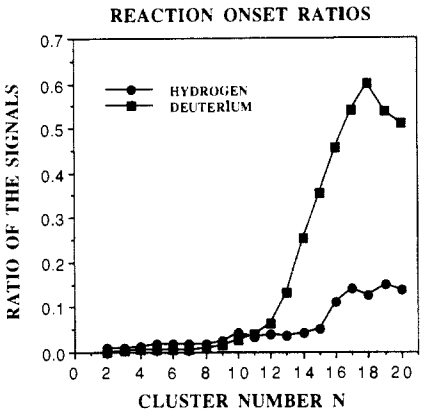

Figure 2. The ratio of the signals $Cs[CH_3OH]_{N-2}[H_2O]^+$:$Cs[CH_3OH]_N^+$ and $Cs[CD_3OD]_{N-2}[D_2O]^+$:$Cs[CD_3OD]_N^+$ are plotted vs cluster number N. These ratios were chosen because two methanols are consumed in reaction 2.

of the Cs^+ does not play a role in the intracluster chemistry.

We do not observe any signals in the mass spectrum due to clusters of the form $Cs[CH_3OH]_N[CH_3OCH_3]^+$. This is somewhat surprising in view of the high-pressure mass spectrometry data for the enthalpy of formation of Li^+ with H_2O (34 kcal/mol), CH_3OH (38.1 kcal/mol), and CH_3OCH_3 (39.5 kcal/mol).[8] If the same trend were present for Cs^+, one would expect to see preferential loss of H_2O over CH_3OCH_3 given comparable ion–neutral distances. However, in large protonated clusters containing one molecule of $(CH_3)_2O$ in CH_3OH[1,9] or H_2O,[9] elimination of $(CH_3)_2O$ appears to be the most favored evaporative process. One interpretation of these results is that the CH_3 groups disrupt the hydrogen bonding within large clusters and lead to looser structures.[10] The possibility of the reaction occurring and neither the H_2O or the CH_3OCH_3 leaving has been investigated by depletion spectroscopy.[3,11] Clusters of the form $Cs[CH_3OH]_{N-2}[H_2O]$-$[CH_3OCH_3]^+$ have the same mass-to-charge ratio as Cs-$[CH_3OH]_N^+$. Laser scans in the region of 938 cm^{-1}, where CH_3OCH_3 has a monomer absorption,[12] detected no depletion.

The size dependence for reaction 2 may be seen in the reaction onset curves in Figure 2, which remain flat until a cluster size of $N = 10$. At this point a sharp increase in the curve is observed, which levels off in the region of $N = 17$–18. These cluster sizes are significant in that 10 methanols fill the first solvation shell of the Cs^+ and the 17th methanol is the last to enter the second solvent shell.[3] Therefore the reaction seems to be occurring most frequently for clusters that have some methanols in the second solvent shell.[13]

This reaction is unique in that first the cluster ion is generated by impact of a Cs^+ into a cluster of methanols, not by electron impact[2] or multiphoton ionization[1] and second the Cs^+ is the center of charge, not a proton. The bonding properties of these two species are vastly different as seen by comparison of the enthalpy of association of the Cs^+ with methanol (~13 kcal/mol[14]) to the proton affinity of methanol (184.9 kcal/mol[15]). Clearly, the proton achieves a much greater degree of covalency than does the Cs^+. Furthermore, the difference in bonding properties of Cs^+ and H^+ suggests the possibility of a catalytic effect. Current research is underway to determine if such reactions are present when other alkali ions act as the charge center.

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Ultraviolet Resonance Raman Spectroscopy of Bacteriorhodopsin: Evidence against Tyrosinate in the Photocycle

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Ultraviolet resonance Raman (UVRR) spectroscopy is a powerful technique for probing the structure of proteins.[1,2] Vibrational scattering from different aromatic residues may be selectively enhanced by choosing appropriate excitation wavelengths. This raises the attractive possibility of using UVRR to study dynamic structural changes in proteins such as bacteriorhodopsin (BR), which functions as a light-driven proton pump.[3,4] Indeed, the feasibility of UVRR experiments on BR has recently been demonstrated.[5,6] It was previously proposed that light-adapted bacteriorhodopsin (BR_{568}) contains an ionized tyrosine (Tyr-185) which protonates upon light absorption and when the protein relaxes to its dark-adapted state (BR_{DA}).[7-10] The presence of tyrosinate in BR_{568} has also been suggested by recent UVRR experiments.[6] In this communication, we present UVRR spectra of BR_{568} and BR_{DA} which indicate that tyrosinate does *not* play a role in the photocycle.

