_e/k_\alpha = 1.0$ at 30 °C.

These experiments suggested that a large group attached to the amino nitrogen is a minimum requirement for the observed exchange with retention. Additionally, a role for the potassium ion was demanded by the observation that for both **1b** and **1c** the stereospecificity disappeared when 18-crown-6 ether was included in the reaction mixture; $k_e/k_\alpha = 0.9$ and $k_e/k_\alpha = 1.0$, respectively. An attempt to magnify the observed stereospecificity by replacing K^+ with Na^+ proved ineffective; $k_e/k_\alpha = 2.0$ for **1b** in NaO-*t*-Bu/DO-*t*-Bu.

The dependence of this effect on the size of the substituent attached to the α-amino group suggests that there is conformational selection in the act of proton removal, leading to a nonequilibrium set of enolate conformers. The conformational inbalance would reflect the preferred conformation for the proton-removal transition state and would, in effect, provide molecular memory of the starting configuration. With large groups attached to the reaction center, high rotational barriers would slow conformational relaxation relative to protonation rate and favor return to starting compound through an enantiomerically identical transition state.

The situation suggested would be similar to that reported by Murr and co-workers for nucleophilic substitution in sterically congested trityl cations.[5] Walborsky and Motes have also offered this type of explanation for their observation of $k_e/k_\alpha = 1.9$ for 2-methyl-3,3-diphenylpropionitrile in methoxide/methanol.[6]

In order to see whether the effect could be attenuated by increasing the size of the group on the α nitrogen, **1f** and **1g** were prepared and studied in the same solvent–base system. In the case of the *N*-diphenylmethyl derivative **1f**, the rate of exchange was slowed relative to **1a** by a factor of 150 even at 62 °C. For the triphenylmethyl derivative **1g**, the half-life was of the order of weeks at 120 °C. In both cases, **1f** and **1g**, the analysis was complicated by exchange at sites other than the α carbon, but it was nevertheless possible to show the $k_e/k_\alpha = 1.0$ in these systems. Thus despite the clear indictions of steric restrictions on transi-

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