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UV resonance Raman study of cation– π interactions in an indole crown ether

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Abstract

UV resonance Raman (UVR) spectroscopy is used to probe changes in vibrational structure associated with cation– π interactions for the most prevalent amino acid π –donor, tryptophan. The model compound studied here is a diaza crown ether with two indole substituents. In the presence of sodium or potassium sequestered in the crown ether, or a protonated diaza group on the compound, the indole moieties participate in a cation– π interaction in which the pyrrolo group acts as the primary π –donor. Systematic shifts in relative intensity in the 760–780 cm^{-1} region are observed upon formation of this cation– π interaction; we propose that these modifications reflect shifts of the delocalized, ring-breathing W18 and hydrogen-out-of-plane (HOOP) vibrational modes in this spectral region. The observed changes are attributed to perturbations of the π –electron density as well as of normal modes that involve large displacement of the hydrogen atom on the C2 position of the pyrrole ring. Modest variations in the UVR spectra for the three complexes studied here are correlated to differences in cation– π strength. Specifically, the UVR spectrum of the sodium-bound complex differs from those of the potassium-bound or protonated-diaza complexes, and may reflect the observation that the C2 hydrogen atom in the sodium-bound complex exhibits the greatest perturbation relative to the other species. Normal modes sensitive to hydrogen-bonding, such as the tryptophan W10, W9, and W8 modes, also undergo shifts in the presence of the salts. These shifts reflect the strength of interaction of the indole N–H group with the iodide or hexafluorophosphate counteranion. The current observation that the W18 and HOOP normal mode regions of the indole crown ether compound are sensitive to cation–pyrrolo π interactions suggests that this region may provide reliable spectroscopic evidence of these important interactions in proteins.

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Supporting information

Supporting information may be found in the online version of this article.

Keywords

tryptophan; noncovalent interactions; vibrational spectroscopy

Introduction

The functional, three-dimensional structure of a protein reflects a careful balance of diverse noncovalent interactions. Stabilizing forces such as hydrogen bonds, salt bridges, and hydrophobic effect have been studied extensively. In contrast, much less is known about cation- π interactions in proteins, in part because of the challenges inherent to rigorous characterization of these interactions. For example, fluorescence and absorption spectra exhibit subtle and non-systematic changes and, therefore, do not provide a reliable measure of the presence or strength of cation- π interactions. Despite the lack of clear spectroscopic measurables, the need for detailed investigations of biological cation- π interactions is unquestioned; statistical analysis of the Protein Data Bank revealed that one favorable cation- π interaction exists for every 77 residues^[1] These interactions are proposed to be comparable in strength to a hydrogen bond^[2] and are thus important factors in protein structure and function, such as ligand binding, ion channel selectivity, and molecular recognition^[1,3,4]

Tryptophan forms cation- π interactions more often than any other aromatic amino acid, with an estimated 26% of all tryptophan residues participating in this favorable interaction^[1] The reason this amino acid is especially prevalent in cation- π interactions is because the indole ring of tryptophan exhibits the largest and most favorable electrostatic potential for binding of cations^[5] Tryptophan has been shown to form these stabilizing interactions with alkali-metal ions,^[6,7] positively charged amino acids,^[8,9] and even a transition metal ion^[10,11] Furthermore, tryptophan is an amphiphilic residue capable of forming hydrogen bonds in both hydrophobic and hydrophilic environments. Collectively, these properties enable tryptophan to simultaneously participate in several stabilizing interactions, including hydrophobic interactions, hydrogen-bond formation, and cation- π interactions. This versatility is especially advantageous in the case of membrane proteins and peptides where the environment of the lipid bilayer is heterogeneous^[12]

Here, we report a detailed UV resonance Raman (UVR) study of the cation- π interactions between cations and tryptophan using a model compound described in our previous publications. *N, N'*-Bis(2-(3-indolyl)ethyl)-4,13-diaza-18-crown-6 is a bibracchial lariat ether that is able to complex alkali metal ions as well as form a protonated species^[13] Side arms connect the crown ether to indole moieties, which interact favorably with the cation (Fig. 1). This simple molecule allows for direct spectral characterization of a cation- π interaction involving tryptophan.