Figure 1 presents UVRR spectra of BR_{568} and BR_{DA} excited at 253 and 240 nm. Lines at 1618, 1578, 1554, 1460, 1360, and 1340 cm^{-1} are due to tryptophan whereas tyrosine contributes scattering at ~1615 cm^{-1}.[11,12] No vibrational bands from tyrosyl radicals at 1393, 1502, and 1552 cm^{-1} or photoproducts of Trp at 1522, 1593, and 1646 cm^{-1} were detected.[13] The Raman signals were linear in laser power from 1.5 to 15 mJ/cm^2 per pulse. Using

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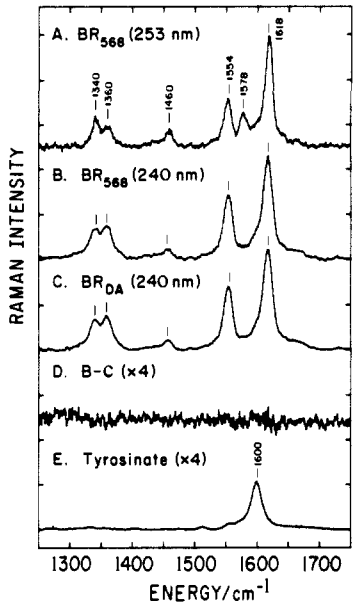

Figure 1. (A) UVRR spectra of BR_{568} excited with 0.5 mW at 253 nm, (B) BR_{568} excited with 0.5 mW at 240 nm, (C) BR_{DA} excited at 240 nm, (D) B - C expanded 4-fold, and (E) tyrosinate excited at 240 nm and scaled to the intensity expected for 0.67 equiv of tyrosinate (see Figure 2C). All 240-nm spectra have been scaled to the same height at 1554 cm^{-1}. A 10-mL aqueous purple membrane suspension (6 OD/cm at 568 nm) in 0.2 M NaCl and 10 mM HEPES at pH 7 and 4 °C was flowed through a wire-guided drip jet. Excitation at 240 and 253 nm (~0.5 mW) from a H_2-shifted 20-Hz Nd:YAG laser was spherically focused to a ~1-mm-diameter spot. Backscattered Raman light was collected with quartz optics, dispersed by a Spex 1401 double spectrograph (10–12 cm^{-1} slit width), and detected with a PAR 1420 intensified reticon. A buffer Raman background spectrum was subtracted from all spectra, and fluorescence backgrounds were also removed. For the BR_{568} spectra, the sample was light adapted by constant illumination of the reservoir. The λ_{max} of the BR_{DA} sample was monitored throughout the experiment; it stayed at ~561 nm, characteristic of fully dark adapted BR.

a typical power density of ~3 mJ/cm^2 per pulse, we calculate that fewer than 30% of the protein molecules absorbed a photon. UV-pump, green-probe Raman experiments have shown that only a small fraction of this excitation is transferred to the chromophore.[5] Furthermore, the samples employed here have been fully characterized; they exhibit native absorption spectra, visible Raman spectra, and photocycle kinetics. Previous UVRR spectra of BR_{568} excited at 253 nm exhibited a strong line at 1600 cm^{-1} assigned to tyrosinate.[6] We see no such band in our 253-nm spectrum, although we have observed that similar bands can sometimes be generated as a result of stray light or rotational lines of H_2. Another possibility is that the ~100-fold-higher power density (200–400 mJ/cm^2 per pulse) in the earlier work together with the use of a rotating cell may have caused the buildup of photocycle intermediates and UV photoproducts. *Our 253-nm UVRR spectrum of BR_{568} does not provide any obvious evidence for tyrosinate.*

