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Solvent Effect on Anthranilic Acid Spectroscopy

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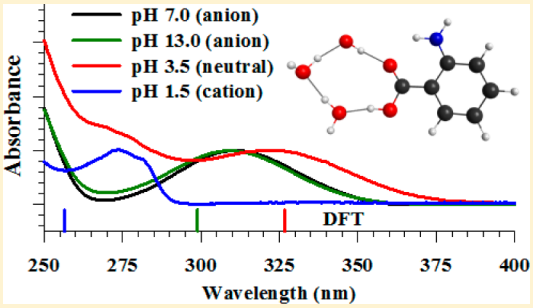
ABSTRACT: The spectroscopy of anthranilic acid (AA) was examined in neat and binary solvents of varying polarity and hydrogen bonding strength in order to understand the role of water in solvating the polar sites of the molecule. With the exception of water, the Stokes shift of AA in different solvents was found to be linearly correlated with the normalized molar transition energy of solvent polarity (E_T^N), indicating the major role of the hydrogen bonding effect in solution. Analysis of the absorption and fluorescence spectra reveals that AA exists as an anion in neutral water. The pK_a (4.50) and pK_a* (4.44) values were estimated from the spectral shift in the absorption and fluorescence spectra measured in different pH solutions. The shortest fluorescence lifetime was measured in cyclohexane and is attributed to intramolecular hydrogen dislocation/transfer in the excited state. The lifetime values in polar solvents point to the dominant effect of the hydrogen-bond donating strength (α value) of the solvent. The number of water molecules solvating the polar region of the neutral form of AA was estimated to be three from the absorbance change in dioxane/buffer (pH 3.5) binary mixtures. The structures of AA:water complexes were calculated from density functional theory using the B3LYP method with a 6-311++G(2d,p) basis set. A stepwise addition of water molecules (1−3) to the polar region of AA leads to a preferential solvation of the COOH group of the molecule in a closed-cyclic geometry. It is worth noting that the spectral shift as a function of pH suggests the suitability of AA as a probe to estimate the local acidity of binding sites in macromolecules in the pH range 3.0−7.0.



INTRODUCTION

Anthranilic acid (AA) is frequently used as a fluorescent label in peptides due to its convenient size, absorption and fluorescence spectral regions, long fluorescence lifetime and its high fluorescence quantum yield (Φ = 0.60) in aqueous solution.[1−3] The absorption and fluorescence maxima of AA are well shifted from those of peptides, and its small size is not expected to cause a significant perturbation of the macromolecular structure to which it is attached. The functional groups in AA are also similar to the functional groups in natural amino acids, which allows for the possibility of connecting the molecule to the N^{α} amino group in proteins without a significant change in its spectroscopic character.[4] A change in the fluorescence properties of AA serves to indicate a change in the local environment of the protein under study.[3] When AA moves from an aqueous to a nonpolar environment, its fluorescence quantum yield increases, accompanied by a blue shift in its fluorescence band. It has also been observed that the fluorescence quantum yield of AA decreases by one order of magnitude when it is attached to specific amino acids in peptide chains.[4−6] This was attributed to either a side reaction taking place or the loss of planarity[4] as in the case of AA−proline.[5,6] Fluorescence quenching in the AA−tryptophan pair was also found to be due to the specific interaction between AA and the indole ring of tryptophan.[4]

AA has two rotamers, as shown in Figure 1, which differ by 180° rotation of the carboxylic group. Rotamer I was found to be more stable than rotamer II by 3.16 kcal mol^{-1} using density functional theory (DFT) calculations.[7] The stability of the former tautomer was attributed to the stronger intramolecular hydrogen bond between the carbonyl group and the amino group than that between the carboxyl group and the amino group in the latter tautomer. AA also shows a strong tendency to form dimers that are stabilized by two intermolecular

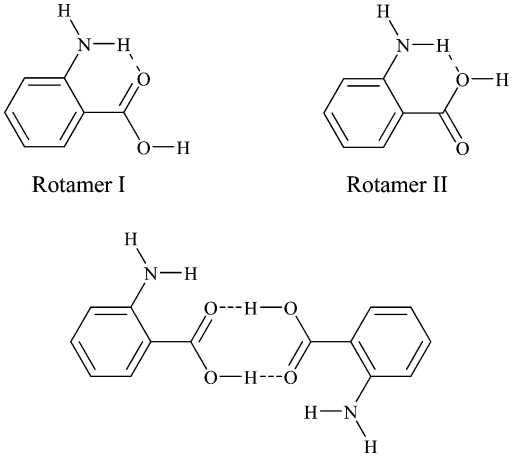


Figure 1. The different rotamers (upper) and the most stable dimer (lower) of AA.

