

Resonance Raman characterization of a stable tryptophan radical in an azurin mutant

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Calculations

Calculations were performed using the Gaussian 03W quantum chemistry program operating on a Windows platform.¹ The geometry and harmonic vibrational frequencies of 3-ethylindole were calculated using spin-restricted DFT with the hybrid B3LYP functional. Calculations for the 3-ethylindole neutral radical and 3-ethylindole cation radical were performed using spin-unrestricted DFT with the B3LYP functional. A 6-31G(d) basis set was selected for all calculations.

Mode assignments

Mode assignments were made by comparing results from calculations of closed-shell 3-ethylindole (3EI) to 3-ethylindole neutral radical (3EI•) with the use of software to visualize atomic displacements (Table S1 and Figure S1).² Nomenclature used here follows standard mode descriptions in literature.³ Modes W18, W17, and W16 are well-matched to modes predicted for the radical, and are observed experimentally at frequencies that agree with prediction. Mode W13• is expected to downshift by 39 cm⁻¹ relative to W13, and a similar experimental shift is observed. Modes W12 and W10 share only partial similarity with their counterparts W12• and W10•; these are C-H bending modes with minor N-H contribution. Mode W12• is predicted to increase by 21 cm⁻¹ upon formation of 3EI•, and the assignment of the radical peak at 1177 cm⁻¹ agrees well with the predicted value and expected increase for this mode. The peak at 1152 cm⁻¹ is assigned to W10•, and the expected shift of -67 cm⁻¹ upon formation of 3EI• is consistent with the experimentally observed shift of -87 cm⁻¹. Both the W8 and W8• peaks show weak intensity; the predicted frequency shift is less than what is observed but due to the direction of the shift we support this assignment. Modes W14 and W6 consist of large N-H bending displacements,³ and analogous normal modes are not found for the deprotonated tryptophan radical.

The W1• and W2• modes are primarily benzene ring-breathing modes and show a similar change in frequency as predicted upon formation of 3EI•. These modes have moderately high similarity to W1 and W2, especially for the displacements of the benzene rings, and show much higher intensity in the radical spectra. This intensity change may be due to greater resonance enhancement for the radical species.

The W4• and W5• modes are predicted to occur in the reverse order than has previously been assigned for tryptophan.³ The peak positions calculated relative to the closed-shell species agree with previous theoretical studies.⁴⁻⁶ Further evidence supporting our assignment of W4•

and W5• is found in the expected solvent dependence⁴ of W4 and W4• frequencies in UVRR spectra of Az108W and RR spectra of ReAz108W• as well as the relative independence of the W5 mode to solvent effects; these changes are both predicted and observed in the closed-shell species.

W7 is a relatively strong mode of closed-shell tryptophan. This mode consists of C₂-C₃ and N-C₈ stretching character as well as some contribution from C-H bending in the benzene ring. However, a well-matched mode is not found in calculations of 3EI•. Instead, we propose that elements of the W7 mode become significantly shifted for W7•, perhaps to a calculated mode at 1278 cm⁻¹ that shares similar pyrrole displacements to W7. Further experiments are required to confirm this mode assignment.

The W3• mode is expected to undergo significant shifts upon tryptophan oxidation due to large calculated changes in the pyrrole ring bond order, especially between C₂ and C₃.^{4,6} Predictions for the frequency range from 1152 cm⁻¹ to 1334 cm⁻¹.^{4,6} However, according to our calculations a well-matched mode with both C₂-C₃ stretching and pyrrole ring breathing motion (Figure 1B) does not exist for the tryptophan neutral radical.² Instead, there are three modes with significant C₂-C₃ stretching character: a low frequency mode at 1020 cm⁻¹, a mode at 1376 cm⁻¹, and a mode at 1509 cm⁻¹. Because the pyrrole displacements for the 1376 cm⁻¹ mode most closely resemble those of W3, the assignment of this peak (experimentally observed at 1345 cm⁻¹) is preferred for W3•. It is possible that the 1345 cm⁻¹ band has contributions from a W7•-like mode, which should also consist of some C₂-C₃ stretching character.