Materials and Methods

Chemicals

Acetonitrile, acetonitrile- d_3 , sodium iodide, potassium iodide, skatole, and potassium hexafluorophosphate were purchased from Fisher Scientific. Ammonium hexafluorophosphate was a gift from Professor Clifford Kubiak at UCSD. Compounds were used as received without further purification. Salts were dried in an oven at 100 °C for 24 h. The indole crown ether (IC) *N, N'*-bis(2-(3-indolyl)ethyl)-4,13-diaza-18-crown-6 was synthesized as described previously^[13] Concentrations of IC were 10 μ M for absorbance and fluorescence experiments, 500 μ M for resonance Raman experiments, and 5 mM for NMR measurements.

NMR spectroscopy

1 H-NMR spectra were acquired on a 500-MHz Jeol ECA spectrometer using acetonitrile- d_3 as the solvent. Spectra with successive addition of salt up to 1 : 1 stoichiometric ratio of IC/salt are shown in Fig. S1 (Supporting Information).

Steady-state absorption and fluorescence spectroscopy

Absorption spectra were acquired on an Agilent 8453A ultra-violet–visible (UV–vis) spectrometer. Absorption and difference absorption spectra of IC in the presence of KPF₆ and NH₄ PF₆ are shown in Fig. 2; reliable difference spectra could not be obtained for the iodide salts because of strong absorption by I[−]. Tryptophan fluorescence spectra were obtained on a Jobin Yvon Horiba Fluorolog-3 spectrofluorometer. The excitation wavelength was 280 nm, and the entrance and exit bandpass were 3 nm. Fluorescence spectra were recorded at a constant temperature of 20 °C.

UV resonance Raman spectroscopy

The UVRR setup has been described elsewhere^[14] Briefly, vibrational spectra were obtained by setting the fundamental laser wavelength to 860, 884, 912, 916, or 920 nm to generate 215, 221, 228, 229, or 230 nm excitation beams, respectively. A typical sample volume of 4 ml was flowed through a 200- μ m i.d., 350- μ m o.d. vertically mounted fused-silica capillary at a rate of 0.40 ml/min to ensure fresh sample for each laser pulse. The UV power was $\sim$ 1 mW at the sample. Ten 1-min spectra were collected and summed for all samples. UVRR spectra of all appropriate blank solutions were also collected and subtracted from the corresponding IC spectra. Artifacts due to self-absorption were corrected on the basis of the relative intensities of the acetonitrile peaks at 920 and 1375 cm^{-1} . Overlapping peaks were decomposed into Gaussian bands using a least-squares fitting routine. Intensities of the UVRR spectra were normalized using the most intense peak found between 750 and 770 cm^{-1} . Accuracy and precision were determined using standard ethanol peaks and were found to be $\pm 2 \text{ cm}^{-1}$. The bandpass for the Raman experiment was less than 11 cm^{-1} .

Normal mode calculations

Calculations were performed using the Gaussian 03W quantum chemistry program operating on a Windows platform^[15] The geometry and harmonic vibrational frequencies of

3-ethylindole and IC in the absence and presence of K^+ were calculated using density functional theory (DFT) with the hybrid B3LYP functional. A 6–31G(d) basis set was selected for all calculations.

Results

The absorption and difference spectra of IC in acetonitrile indicate subtle blue shifts of less than 1 nm for the $L_{a,b}$ and B_b bands (Fig. 2) in the presence of KPF_6 and NH_4PF_6 . These shifts result in decreased resonance enhancement of indole at wavelengths optimal for study of tryptophan, e.g. 228–230 nm excitation. The absorption spectra reported here contrast with those of a prior study of an IC– K^+ complex in methanol in which a subtle increase in absorption at 230 nm in the presence of cation was reported^[16] We attribute the contrasting results to the use of different solvents, as described below. The fluorescence spectra showed similar minor changes; for example, the emission maxima are 353 and 350 nm in the absence of K^+ and presence of a 1 : 1 stoichiometric ratio of K^+ to IC, respectively (data not shown).