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hydrogen bonds. The most stable dimer was found to form between two monomers of rotamer I as shown in Figure 1.[8]

Microscopic solvation of the AA molecule by water was investigated theoretically and in supersonic jets.[9,10] One water molecule results in different 1:1 AA:water complexes. The most stable isomer was found in the complex where the water molecule resides in the pocket of the carboxyl group and binds between the carbonyl oxygen and the acid hydrogen. In the 1:2 complex, a cyclic ring structure involving three hydrogen bonds was found to be the most stable isomer, with both water molecules bonded to the carboxyl group.

In this paper, we employ steady state spectroscopy and fluorescence lifetime measurements to examine the spectra of AA in solvents of varying polarity and hydrogen bonding strength. The selected solvents in this work give a wide range of polarity that allows a comprehensive study of the effect of changing the local environment around the AA molecule, particularly the effect of water on the spectroscopy of AA. We show that AA is ionized in neutral water. We quantify the number of water molecules that solvate the polar sites of AA by studying the spectral change in binary mixtures of buffered water and dioxane. Microsolvation of AA by water is further investigated by calculating the structures of AA:H_2O complexes and the Franck−Condon transitions using DFT.

EXPERIMENTAL SECTION

AA was obtained from Sigma-Aldrich (>99%) and was used as received without further purification. Acetonitrile (spectroscopic grade), anhydrous 1,4-dioxane and methanol were obtained from Sigma-Aldrich. Dimethyl sulfoxide (DMSO) was purchased from Fluka. Anhydrous ethanol was received from Acros Organics. Spectroscopic grade cyclohexane was purchased from Sigma-Aldrich. Deionized water (Millipore) was used.

For acidic media, fuming HCl (36.5−38.0%, analytical grade from Sigma-Aldrich) was used to adjust the pH of the solution, while 1.0 M NaOH (Pellets, >99%, Sigma-Aldrich) was used for the basic media. The pH study in the range 2.0−12.0 was performed using a universal buffer[11] that is composed of 0.1 M each of citric acid, potassium phosphate, sodium tetraborate, Tris, and potassium chloride (all obtained from Aldrich as research grade and used as received). The concentration of AA in all solvents was fixed at 0.05 mM.

Absorption spectra were obtained using an HP 845x diode array spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectrofluorophotometer. Lifetime measurements were performed using a TimeMaster fluorescence lifetime spectrometer obtained from Photon Technology International. Excitation was at 340 nm using a light-emitting diode. The system response time as measured from the scattered light was estimated to be approximately 1.5 ns (fwhm). The measured transients were fitted to multiexponential functions convoluted with the instrument response function (IRF). The fit was judged by the value of the reduced chi-squared (χ^2). The experimental time resolution (after deconvolution) was approximately 100 ps, using stroboscopic detection.[12] In all the experiments, samples were contained in a 1 cm path-length quartz cell and the measurements were conducted at 23 ± 1 °C.

Geometry optimization and time-dependent calculations for the different tautomers of AA uncomplexed and complexed with water molecules were carried out using the GAMESS program.[13] Polarizable continuum model (PCM) calculations were carried out using Gaussian 03.[14]

RESULTS AND DISCUSSION

Spectral Characterization and Dynamics of AA in Different Solvents. The absorption and fluorescence spectra of AA in different solvents are shown in Figure 2, and the spectroscopic parameters are summarized in Table 1. When compared to its counterpart salicylic acid, the absorption peak in all solvents arises from the lowest $\pi-\pi^*$ transition in AA.[15] A slight blue shift is observed in protic solvents which is much more pronounced in water. The corresponding fluorescence spectra show a small red shift in polar solvents, and a spectral broadening is clearly observed in protic solvents. This observation, along with an increase in the Stokes shift ($\Delta\nu$), points to a specific intermolecular hydrogen bonding interaction between AA and the protic solvent in the excited state potential energy surface. The large Stokes shifts of AA in solution indicate a large geometrical change upon excitation which has been reported in the gas phase of bare AA and of AA complexed with water.[7−10,16] The large geometrical change in the excited state promotes a hydrogen dislocation that induces amine−imine tautomerization.[7]

