Ultraviolet Resonance Raman Evidence for a Change of Hydrophobicity of the Retinal Pocket in the M State of Bacteriorhodopsin

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Received January 16, 1992

Ultraviolet resonance Raman (UVRR) studies have recently been reported by two laboratories on bacteriorhodopsin (bR) in its resting states: light-adapted and dark-adapted states.[1,2] The retinal conformation and protonation state of the Schiff base are well characterized in the different intermediates of the photocycle of bR.[3] In this communication, we present UVRR spectra of the M state of bR using a pump probe experiment and bring evidence for an increase in the hydrophobicity of the retinal pocket of bR in the M state.

UVRR spectra of bR taken with 240-nm excitation are presented in Figure 1. The utilization of a system developed in this laboratory[4] allows each laser pulse to strike a completely new sample. A defocused beam (0.5 mW average power, 1 mm diameter) helps prevent the formation of photoproducts.[2] No changes are observed in the absorption spectra or the UVRR spectra (using the same sample twice in a UV experiment) when this type of setup is used. The 240-nm excitation line is preresonant with the B_b excited state of tryptophan (Trp).[5] Raman bands at 1178, 1209, and partial 1618 cm^{-1} are due to tyrosine (Tyr). Bands at 1012, 1343–1360, 1554, and 1618 cm^{-1} are due to Trp. Because of the Trp preresonance condition used in this study, one can expect that the Trp bands in the spectra correspond to the summation of the signals of the eight Trp molecules of bR.

To generate the M state, a 488-nm line of an Argon laser (200 mW on the sample) is defocused to a spot about 1.5 mm in diameter that is centered around the UV beam. Under these conditions, the M state is expected to build up because it is the longest lived intermediate in the photocycle of bR.[6] The UVRR Raman spectrum of the M state shows significant and reproducible changes in the 1300–1400 cm^{-1} region. The reversibility of these changes has been checked using the same sample a second time in light-adapted conditions, and a spectrum identical to the middle spectrum in Figure 1 has been obtained. Moving the visible illumination away from the UV probe to create a delay of approximately half a second (long enough to permit the depopulation of the M state) results in a UVRR spectrum similar to the middle spectrum in Figure 1. To estimate the population of the M state, the visible resonance Raman spectrum of the sample has been obtained by switching the photomultiplier and using the 488-nm pump beam as pump and probe at the same time.

The 1500–1600 cm^{-1} region of this spectrum is shown in Figure 2. Four major bands are observed at 1529, 1545–1555, and 1569 cm^{-1} and can be attributed to the C=C stretching of the retinal in the light-adapted, L, and M states, respectively. Alshuth and Stockburger[7] calculated that at 475 nm the visible RR cross section of M is about 1.5 times smaller than the cross section of light-adapted bR. The visible RR cross section of the L state is 1.25 times bigger.[7] Because of the relative intensities of these bands, and the red shift of our excitation, it can be estimated that around 70% of the M state, 20% of the bR, and 10% of the L state are accumulated within the sample. The intensity of the UVRR band

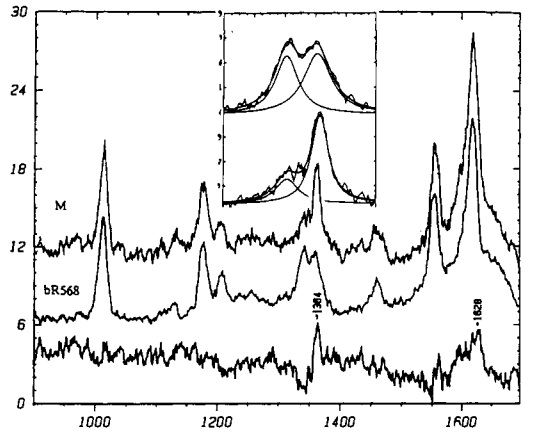

Figure 1. UVRR spectra of bacteriorhodopsin at 240 nm in the light-adapted state (bR, middle spectrum) and in the M state (top spectrum). The bottom spectrum is the computer-generated difference spectra. These spectra were taken from an aqueous suspension of purple membrane (5 OD/cm at 568 nm) in 100 mM NaCl, pH 7, 3 deg, that was flowing through a quartz capillary of 0.7 mm diameter at a rate of 1 cm/s. Excitation at 240 nm (0.5 mW) from an H_2-Raman-shifted pulsed (10 Hz) Nd:YAG laser is spherically focused to a 1 mm diameter spot, preventing any possibly saturation effect. Backscattered Raman light is collected with quartz optics, dispersed by a Spex 1401 double spectrograph, and detected with a solar blind photomultiplier. The bR is kept in the light-adapted state by continuous illumination of the syringe. The M state is generated by a 488-nm line of an argon laser (200 mW) focused to a 1.5 mm spot. The visible pump beam and the UV probe beam are collinear, but the two beams approach the capillary on opposite sides. Insert: An enlargement of the spectra in the 1300–1400 cm^{-1} region showing curve fitting of the two peaks in the light-adapted state (top) and M state (bottom).