The UVRR spectrum of IC is nearly identical to that of *N*-acetyl-tryptophan ethyl ester (NATEE, Fig. S2). The W18 and W16 regions of the resonance Raman spectra (228 nm excitation) of IC with 0 : 1, 0.5 : 1, and 1 : 1 stoichiometric ratios of NaI, KI, and NH_4PF_6 are shown in Fig. 3, with extended spectra shown in Fig. S2. The W18 peak in the 0 : 1 IC spectrum is a doublet centered at ~ 755 and ~ 764 cm^{-1} . Upon addition of K^+ and NH_4^+ , the W18 peak broadens and the intensity profile of the doublet changes; the peak at 755 cm^{-1} decreases in intensity and the peak at 764 cm^{-1} increases in intensity. The W16 peak region remains unchanged; however, the intensity ratio of the W16 and W18 peaks (I_{W16}/I_{W18}) is greater than 1 for the IC molecule. Typically this ratio is less than or equal to 1 for model tryptophan compounds and proteinaceous tryptophans^[17] The W18 region is also perturbed in the presence of Na^+ , but in a manner qualitatively different from that observed with K^+ and NH_4^+ . The W18 peak exhibits some broadening, but the high wavenumber shoulder near 764 cm^{-1} becomes less intense in the presence of Na^+ relative to the peak at 755 cm^{-1} . These wavenumber and intensity shifts are illustrated in the difference spectra shown in Fig. 3, where relative maxima can be found at 742, 764, and 777 cm^{-1} for NH_4^+ , 742, 764, and 775 for K^+ , and 744, 759, and 779 cm^{-1} for Na^+ . Relative minima are found at 753, 770, and 785 cm^{-1} for NH_4^+ , 755, 770, and 785 cm^{-1} for K^+ , and 755 and 768 cm^{-1} for Na^+ . These changes in the W18 region are reproduced with different excitation wavelengths (Figs S3 and S5). Raman scattering from the crown ether portion of the molecule made no contributions to the UVRR spectrum of IC (data not shown).

UVRR and 1H -NMR spectra were measured for skatole to determine whether the cation and indole groups interact in the absence of the crown portion. No significant shifts in the NMR spectra were detected (Fig. S1). The UVRR spectra were also largely unaltered, with the exception of a slight decrease in the W16 intensity (Figs S2 and S4).

The W10, W9, W8, and W7 regions of the 228 nm excitation UVRR spectra are shown in Fig. 4. In all IC spectra, Gaussian decompositions indicate increases in the intensity ratio of the W10 and W9 modes ($R_{W10} = I_{\sim 1230}/I_{\sim 1250}$)^[17] upon complexation of the cations. The

W8 mode exhibited a small shift from 1304 to 1306 cm^{-1} in the presence of cations, and this shift is most obvious in the case of NH_4^+ in Fig. 4. Finally, the intensity of the W7 mode also increased, although it is difficult to interpret quantitative changes in the W7 mode because of strong overlap with a solvent band.

Crystal structures of IC in the presence of NaI, KI, and NH_4PF_6 are superimposed in Fig. 5 to compare key structural differences of the IC molecule when bound to each cation^[18] The three complexes were overlaid such that atoms C11 and N12 of each side arm overlap. Each complex folds to form a bound structure upon addition of cations, but there are important differences among the three structures. The cation in the Na^+ and the K^+ structures is located in the center of the crown ether, as expected. However, the addition of NH_4^+ transfers protons to the macroring nitrogens of the crown, creating a dication structure with no central cation present in the crown ether^[18] The consequences of this difference are that the protonated complex may possess up to two positive charges instead of one and the distances between the cationic region and the indole ring vary. The distance from the Na^+ , K^+ , or center of the crown to the center of the indole pyrrole ring in the folded IC molecule is 3.50, 3.45, and 3.51 Å, for the Na^+ , K^+ , and NH_4^+ complexes, respectively^[18] The distance from the protonated nitrogen in the case of NH_4^+ salt to the center of the pyrrole ring is 3.88 Å^[18] However, the distance between the relevant proton on the macroring to the pyrrole center is presumably shorter than 3.88 Å. In addition to these variations in distance, the small size of the Na^+ cation results in a variation in the angle between the indole ring and the crown ether plane. This variation in angle can be quantified by measuring the torsion angles defined by C2, C3, C10, and C11. The torsion angle for the unbound IC molecule is 101°, and the angles are 120, 111, and 110° for the bound Na^+ , K^+ , and NH_4^+ complexes, respectively.