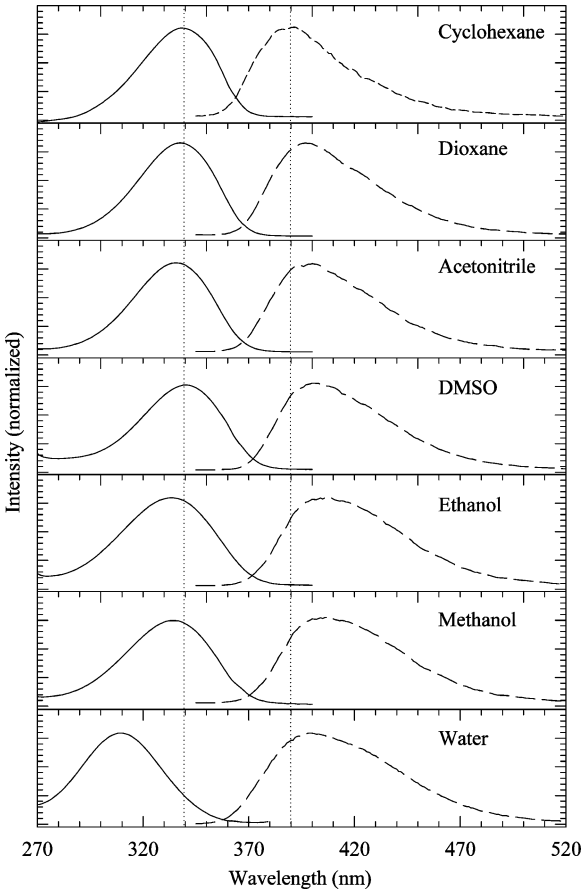

Figure 2. Absorption (solid lines) and fluorescence (dashed lines) spectra of AA in different solvents. λ_{ex} = 340 nm.

The fluorescence behavior cannot be directly correlated to solvent polarity. In order to understand the spectral change in fluorescence, we need to discuss the solvent properties beyond the average values of their dielectric constants (ε),[17] particularly in terms of their hydrogen bond ability. Table 1 shows the ε

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Table 1. Spectroscopic Parameters and Lifetimes of AA in Different Solvents

solvent	ε^a	π^{*a}	E_T^{Na}	α^a	β^a	λ_{abs}^{max} (nm)	λ_{flu}^{max} (nm)^b	$\Delta\nu$ (cm^{-1})	τ (ns)^c	χ^2
cyclohexane	2.02	0.00	0.01	0.00	0.00	338	392	4076	4.89	1.08
dioxane	2.21	0.49	0.16	0.00	0.37	338	395	4269	6.62	1.12
acetonitrile	35.94	0.66	0.46	0.19	0.40	336	398	4636	6.78	1.29
DMSO	46.45	1.00	0.44	0.00	0.76	341	402	4450	6.93	1.02
ethanol	24.55	0.54	0.65	0.86	0.75	335	406	5220	7.09	1.09
methanol	32.66	0.60	0.76	0.98	0.66	334	408	5430	7.94	1.07
water	78.30	1.09	1.00	1.17	0.47	310	396	7006	8.70	1.29

^{a}Obtained from ref 18. $^b\lambda_{ex}$ = 340 nm. ^{c}Uncertainty in measurements is ±0.05−0.10 ns.

values, along with the empirical parameters of solvent polarity (π^* and E_T^N).[18] A solvent such as dioxane, which appears to be nonpolar according to its static dielectric constant (ε = 2.21), has a high solvent polarity parameter (π^* = 0.49) and an E_T^N value of 0.16.[17] Dioxane has two CH_2-O-CH_2 groups opposite to each other, which results in a net zero dipole moment. Hence, it is considered a nondipolar solvent. However, dioxane exhibits a large quadrupole moment[19,20] which is reflected in its π^* parameter that mainly takes into consideration the polarizability and the dipolarity of the solvent.[21] The corresponding E_T^N value indicates that dioxane exhibits only 16% of the solvent polarity of water, which classifies dioxane as an apolar, non-hydrogen-bond donor solvent, which is reflected in its hydrogen-bond donor inability (α = 0 in Table 1).[18] On the other hand, dioxane's hydrogen-bond acceptor ability (β = 0.37) is close to that of acetonitrile (β = 0.40), which may explain the same fluorescence peak shift and shape of AA in both solvents (Figure 2).