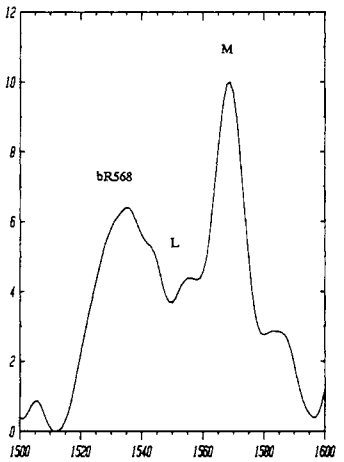

Figure 2. Resonance Raman spectrum (1500–1600 cm^{-1} region) of bR using the 488-nm pump beam as probe. Nothing in the experimental setup of the obtention of the UVRR spectrum of the M state has been modified except that the solar blind photomultiplier has been switched to a visible sensitive one.

at 1361 cm^{-1} increases linearly with the increase of intensity of the ethylenic band of the M state. For these reasons, the differences observed in the UVRR spectra obtained with or without the pump beam are the differences between the M and light-adapted states. The increase in intensity of the 1361 cm^{-1} band is also observed when light at 514.5 nm or white light is used to excite the photocycle.

The strongest modification in the UVRR spectrum is observed for the 1340/1360 cm^{-1} doublet that has been assigned to a Fermi resonance interaction in the Trp side chain.[8] The relative intensities of this doublet are drastically changed in going from the light-adapted to the M state, but the total intensity as measured

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by the total area of the doublet in both spectra is identical in the two states. The ratio of the integrated intensity of this Trp doublet to the intensity of the Tyr signal is the same in both states. For this reason, the dramatic change in the relative strength of the two components of the doublet must be interpreted in terms of changes in the environment of the Trp residues rather than modification of the resonance condition. The insert in Figure 1 shows an enlargement of the spectra in the region of the Trp doublet and the resolution of the two peaks using a fitting program to define the relative contribution of the bands at 1341 and 1361 cm^{-1}. From these areas, one can calculate that approximately 50% of the low-frequency intensity is transferred to the high-frequency band upon going into the M state. Given the fact that this signal originates from eight Trp residues and assuming that the Raman signal of these eight residues is equally enhanced, the change observed implies that approximately four Trp residues are perturbed by the bR → M reaction. The relative intensities of this doublet in proteins have been shown to be altered upon Trp exposure to the aqueous environment.[9] The retinal pocket includes four Trp residues (W86, W138, W182, W189).[10,11] These are the Trp residues likely to be perturbed by the bR → M reaction, and we propose that their environment is becoming more hydrophobic in the M state. The bR568 → M reaction corresponds to the deprotonation of the Schiff base that transfers its proton to the nearby aspartate D85. The increase of the retinal pocket hydrophobicity indicated by our results could be due to the disappearance of charges in the retinal pocket.

Other features are found in the 1550–1630 cm^{-1} region of the UVRR difference spectrum (Figure 1). Because the intensities of the 1550 and 1620 cm^{-1} bands of Trp vary with the environment, these bands are very likely to contribute to the difference spectra. However, because of the complexity of this region where three Trp and two Tyr bands contribute, these changes are difficult to assign and interpret at the present time.

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Palladium(II) Catalysts for Living Alternating Copolymerization of Olefins and Carbon Monoxide

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Received March 19, 1992

Several Pd(II) catalyst systems have been reported[1,2] which effect perfectly alternating copolymerization of olefins with carbon monoxide to yield polyketones $[C(O)CH(R)CH_2]_n$. There has been increasing interest in these polymers, particularly the

(1) For reports of $(CH_2CH_2CO)_n$, see: (a) Sen, A.; Lai, T.-W. *J. Am. Chem. Soc.* **1982**, *104*, 3520–22. (b) Sen, A.; Lai, T.-W. *Organometallics* **1984**, *3*, 866–70. (c) Sen, A. *Adv. Polym. Sci.* **1986**, *73/74*, 125. (d) Drent, E.; van Broekhoven, J. A. M.; Doyle, M. J. *J. Organomet. Chem.* **1991**, *417*, 235–51. (e) Klabunde, U.; Ittel, S. D. *J. Mol. Catal.* **1987**, *41*, 123–34. (f) For leading references to earlier work and the extensive patent literature, see 1c,d.
(2) For reports of $(CH(C_6H_5)CH_2CO)_n$ and related polymers, see: (a) Drent, E. Eur. Pat. Appl. 0229408, 1986. (b) Drent, E. U.S. Patent 4,788,279, 1988, and references therein. (c) Corradini, P.; De Rosa, C.; Panunzi, A.; Petrucci, G.; Pino, P. *Chimia* **1990**, *44*, 52–54.