DFT calculations predict the 3-ethylindole W18 mode to appear at 784.3 cm^{-1} , and two hydrogen out-of-plane (HOOP) modes at 784.1, and 822.2 cm^{-1} . The experimentally observed UVRR spectrum of NATEE exhibits a large W18 peak at 761 cm^{-1} and two shoulders at ~780 and 809 cm^{-1} . The predicted W18 and HOOP modes for IC in the absence of salt are 766.4, 781.6, and 788.5 cm^{-1} , respectively. In the presence of K^+ , these peaks shift to 775.3, 780.0, and 800.3 cm^{-1} . DFT results are presented in Figs S6 and S7.

Discussion

Spectral changes in the electronic spectra of the indole chromophore may arise because of variations in noncovalent interactions and local environment. It is now well accepted that systematic shifts up to ~50 nm in emission maxima, large changes in fluorescence quantum yield, and alterations in absorption maxima and extinction coefficients are correlated to local environment and hydrogen bonding^[19–21] In contrast, only subtle alterations in the electronic spectra of indole have been attributed to cation– π effects, but no clear correlations have been established^[10,16,22] For example, the absorption spectrum of IC– K^+ in acetonitrile presented here differs from that of a previous study of the same complex in methanol^[16] Methanol is a stronger hydrogen-bond acceptor than acetonitrile, with Kamlet–Taft hydrogen-bond acceptor basicity (β) values of 0.62 and 0.31 for methanol and acetonitrile, respectively^[23] Therefore, it is not surprising that the strengths of cation– π interaction in these two solvents differ and give rise to variations in the absorption

difference spectra. In addition to the lack of systematic shifts in absorption spectra, the magnitude of change is extremely small and likely undetectable in a protein where numerous other chromophores contribute to the deep-UV absorption. Circular dichroism is a promising tool for detection of cation- π interactions in small peptides,^[11] but this technique may not be reliable in studies of large peptides or proteins because of the strong signal from secondary structure elements. The combination of subtle spectroscopic changes for cation- π interaction and large competing effects of other environmental factors makes electronic spectroscopy a difficult tool for the detection of these important cation- π interactions.

In contrast to electronic spectroscopy, UVRR is able to report on specific changes in structure because it is a highly specific and sensitive vibrational technique.^[24–26] In fact, several tryptophan UVRR modes have been shown to independently reflect properties such as local polarity, structure, and hydrogen-bond environment^[17,27] Here, we report a UVRR investigation of a model compound capable of forming strong cation- π interactions between alkali metal ions and the indole ring side chain that constitutes the tryptophan residue in proteins^[13]

Previous UVRR studies of cation- π interactions involving tryptophan have proposed several vibrational modes that reflect cation- π interactions, including W18, W16, W10, W7, and W3; the observed shifts in these modes depend on the excitation wavelength^[10,16,17,28,29] Here, the primary reproducible changes in the UVRR spectra of IC are in the $\sim 760\text{ cm}^{-1}$ W18 region. Our calculations of IC also support significant vibrational wavenumber shifts upon formation of cation- π interaction in this spectral region. The W18 region in the UVRR spectra of IC is broadened and consists of several peaks that involve the W18 mode as well as other modes; we propose on the basis of DFT calculations and NMR results (see below) that these additional modes are HOOP vibrations. According to normal mode calculations of 3-ethylindole and the IC molecule, the W18 mode can be described as an in-plane breathing mode delocalized over all atoms of the indole ring and is thus sensitive to changes in the π -electron density that participates in cation- π interaction^[24] In addition to this large Raman active mode, three indole HOOP normal modes are predicted to appear in the region between the W18 ($\sim 760\text{ cm}^{-1}$) and W17 ($\sim 880\text{ cm}^{-1}$) peaks in IC; these HOOP modes consist of large out-of-plane displacements of the hydrogen atom (H2) bonded to C2 as well as benzyl protons on the indole ring of IC (Fig. S6). Similar HOOP modes are predicted to appear in the same spectral region for 3-ethylindole, and on- and off-resonance Raman spectra of model compounds such as *N*-acetyl-tryptophanamide (NATA) and NATEE support the presence of these additional modes between the W18 and W17 peaks^[17] We acknowledge that unambiguous identification of the HOOP modes requires experiments with isotopically labeled compounds; however, the combination of NMR, UVRR, DFT, and crystal structures presented here supports the current assignment of the additional peaks in the W18 region to HOOP vibrations.