It is worth noting that a linear correlation between the Stokes shift and the empirical parameter π^* was observed in aprotic solvents, whereas deviation from linearity was evidenced in protic solvents.[22] In our case, the Stokes shift for AA in the solvents studied here correlates with E_T^N, except for water, as shown in Figure 3. This correlation was not obtained for the

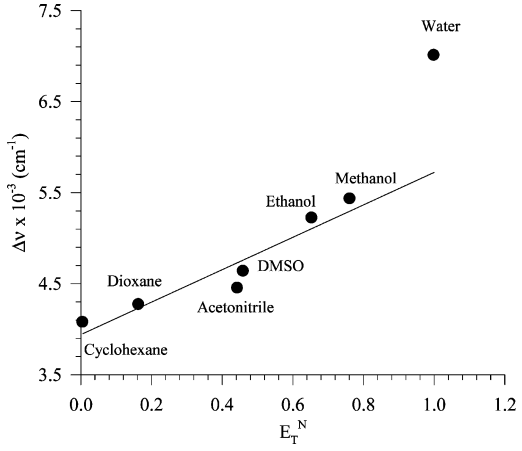

Figure 3. Stokes shift versus the empirical polarity parameter E_T^N for AA in different solvents as indicated in the graph.

other parameters (ε and π^*) which confirms the major effect of hydrogen bonding on AA spectroscopy. In a more comprehensive study, Stalin and Rajendiran found that both hydrogen bonding and solvent polarity effects must be considered in order to correlate the Stokes shift in AA with the solvent properties.[23]

The deviation of $\Delta\nu$ from linearity in water (Figure 3) may stem from the amphoteric nature of water that has a strong hydrogen bond donor and acceptor property and thus can behave as either acid or base depending on the solute.[18] Figure 4 shows the absorption spectra of AA (in the spectral region

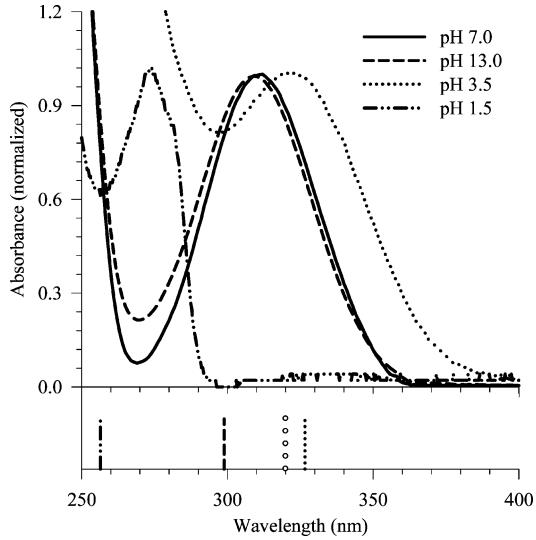

Figure 4. Absorption spectra of AA in different pH aqueous solutions as indicated in the graph. Vertical lines at the bottom represent the DFT calculations for the Franck−Condon $S_1 \leftarrow S_0$ transitions using a PCM for the neutral, anion and cation. The DFT calculation for the gas phase neutral AA is shown by open circles.

250−400 nm) in aqueous solution of pH 7.0 (equivalent to the absorption spectrum in Figure 2), and in aqueous pH 13.0, 3.5, and 1.5. It is clear that AA exists in the anion form at pH 7.0 as previously reported.[24] This explains the deviation from linearity in Figure 3. The ability of water to ionize AA may be a consequence of the large dipole moment of AA in the ground state (μ_g = 5.638 D),[23] which is expected to induce a large acidity in the carboxylic group. The absorption peak in aqueous pH 3.5 represents the neutral form of AA (pK_a (COOH) = 4.95),[25] whereas that in aqueous pH 1.5 is for the cationic form of AA (pK_a (NH_3^+) = 1.8−2.1).[23,25]

The corresponding fluorescence spectra in different pH solutions are displayed in Figure 5 for two excitation wavelengths. The results indicate that there is only one emission peak for each of the basic (pH 13.0), neutral (pH 3.5) and cationic (pH 1.5) species of AA. In aqueous solution of pH 7.0, excitation at 340 nm (equivalent to that in Figure 2) shows a shoulder that is absent for excitation at 310 nm. This shoulder is located in the same spectral region as that of the neutral form