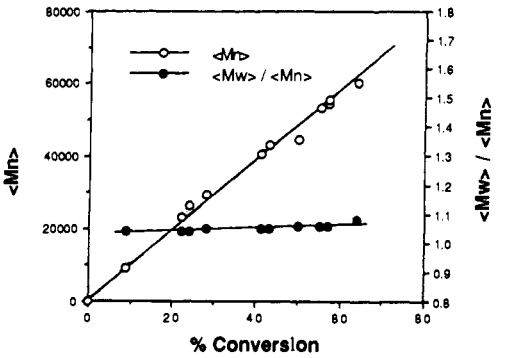
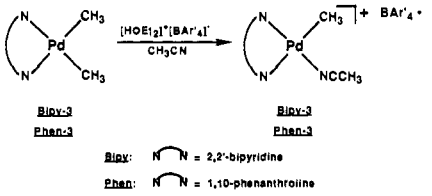


Figure 1. Molar masses and molar mass distribution as a function of conversion for 4-*tert*-butylstyrene and carbon monoxide. (M_n) values were determined by GPC relative to polystyrene standards, and % conversion was determined by ^1H NMR spectroscopy. Conditions: 0.2 mmol of Phen-3 and 20 g of 4-*tert*-butylstyrene in 80 mL of chlorobenzene under 40 psi of CO, 25 °C.

C_2H_4/CO copolymer (T_m = 257 °C),[1d] due to their unusual properties, the low cost of monomers, the presence of the carbonyl functionality, and the potential for further functionalization.[1c,d,3a] The catalyst systems used have typically employed Pd(II) salts in methanol or chloroform in combination with acids, mono- or bidentate phosphines, or bidentate nitrogen ligands and frequently oxidants such as benzoquinone. Little has been reported concerning molecular weights or molecular weight distributions;[3b] however, the nonliving nature of these systems can be inferred from the facile chain termination steps that have been identified, including alcoholysis of acyl intermediates.[1b-d,4] We report here the synthesis of well-defined Pd(II) catalysts that operate in aprotic solvents to yield living alternating copolymers of olefins and CO. In situ spectroscopic studies have established mechanistic details including the identity of the catalyst resting state. We illustrate our investigations with 4-*tert*-butylstyrene/CO, which yields the soluble polymer $[CHArCH_2C(O)]_n$, **1**, where Ar = 4-$((CH_3)_3C)C_6H_4$.

Treatment of (2,2′-bipyridine)$Pd(CH_3)_2$, Bipy-**2**, or (1,10-phenanthroline)$Pd(CH_3)_2$, Phen-**2**, with 1 equiv of $Et_2OH^+Ar'_4B^-$ [Ar′ = 3,5-$(CF_3)_2C_6H_3$] in acetonitrile followed by solvent removal gives the monomethyl salts **3** as colorless solids[5] (eq. 1). Exposure



(1)

of solutions of either Bipy-**3** or Phen-**3** (0.1 mmol) and *tert*-butylstyrene (62 mmol) in chlorobenzene (40 mL, 25 °C) to 1 atm of CO results in uptake of CO and formation of **1**. The initial rate of CO uptake (ca. 0.2 turnover/min under these conditions) is proportional to *tert*-butylstyrene concentration and suggests chain growth is first order in olefin.

The copolymer can be precipitated from methanol as a white solid (T_g = 158 °C), which has been characterized by ^1H and ^{13}C NMR spectroscopy.[6] Both the ^1H and ^{13}C spectra of **1** suggest

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(5) See supplementary material for preparation and complete characterization.
(6) **1**: ^1H NMR (CD_2Cl_2) δ 1.23 (s, 9 H), 2.63 (br dd, 1 H), 2.98 (br dd, 1 H), 4.08 (br q, 1 H), 6.57 (d, 2 H), 6.98 (d, 2 H); $^{13}C(H)$ NMR (CD_2Cl_2) δ 31.5 ($(CH_3)_3C$), 34.6 ($(CH_3)_3C$), 43.4 (CH_2), 53.0 (CH), 125.9 (C_{ortho}), 128.3 (C_{meta}) 134.7 (C_{ipso}), 150.2 (C_{ipso}), 206.9 (CO); ν_{CO} (CH_2Cl_2) 1708 cm^{-1}. Anal. Calcd for $C_{13}H_{16}O$ (found): C, 82.93 (82.36); H, 8.57 (8.53).

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