The W3 peak is known to indicate torsional angle of the indole ring within a protein,⁴ and UVRR spectra of Az108W indicate that this angle remains constant despite different solvent conditions. We expect that, for a given tryptophan radical, the frequency of W3• should also remain constant in different solvent conditions if the structure is unchanged. The ReAz108W• peak at 1345 cm⁻¹ remains at the same frequency despite changing buffer conditions, providing further evidence that this peak is due to the W3• mode.

Ethyl indole closed-shell (3EI)**Ethyl indole radical neutral (3EI•)**

Trp mode	Frequency 3EI (Calc)	Frequency Az108W (Obs)	Scaling factor	Frequency 3EI• (Calc)	Frequency ReAz108W• (Obs)	Scaling factor	Mode similarity
W18	784.3	764	-2.66%	779.3	760	-2.54%	98.1%
W17	889.7	880	-1.10%	859.5	875	1.77%	87.2%
W16	1042.2	1012	-2.99%	1038.5	995	-4.37%	90.9%
W14	1119.3	1079	-3.73%	--	--		
W13	1155.3	1129	-2.33%	1116.2	1082	-3.16%	47.4%
W12	1185.6	1149	-3.19%	1206.8	1177	-2.53%	37.6%
W10	1251.8	1239	-1.03%	1185.3	1152	-2.89%	37.8%
W8	1330.3	1315	-1.16%	1312.3	1270	-3.33%	35.3%
W7'	Fermi doublet	1364					
W7	1350.6	1352	0.10%	1277.4	1224	-4.36%	--
W6	1464.2	1435	-2.03%	--	--		
W5	1539.8	1462	-5.32%	1503.8	1498	-0.39%	71.3%
W4	1501.1	1498	-0.21%	1469.0	1462	-0.48%	50.8%
W3	1611.7	1553	-3.78%	1375.6	1345	-2.28%	27.2%
W2	1634.9	1583	-3.28%	1623.6	1562	-3.94%	51.5%
W1	1678.5	1621	-3.55%	1647.8	1595	-3.31%	42.3%
		<i>Average</i>	<i>-2.42%</i>		<i>Average</i>	<i>-2.45%</i>	

Table S1. Tryptophan mode assignments³ and frequencies from calculations on 3-ethylindole (3EI) and 3-ethylindole radical (3EI•) along with experimentally observed frequencies. Percentage difference between experimental and calculated frequencies is given as the scaling factor. Mode similarities were calculated using an internet-based application to analyze vibrational modes.² Italicized text represents modes with ambiguous assignments. See above text for details.