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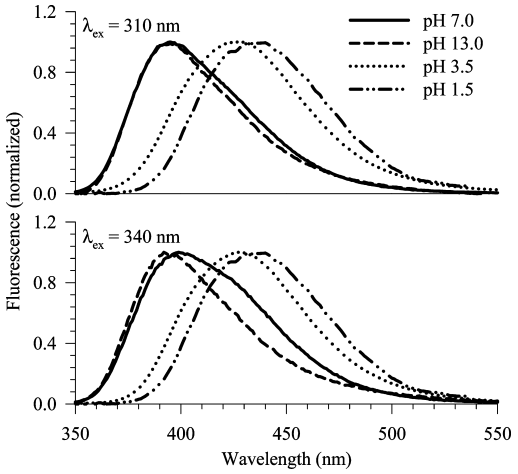

Figure 5. Fluorescence spectra of AA in different pH aqueous solutions after two excitation wavelengths as indicated in the graph.

of AA (pH 3.5). It is clear that some traces of neutral AA exist along with the basic form in aqueous solution of pH 7.0.

We performed a pH titration procedure in order to determine the pK_a value in the ground and excited states using the spectral shifts in the absorption (pK_a) and fluorescence (pK_a*) spectra. The results (Figure 6) indicate

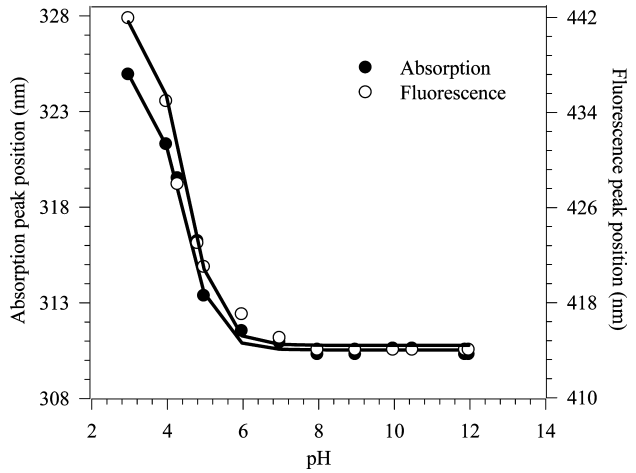

Figure 6. Titration curves showing the shift in the absorption and fluorescence (λ_{ex} = 310 nm) peaks of AA monitored at different pH conditions.

that dissociation of the carboxylic group in the ground state has a pK_a value of 4.50 (the reported value is 4.95[25]) and a pK_a* value of 4.44. The titration curves in Figure 6 show that the spectral position of AA should be useful in estimating the pH of the local environment of the molecule. By simply measuring the spectral position, the local pH of binding sites in macromolecules such as proteins and enzymes can be estimated in the pH range 3.0−7.0 when AA is used as a probe. We have recently used 2-(2′-hydroxyphenyl)benzoxazole (HBO) to estimate the pH medium of a drug-binding site in the human serum albumin protein, and we showed that HBO can be used to estimate the local pH in the range 7.0−11.0.[26,27] The present study on AA complements this range and extends it to the acidic region, which should be useful in studying the nature of binding in biological macromolecules. Replacing the OH group of AA by hexadecylamine did not affect the sensitivity to local pH when the molecule was used to probe surfactants in premicellar and micellar forms as indicated by Marquezin et al.[28]

Fluorescence lifetime measurements for AA in the different solvents are summarized in Table 1. The results are in substantial agreement with previously reported values for some of the solvents.[4,22,24] The shortest lifetime was observed in cyclohexane, which can be explained as a consequence of the quenching action of the strong hydrogen bond that leads to active intramolecular hydrogen dislocation/transfer in the excited state (amine−imine tautomerization). This dynamic process is enhanced in hydrophobic solvents that lack any direct contact with the polar sites of the AA molecule.

The measured fluorescence lifetimes in dioxane, acetonitrile and DMSO are close to each other, and longer by about 40% than that observed in cyclohexane. The three solvents have a strong hydrogen-bond accepting power as indicated by their β values, and no (dioxane and DMSO) or very weak (acetonitrile) hydrogen-bond donating power, which is reflected in their α values (see Table 1). Considering the most stable tautomer of AA (rotamer I in Figure 1), the most likely interaction between these solvents and AA is by forming a hydrogen bond with the hydroxyl group. A strong interaction with the OH group of AA is expected to affect its free rotation, hence reducing the nonradiative decay due to this low-frequency motion and resulting in longer lifetime values as shown in Table 1. This mechanism of solvation also explains the larger quantum yield values observed in DMSO and acetonitrile compared to those measured in nonpolar solvents.[22] Interaction between polar, aprotic solvents with the amino group of AA is also possible which may reduce the ability of tautomerization.