To more sensitively look for tyrosinate, we used 240-nm excitation, which optimizes tyrosinate scattering and reduces interference from the 1578-cm^{-1} Trp line. BR_{568} is a homogeneous pigment species containing an *all-trans*-retinal chromophore, while BR_{DA} is a mixture containing 33% BR_{568} and 67% of a species called BR_{555} which contains 13-*cis*-retinal.[14] Previous work has suggested that a tyrosinate in BR_{568} protonates upon formation of BR_{555}.[8] Figure 1 presents the 240-nm spectra of BR_{568} and BR_{DA} and the difference between them. *The lack of a residual peak at 1600 cm^{-1} in Figure 1D shows that no tyrosines change protonation state in the $BR_{568} \rightarrow BR_{555}$ transition.* Tyrosinate should exhibit an intense ν_{8a} mode at 1600 cm^{-1}.[11] This is illustrated in Figure 1E, which presents a tyrosinate spectrum scaled

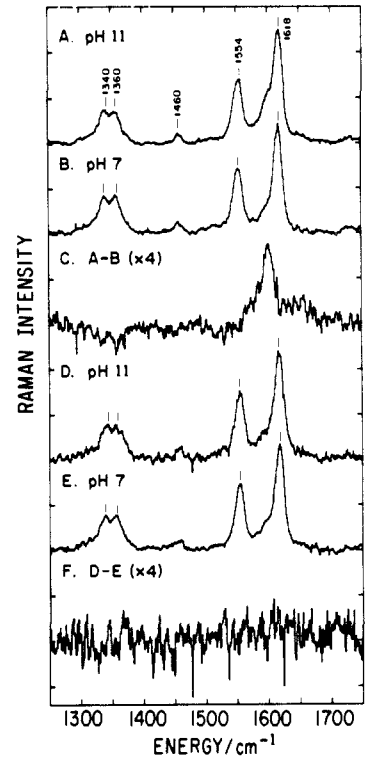

Figure 2. UVRR spectra of BR excited with 0.5 mW at 240 nm: (A) 95 μM BR_{568} + 95 μM tyrosine at pH 11; (B) 95 μM BR_{568} + 95 μM tyrosine at pH 7; (C) A - B expanded 4-fold; (D) BR_{568} at pH 11; (E) BR_{568} at pH 7; (F) D - E expanded 4-fold. Other experimental conditions are the same as in Figure 1.

to the intensity expected for the conversion of 0.67 equiv of tyrosinate to tyrosine (see below).

Figure 2 presents UVRR spectra that demonstrate the feasibility of detecting Raman scattering from one tyrosinate residue in BR. UVRR spectra of BR_{568} with 1 equiv of tyrosine in the buffer at pH 11 and 7 are presented in Figure 2, parts A and B, respectively. The difference (Figure 2C) shows residual scattering from the 1600-cm^{-1} mode of tyrosinate. Parts D and E of Figure 2 present UVRR spectra of just BR_{568} at pH 7 and 11, respectively, to control for possible changes in protein structure induced by pH. The difference (Figure 2F) shows that pH 11 does not induce a significant change in tyrosine or Trp environment. It is evident that an individual tyrosine deprotonation can be readily detected in these UVRR difference spectra.

These data also permit us to examine whether there is any tyrosinate in BR_{568}. In Figure 2A, a shoulder is observed at ~1600 cm^{-1}, due to tyrosinate scattering from the buffer, which is nearly as intense as the 1554-cm^{-1} mode. Only a much weaker shoulder is observed in spectra of just BR_{568} (Figure 1B), which has the intensity expected for the ν_{8b} mode from the 11 tyrosines in BR. Although it is possible that an ionized tyrosinate in BR is in an unusual environment which shifts its ν_{8a} mode under the 1618-cm^{-1} Trp line or decreases its intensity, the simplest interpretation is that there is no tyrosinate in BR_{568}.

It has been proposed that tyrosinate plays a prominent role in the proton-pumping photocycle of BR. Our UVRR results show that there is no tyrosine protonation change between BR_{568} and BR_{555}. This result is consistent with recent solid-state NMR studies.[15] Also, no tyrosine deprotonation was observed at pH 11 in Figure 1D, indicating that the tyrosines in BR have their pK_a elevated significantly from aqueous solution values. This is consistent with the solid-state NMR observation that titration to pH 13 is necessary to see tyrosinate. The disagreement between these UVRR results and the previous FTIR studies[7-10,16] may be