The broad and complex W18 region of IC is sensitive to cation- π interactions. Previous X-ray structural studies of IC indicated that the pyrrolo, and not benzyl, portion of IC interacts directly with bound cations^[18] This pyrrole-cation interaction was also found in the solution phase via ^1H -NMR spectroscopy, which indicated that the signal from H2 experienced the greatest shift of $\sim 0.3\text{ ppm}$ upon addition of a stoichiometric amount of Na^+ ^[13] Systematic

shifts of the H2 proton are also observed for the K^+ complex, though to a lesser degree (Fig. S1). These shifts are consistent with the distances between the cationic moiety and C2; in the case of the Na^+ complex, the distance is 3.23 Å while that of the K^+ complex is 3.32 Å. The analogous distance for the NH_4^+ complex is difficult to determine since the cationic moiety is a protonated nitrogen on the macrocyclic ring; however, the distance is less than 3.61 Å, which is the separation from N12 to C2. Based on the observed NMR shifts, it is expected that normal modes that involve displacement of H2 would also be perturbed. Our vibrational spectra are consistent with this analysis. Specifically, the observed systematic changes in the W18 and HOOP regions of the IC spectra support a pyrrole–cation complex which results in perturbation of normal modes that involve general ring breathing modes (W18) and the H2 proton^[13,18] One other prior UVRR study of IC reported similar changes in the W18 region, though this previous work did not provide extensive analysis of the normal modes or spectra^[16] The consistency between the NMR and UVRR data support the notion that electrostatics due to the presence of the cation is primarily responsible for the observed spectral shifts. We do not eliminate the possibility that structural changes contribute to the differences in the W18 region of the vibrational spectra; however, the majority of the other UVRR peaks, even the W3 mode (see below), are unaltered between the closed and open forms of IC, indicating that changes in structure do not significantly modify the UVRR spectra.

Slight variation in the UVRR spectra for the Na^+ , K^+ , and NH_4^+ may reflect the different strengths of cation– π interaction in these complexes. The crystal structures of the three cation-bound complexes in Fig. 5 suggest that the indole ring of the Na^+ complex is in a slightly different orientation than in the NH_4^+ or the K^+ complexes. This variation may arise due to the differential affinities for Na^+ and K^+ of the crown and aromatic systems. While the parent crown ether molecule of IC, 4,13-diaza-18-crown-6 ether, selectively binds K^+ over Na^+ ,^[30] gas-phase experiments and calculations indicate that Na^+ binds indole stronger than K^+ because of more favorable electrostatic interactions^[3] The close interaction between the Na^+ and C2 as shown in the crystal structure and NMR spectra accounts for a greater perturbation of H2 relative to the K^+ and NH_4^+ complexes, resulting in modifications of the H2–OOP vibrational wavenumbers. On the other hand, Na^+ has a smaller radius than K^+ and, therefore, may result in weaker cation– π interactions in this confined structure; the variability in cation– π strength may impact the delocalized W18 mode. We are continuing to investigate these and other possible origins of the differences among the UVRR spectra of the complexes.

The W16 normal mode is composed of breathing motion of the benzyl ring of indole and, therefore, this band was also expected to be sensitive to cation– π interactions because of perturbation of the aromatic π electrons upon binding of cations. We and others have previously reported subtle changes in the W16 region of proteins^[10,17] However, there are no observable shifts in W16 wavenumber or intensity in the UVRR spectra of IC (Fig. 3). The lack of changes in the W16 peak may be attributed to the observation that the pyrrole, and not benzyl, ring of indole interacts with the Na^+ and K^+ cations in the complex. For the NH_4^+ complex, the position of the indole rings is nearly identical to that of the K^+ complex, and the protonated crown nitrogens may not interact strongly with the benzyl subunit because of the relatively large distance^[18] Synthetic efforts have shown that connection of

the indole side arm via the benzyl or pyrrolo ring results in cation– π interactions with the benzyl or pyrrolo portions, respectively. Therefore, the position of the cation above the pyrrolo ring in the case of IC is largely a result of steric effects and not solely electrostatic interactions^[31]