In protic solvents, the fluorescence lifetimes are longer than those measured in other solvents. This is a manifestation of the effect of both hydrogen-bond donating and accepting properties of these solvents, which is consistent with the observed large Stokes shifts in protic solvents. The measured lifetime values in ethanol, methanol and water indicate that the dominant effect is due to the hydrogen-bond donating strength (α value). It is clear that as the α value increases, the lifetime is longer although the β value decreases in the same order.

Water Solvation of AA. The tendency of water molecules to strongly associate with each other through intermolecular hydrogen bonds allows more than one molecule of water to form a solvent network that is capable of solvating the hydrogen donor and acceptor sites of the solute by forming a cyclic complex.[29−33] We have recently estimated the number of water molecules solvating the hydrogen bonding centers of several systems experimentally in binary mixtures of dioxane/water[34−37] and theoretically using ab initio methods.[35−37] The choice of dioxane is convenient because it is miscible with water in all proportions and thus provides an opportunity to study the effect of a broad range of solvent polarity. Mixtures of dioxane and water were also proposed as media to study probes in nanoenvironments similar to those encountered in vesicles and at interfaces.[34,35,38−40]

As mentioned above, AA exists in the neutral form in dioxane, whereas the molecule is ionized in neutral water. Studying the spectroscopy of AA in dioxane/water mixtures will be complicated because the spectral change will be due not only to the solvent effect but also to the structural change of AA. In order to maintain the neutral state of AA, we replaced water in the binary solvent mixtures by an aqueous buffer of pH 3.5

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(vide supra). Figure 7 shows selected absorption spectra of AA in the dioxane/buffer binary mixtures. As the buffer content

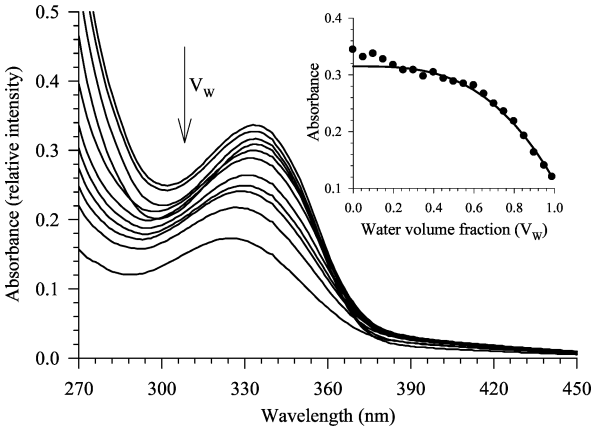

Figure 7. Selected absorption spectra of AA in dioxane/buffer (pH 3.5) binary mixtures. The inset shows the absorbance change of the peak at 330 nm as a function of water volume fraction in the binary mixtures (V_W). The solid line represents the best nonlinear regression fit to eq 1.

increases in the mixture, there is a decrease in absorbance and a slight blue shift.

The number of water molecules solvating the polar sites in different molecular systems can be estimated by fitting the spectral intensity change in the binary mixtures to a binding isotherm that takes the form[34−37,41]

$$A_{obs} = \frac{A_B + K_{eq} A_{BS_n} [S]^n}{1 + K_{eq}[S]^n} \quad (1)$$

where A_{obs} is the observed absorbance, A_B is the absorbance of unsolvated AA, A_{BS_n} is the absorbance of solvated AA by water, K_{eq} is the equilibrium binding constant of the complex formation between AA and water molecules, [S] is the water concentration in the mixture, and n is the number of water molecules solvating the polar sites in AA. Equation 1 represents a binding isotherm between species in equilibrium. The measured change in the absorbance of AA as a function of water volume fraction (V_W) is displayed in the inset in Figure 7 (assuming the density of water = 1.0 g mL^{-1}, V_W = [S] × molecular weight of water). The best fit to eq 1 is also shown in the figure. The calculations from the best fit show that three water molecules locally solvate the polar groups in the AA molecule (n = 3). This number reflects the water molecules in direct contact with the polar sites of AA (i.e., water molecules in the first solvation shell around the AA molecule). The estimated value for K_{eq} from the fit is $(1.8 \pm 0.4) \times 10^{-6}$ M^{-3}. It should be noted here that K_{eq} represents an equilibrium constant for the binding of three water molecules to AA.