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the result of different sample conditions; the FTIR data were taken on hydrated films or pellets which may trap nonnative protein conformations. Another possibility is that the FTIR studies are detecting *tyrosine* environmental changes[17] as opposed to full protonation/deprotonation events. One implication of this study is that if tyrosinate is not involved in the $BR_{568} \rightarrow BR_{555}$ transition, then tyrosinate is not involved in the photocycle. This implication is supported by the absence of a shoulder at 1600 cm^{-1} with sufficient intensity to justify the presence of a tyrosinate in BR_{568} (Figure 1B). We have recently proposed a detailed molecular mechanism (the C-T model) for proton pumping which is consistent with these results on the role of tyrosine in the photocycle.[18-20]

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Electron Spin Polarization Transfer between Radicals

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Electron spin exchange between paramagnetic particles is an elementary bimolecular process that is accessible to both detailed experimental investigation and probing theoretical analysis, allowing study of the elementary processes of collisions and interactions of molecules in fluid media.[1,2] An important parameter for experimentally characterizing the strength of such interactions is the rate constant of spin exchange, k_e, which is commonly obtained indirectly from the dependence of the line broadening of ESR spectra on the concentration of paramagnetic particles. Since exchange involves the overlap of electron orbitals, k_e is expected to be influenced by the same factors that usually affect other important chemical interactions between molecules, such as energy transfer, electron transfer, and frontier orbital interactions that initiate bond formation and bond breaking.[3] Measurements of k_e have been employed to investigate how collisions are influenced by microscopic properties of homogeneous liquids and more recently by inclusion of reactants in microheterogeneous systems, such as micelles.[4]

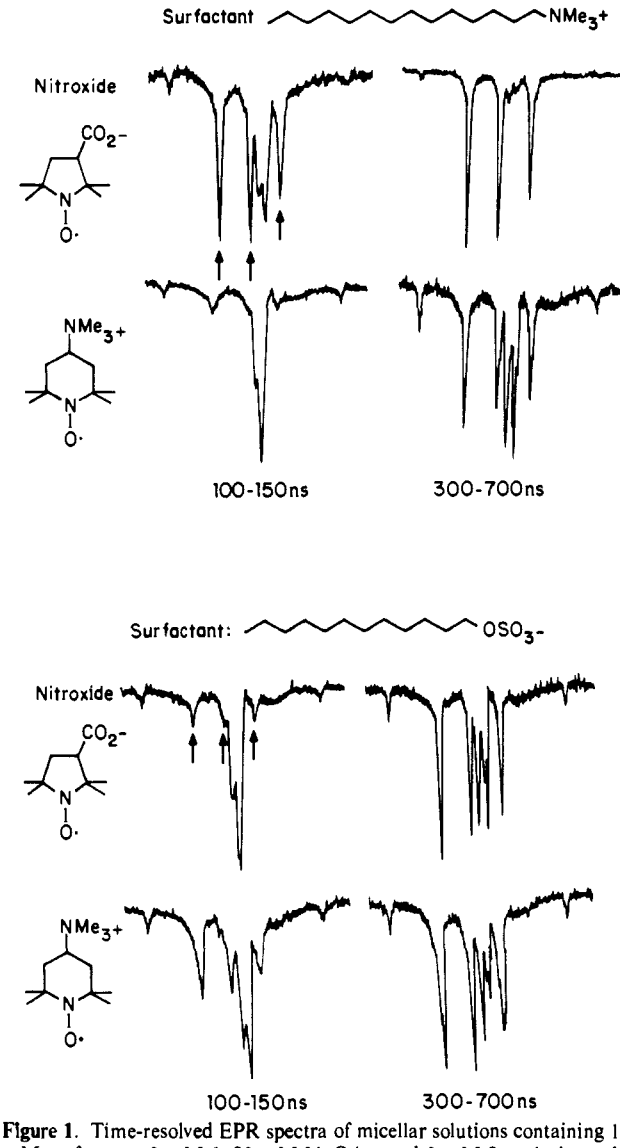

Figure 1. Time-resolved EPR spectra of micellar solutions containing 100 mM surfactant, 3 mM **1**, 20 mM NaOAc, and 3 mM **2** or **4**, titrated to pH 7.0. The three lines of the nitroxide are noted by arrows in the upper left spectrum of each set. The rest of the lines are attributable to the benzoyl and hydroxycyclohexyl radicals.

Electron spin polarization, detected by time-resolved EPR spectroscopy (TREPR), has opened the possibility of direct determination of k_e by polarization transfer. Transfer of triplet mechanism polarization has been demonstrated in triplet to doublet,[5] triplet to singlet,[6] doublet to singlet,[7] and doublet to

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