The question of whether the benzyl or pyrrolo ring of indole interacts directly with cations in a protein has been investigated in detail. Calculations indicate that the most likely subunit is the benzyl ring^[3] and a statistical analysis of single subunit proteins in the Protein Data Bank have confirmed this energetic preference for the benzyl ring of tryptophan^[11] In the UVRR spectra of skatole (Figs S2 and S4), we found subtle decreases in the intensity of the W16 mode in the presence of Na⁺ and K⁺ spectra which were remarkably reproducible across excitation wavelengths 228–230 nm (data not shown). This decrease in W16 intensity has been reported in previous UVRR spectra of a copper–tryptophan interaction in a protein,^[10] proposed tryptophan–lysine/arginine interactions in peptides,^[17,32] and a membrane-bound model compound^[17] Collectively, the spectral changes observed here and elsewhere support W18, HOOP, and W16 modes as reliable markers of cation– π interactions, where preference for binding to the pyrrolo and benzyl portions may be reflected in differential shifts in the intensities/wavenumbers of the W18 and W16 modes, respectively. HOOP modes are likely sensitive to both types of cation– π interactions, with experimentally observed shifts in modes that involve H2 displacement in the case of pyrrole–cation interactions.

Increases in hydrogen bonding of the indole N–H in IC upon addition of cations are indicated by increases in the W10 doublet intensity ratio ($R_{W10} = I_{\sim 1230}/I_{\sim 1250}$) and increases in the W8 wavenumber (Fig. 4), as well as slight increases in the W17 mode intensity (data not shown)^[17] These observations reflect the close proximity of the counteranion (iodide in Na⁺ and K⁺, and hexafluorophosphate in NH₄⁺) to the indole N–H groups of IC in the crystal structures^[18] The distances between the indole N–H group and the counteranion (N...I[−] or N...PF₆[−]) are 3.57 Å for the Na⁺ complex, 3.54 Å for the K⁺ complex, and 3.24 and 3.27 Å corresponding to two closely positioned fluorine atoms for the NH₄⁺ complex. The visible shift of the W8 mode, in particular for the NH₄⁺ bound IC complex, also reflects the close proximity between the indole nitrogen and the anion^[18]

Alterations of other indole modes, such as W3 and W7, were expected but not definitively observed in the current study. The W3 mode involves displacements of all atoms of the indole ring in IC and 3-ethylindole, with dominant vibration of the C2–C3 bond^[24] A previous report quantitatively correlated the W3 normal mode wavenumber to the tryptophan torsion angle defined by C2, C3, C10, and C11 atoms^[27] In the current experiments, however, the peak wavenumber of the W3 mode remains constant at 1552 cm^{−1} for all three cationic complexes despite shifts in dihedral angle of up to ~20°. The lack of observable shifts in the W3 peak suggests that the crystal and solution structures may vary, the trends reported for the smaller tryptophan-based model compounds from earlier studies are not necessarily applicable to the large IC molecule studied here, and/or changes in the W3 mode as a result of variation in structure may be complicated by the presence of cation– π interactions that perturb the pyrrolo ring. Previous UVRR studies also suggested

shifts in the W7 region in the presence of cation- π interactions^[10,28] However, we are unable to investigate the W7 region because of strong overlap with a solvent peak.

Finally, it is important to discuss the relevance of the current results to proteins. Cations that are known to interact with tryptophan in biological systems include alkali metals such as Na⁺ and Cs⁺, transition metals (Cu⁺), and ammonium ions from arginine, lysine, histidine, acetylcholine, and lipid head groups^[1,4,6,7,10,33,34] In the case of the model compound IC, interaction with K⁺ and ammonium ions gave identical results; however, unlike proteins, the cation- π interaction in IC involves primarily the pyrrole, not benzyl ring. Therefore, the specific trends from the current report are likely limited to the IC molecule studied here. Nonetheless, it is reasonable to expect that the tryptophan modes monitored here, such as W18 and W16, as well as HOOP modes, will serve as reliable markers for cation- π interactions in biological systems. We are currently studying native cation- π interactions in proteins with the goal of establishing a library of UVRR markers that report on these ubiquitous biological interactions.