Structures of the AA:H_2O Complexes. Calculating the AA:H_2O structures depends on which rotamer of AA is considered. As mentioned in the Introduction, previous work has identified rotamer I (shown in Figure 1) to be the most stable one in the gas phase.[7] We then started with this rotamer and added water molecules in a stepwise manner in the vicinity of the polar sites of the AA molecule. The structures of AA with one, two and three water molecules were calculated in the ground state using the DFT-B3LYP method with a 6-311++G(2d,p) basis set. As observed by us and reported by others,[9,10,42] the most stable complexes are obtained when the water molecules are located close to the carboxylic group and not the amino group of AA. A similar preference for the carboxylic pocket has been reported in salicylic acids which are structurally similar to AA.[43] Our structures were fully optimized without any symmetry constraint. The structure of the most stable form of AA:$(H_2O)_3$ calculated with the aforementioned basis set is shown in Figure 8.

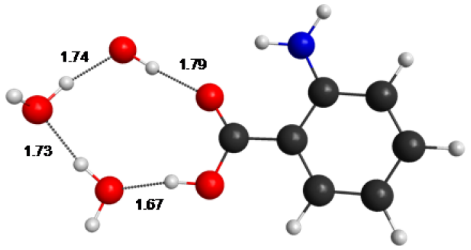

Figure 8. Structure of the most stable ground-state minimum configuration of the AA:$(H_2O)_3$ complex. The structure was obtained from DFT calculations described in the text. The lengths of the hydrogen bonds are shown (in Å).

We started by adding one water molecule in the polar region of rotamer I and optimizing the total structure. The most stable geometry shows the formation of a six-membered ring between H_2O and the carboxylic group of AA. The calculations of the most stable structure of AA:$(H_2O)_2$ show the formation of a closed cyclic structure that involves a hydrogen-bonding network. The obtained structures for the 1:1 and 1:2 complexes are similar to those previously reported by DFT calculations,[9,10,42] and reflect the experimentally observed 1:1 and 1:2 complexes in the gas phase using resonant two/multiphoton ionization spectroscopy.[9,10]

We continued the microsolvation process by adding a third water molecule to the optimized AA:$(H_2O)_2$ structure, followed by a full optimization. The results show that the most stable structure is obtained when the three water molecules form a solvation wire around the COOH group of AA. This structure (shown in Figure 8) was found to be 4 kcal/mol more stable than the structure that involves the third water molecule residing close to the NH_2 group and forming a weak hydrogen bond with one of the amino hydrogen atoms (similar to that obtained by Pachêco and Chaudhuri and shown in their Figure 2).[42] Our results for the AA:$(H_2O)_3$ complex (where only the COOH group of the molecule is solvated) are supported by the infrared results of the NH stretch fundamentals.[10] It was observed that the NH stretch vibrations of the AA:H_2O complex in the ground and excited states were quite similar to those of the bare molecule, indicating that water has little effect on the hydrogen atom dislocation, and hence on the NH_2 group. The three water molecules estimated from the spectral change in the binary mixture experiment can then be explained as a consequence of water solvation of the carboxylic group of AA. We found that when the effect of bulk water, via the PCM, is included in the optimization of the two AA:$(H_2O)_3$ structures described above, the energy difference between them falls to 2 kcal/mol, consistent with a picture in which the positions of individual water molecules are less fixed in solution than in isolated AA:$(H_2O)_n$ clusters.

We calculated the Franck−Condon $S_1 \leftarrow S_0$ transitions for AA in the gas phase, and using a PCM with water as the solvent. The results are displayed in Figure 4 as vertical lines.

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The results show that, for the neutral AA molecule, a slight red shift is observed when moving the molecule from gas phase to aqueous environment. Compared with the absorption spectra, the relative spectral locations for the neutral, anion and cation species follow the same trend.