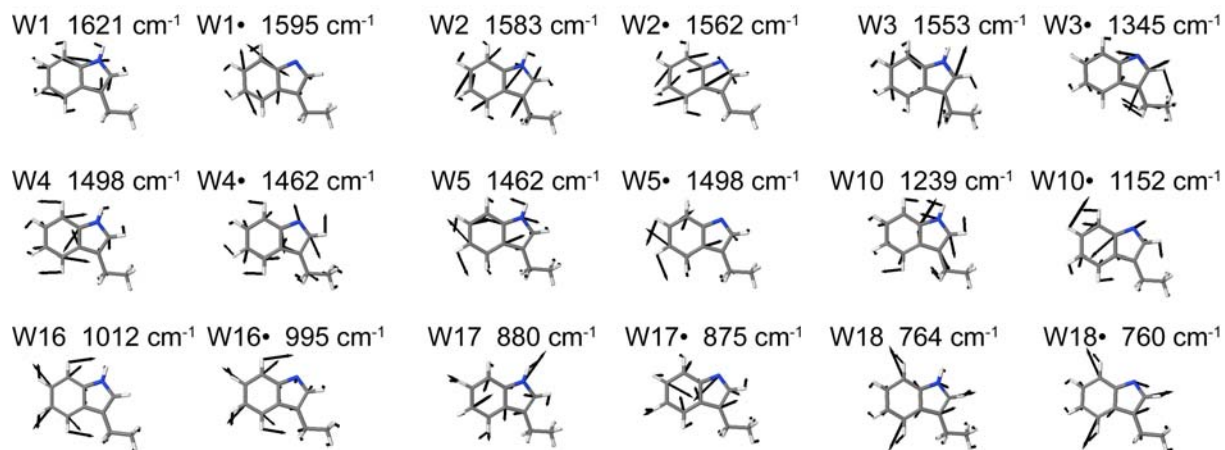


Figure S1. Depictions of calculated 3-ethylindole normal modes for the closed-shell (3EI) and neutral radical (3EI•) species. All displacements are in the plane of the indole ring. Vectors are enlarged five times. Experimentally observed frequencies are indicated.

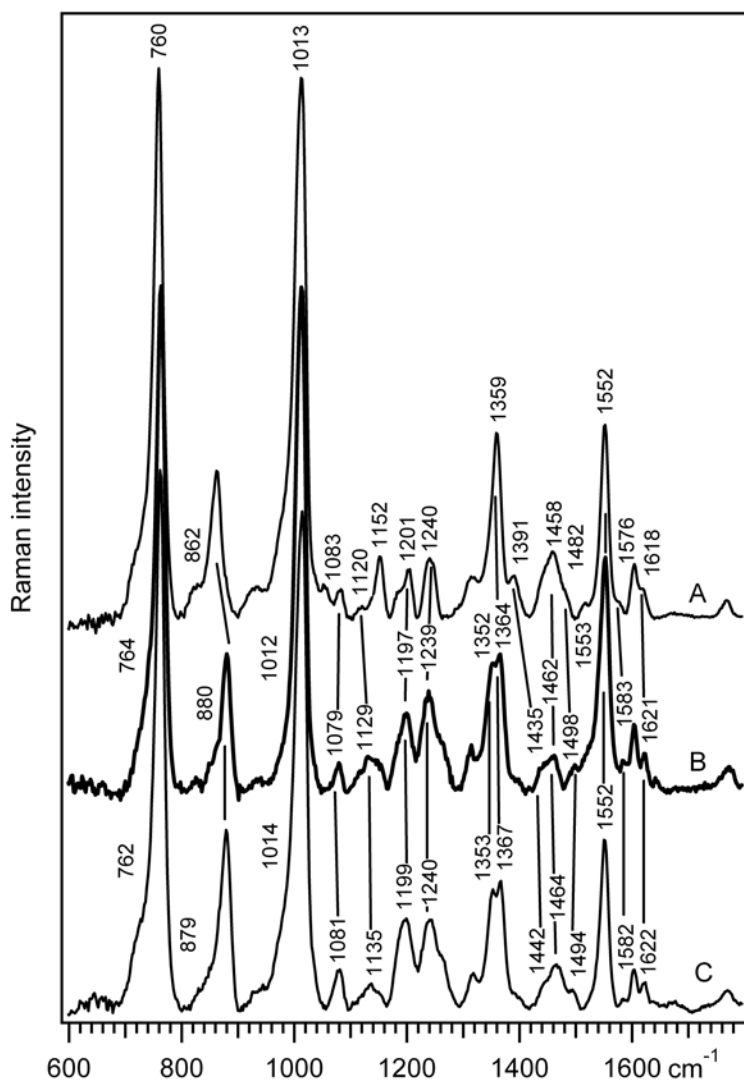


Figure S2. UVRR spectra of 50 μ M Az108W in (A) 50 mM phosphate buffer (D_2O), pD 7.6, (B) 50 mM phosphate buffer, pH 7.2, and (C) 50 mM acetate buffer, pH 4.0. Peak frequencies are listed; mode assignments are given in Tables 1 and 3, and Figure 3.

Supporting References

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