Conclusions

We report UVRR spectra of an indole crown-ether molecule that is known to form cation- π interactions between the indole chromophore and alkali and amine cations. The current study complements previous studies that elucidated UVRR spectral changes that correlate structure, solvent polarity, hydrogen-bond strength, and environment. The unambiguous W18 and HOOP UVRR markers for cation-pyrrole π interactions contribute to a more comprehensive set of molecular markers for crucial noncovalent interactions in proteins. These and other spectral markers can be used to describe important noncovalent interactions that are significant for proteins events such as folding, ligand binding, and membrane association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Gallivan JP, Dougherty DA. Proc. Natl. Acad. Sci. USA. 1999; 96:9459. [PubMed: 10449714]
2. Anslyn, EV.; Dougherty, DA. Modern Physical Organic Chemistry. Sausalito, CA: University Science; 2006.
3. Dougherty DA. Science. 1996; 271:163. [PubMed: 8539615]
4. Ma JC, Dougherty DA. Chem. Rev. 1997; 97:1303. [PubMed: 11851453]
5. Mecozzi S, West AP Jr, Dougherty DA. Proc. Natl. Acad. Sci. USA. 1996; 93:10566. [PubMed: 8855218]

6. Wouters J. *Protein Sci.* 1998; 7:2472. [PubMed: 9828016]
7. Kooystra PJU, Kalk KH, Hol WGJ. *Eur. J. Biochem.* 1988; 177:345. [PubMed: 3191921]
8. De Vos AM, Ultsch M, Kossiakoff AA. *Science.* 1992; 255:306. [PubMed: 1549776]
9. Aleshin AE, Firsov LM, Honzatko RB. *J. Biol. Chem.* 1994; 269:15631. [PubMed: 8195212]
10. Xue Y, Davis AV, Balakrishnan G, Strasser JP, Staehlin BM, Focia P, Spiro TG, Penner-Hahn JE, O'Halloran TV. *Nat. Chem. Biol.* 2008; 4:107. [PubMed: 18157124]
11. Yorita H, Otomo K, Hiramatsu H, Toyama A, Miura T, Takeuchi H. *J. Am. Chem. Soc.* 2008; 130:15266. [PubMed: 18939840]
12. Killian JA, Heijne Gv. *Trends Biochem. Sci.* 2000; 25:429. [PubMed: 10973056]
13. De Wall SL, Meadows ES, Barbour LJ, Gokel GW. *J. Am. Chem. Soc.* 1999; 121:5613.
14. Sanchez KM, Neary TJ, Kim JE. *J. Phys. Chem. B.* 2008; 112:9507. [PubMed: 18588328]
15. Frisch, MJ.; Trucks, GW.; Schlegel, HB.; Scuseria, GE.; Robb, MA.; Cheeseman, JR.; Montgomery, JA., Jr; Vreven, T.; Kudin, KN.; Burant, JC.; Millam, JM.; Iyengar, SS.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, GA.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, JE.; Hratchian, HP.; Cross, JB.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, RE.; Yazyev, O.; Austin, AJ.; Cammi, R.; Pomelli, C.; Ochterski, JW.; Ayala, PY.; Morokuma, K.; Voth, GA.; Salvador, P.; Dannenberg, JJ.; Zakrzewski, VG.; Dapprich, S.; Daniels, AD.; Strain, MC.; Farkas, O.; Malick, DK.; Rabuck, AD.; Raghavachari, K.; Foresman, JB.; Ortiz, JV.; Cui, Q.; Baboul, AG.; Clifford, S.; Cioslowski, J.; Stefanov, BB.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, RL.; Fox, DJ.; Keith, T.; Al-Laham, MA.; Peng, CY.; Nanayakkara, A.; Challacombe, M.; Gill, PMW.; Johnson, B.; Chen, W.; Wong, MW.; Gonzalez, C.; Pople, JA. *Gaussian 03*. Wallingford, CT: 2004.
16. Okada A, Miura T, Takeuchi H. *Biochemistry.* 2001; 40:6053. [PubMed: 11352741]
17. Schlamadinger DE, Gable JE, Kim JE. *J. Phys. Chem. B.* 2009; 113:14769. [PubMed: 19817473]
18. Meadows ES, De Wall SL, Barbour LJ, Gokel GW. *J. Am. Chem. Soc.* 2001; 123:3092. [PubMed: 11457020]
19. Bailey JE, Beaven GH, Chignell DA, Gratzer WB. *Eur. J. Biochem.* 1968; 7:5.
20. Callis, PR. *Reviews in Fluorescence 2007*. Geddes, CD., editor. Vol. 4. New York: Springer; 2009. p. 199
21. Lakowicz, JR. *Principles of Fluorescence Spectroscopy*. 3rd edn. New York: Springer; 2006.
22. Lu W, Qiu W, Kim J, Okobiah O, Hu J, Gokel GW, Zhong D. *Chem. Phys. Lett.* 2004; 394:415.
23. Kamlet MJ, Abboud J-LM, Abraham MH, Taft RW. *J. Org. Chem.* 1983; 48:2877.
24. Harada, I.; Takeuchi, H. *Spectroscopy of Biological Systems*. Clark, RJH.; Hester, RE., editors. Vol. 13. Chichester, UK: John Wiley and Sons; 1986. p. 113
25. Asher SA. *Annu. Rev. Phys. Chem.* 1988; 39:537. [PubMed: 3075468]
26. Austin, JC.; Jordan, T.; Spiro, TG. *Biomolecular Spectroscopy, Part A: Advances in Spectroscopy*. Clark, RJH.; Hester, RE., editors. Vol. 20. England: John Wiley and Sons: West Sussex; 1993. p. 55
27. Miura T, Takeuchi H, Harada I. *J. Raman Spectrosc.* 1989; 20:667.
28. Wen ZQ, Thomas GJ Jr. *Biochemistry.* 2000; 39:146. [PubMed: 10625489]
29. Otomo K, Toyama A, Miura T, Takeuchi H. *J. Biochem.* 2009; 145:543. [PubMed: 19155268]
30. Gatto VJ, Gokel GW. *J. Am. Chem. Soc.* 1984; 106:8240.
31. Hu J, Barbour LJ, Gokel GW. *J. Am. Chem. Soc.* 2002; 124:10940. [PubMed: 12224916]
32. Gable JE, Schlamadinger DE, Cogen AL, Gallo RL, Kim JE. *Biochemistry.* 2009; 48:11264. [PubMed: 19894716]
33. Petersen FNR, Jensen MØ, Nielsen CH. *Biophys. J.* 2005; 89:3985. [PubMed: 16150973]
34. Gaede HC, Yau W-M, Gawrisch K. *J. Phys. Chem. B.* 2005; 109:13014. [PubMed: 16852615]

Figure 1.
Crystal structures of the IC molecule in the absence and presence of K^+ . Numbering scheme for indole ring and linker arm is indicated.

Figure 2.

Absorbance spectra of IC normalized to the absorption at 290 nm. The stoichiometric ratios of salt/IC are 0 : 1 (solid line), 0.5 : 1 (dotted line), and 1 : 1 (dashed line). Corresponding difference spectra for 0 : 1–0.5 : 1 (dotted line) and 0 : 1–1 : 1 (dashed line) are also shown.

Figure 3.

UVRR and difference spectra of the W18 and W16 mode region of IC molecule in the absence and presence of NaI, KI, and NH_4PF_6 . UVRR spectra are normalized to the most intense peak in the W18 region. Stoichiometric ratios of cation/IC are the following: Solid line, 0 : 1; dotted line, 0.5 : 1; dashed line, 1 : 1. Difference spectra are multiplied by 2.

Figure 4.

UVRR and difference spectra of the W10, W9, W8, and W7 mode regions of IC molecule in the absence and presence of NaI, KI, and NH_4PF_6 . UVRR spectra are normalized to the most intense peak in the W18 region. Stoichiometric ratios of cation/IC are the following: Solid line, 0 : 1; dotted line, 0.5 : 1; dashed line, 1 : 1. Difference spectra are multiplied by 2.

Figure 5.
Superimposed crystal structures of NaI (A), KI (B), and NH₄ PF₆ (C) complexed IC molecule. Ions are not shown for clarity. See main text for details.