CONCLUSIONS

In this work, the spectroscopy of AA was examined in neat and binary solvents of varying polarity and hydrogen bonding strength in order to understand the role of water in solvating the polar sites of the molecule. Analysis of the absorption and fluorescence spectra reveals that AA exists as an anion in neutral water. The Stokes shift of AA in different solvents was found to be linearly correlated with the empirical parameter of solvent polarity (E_T^N), indicating the major role of the hydrogen bonding effect in solution. The shortest fluorescence lifetime was measured in cyclohexane and is attributed to intramolecular hydrogen dislocation/transfer in the excited state. The lifetime values in polar solvents point to the dominant effect of the hydrogen-bond donating strength (α value) of the solvent. The pK_a and pK_a* values were estimated to be 4.50 and 4.44, respectively from the spectral shift in the absorption and fluorescence spectra measured in different pH solutions.

The number of water molecules solvating the polar region of the neutral form of AA was estimated to be three from the absorbance change in dioxane/buffer (pH 3.5) binary mixtures. The structures of AA:water complexes were calculated using DFT. A stepwise addition of water to the polar sites of AA leads to solvation of the COOH group of the molecule. The most stable structure for the AA:$(H_2O)_3$ complex was found to involve a water wire that solvates the COOH group in a cyclic geometry.

The spectral shift as a function of pH suggests that AA may be used as a probe to estimate the local acidity of binding sites in macromolecules in the pH range 3.0−7.0.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Niyomrattanakit, P.; Yahorava, S.; Mutule, I.; Mutulis, F.; Petrovska, R.; Prusis, P.; Katzenmeier, G.; Wikberg, J. E. S. Probing the Substrate Specificity of the Dengue Virus T 2 NS3 Serine Protease by using Internally Quenched Fluorescent Peptides. *Biochem. J.* **2006**, *397*, 203−211.

(2) Turchiello, R. F.; Lamy-Freund, M. T.; Hirata, I. Y.; Juliano, L.; Ito, A. S. *Ortho*-Aminobenzoic Acid-Labeled Bradykinins in Interaction with Lipid Vesicles: Fluorescence Study. *Biopolymers* **2002**, *65*, 336−346.

(3) Turchiello, R. F.; Lamy-Freund, M. T.; Hirata, I. Y.; Juliano, L.; Ito, A. S. *Ortho*-Aminobenzoic Acid as a Fluorescent Probe for the Interaction between Peptides and Micelles. *Biophys. Chem.* **1998**, *73*, 217−225.

(4) Ito, A. S.; Turchiello, R. D. F.; Hirata, I. Y.; Cezari, M. H. S.; Meldal, M.; Juliano, L. Fluorescent Properties of Amino Acids Labeled with *ortho*-Aminobenzoic Acid. *Biospectroscopy* **1998**, *4*, 395−402.

(5) Moroder, L.; Lutz, J.; Grams, F.; Böhner, S. R.; Ösapay, G.; Goodman, M.; Kolbeck, W. A New Efficient Method for the Synthesis of 1,4-Benzodiazepine-2,5-Dione Diversomers. *Biopolymers* **1996**, *38*, 295−300.

(6) Hirata, I. Y.; Sedenho, M. H. C.; Boschcov, P.; Garratt, R. C.; Oliva, G.; Ito, A. S.; Spisni, A.; Franzoni, L.; Juliano, L. Stereospecific Synthesis of L-2-amino-3-(7-methoxy-4-coumaryl)Propionic Acid, an Alternative to Tryptophan in Quenched Fluorescent Substrates for Peptidases. *Lett. Peptide Sci.* **1998**, *5*, 1−4.

(7) Southern, C. A.; Levy, D. H.; Florio, G. M.; Longarte, A.; Zwier, T. S. Electronic and Infrared Spectroscopy of Anthranilic Acid in a Supersonic Jet. *J. Phys. Chem. A* **2003**, *107*, 4032−4040.

(8) Southern, C. A.; Levy, D. H.; Stearns, J. A.; Florio, G. M.; Longarte, A.; Zwier, T. S. Spectroscopic Consequences of Localized Electronic Excitation in Anthranilic Acid Dimer. *J. Phys. Chem. A* **2004**, *108*, 4599−4609.

(9) He, Y.; Wu, C.; Kong, W. Theoretical and Experimental Studies of Water Complexes of *p*- and *o*-Aminobenzoic Acid. *J. Phys. Chem. A* **2005**, *109*, 2809−2815.

(10) Stearns, J. A.; Das, A.; Zwier, T. S. Hydrogen Atom Dislocation in the Excited State of Anthranilic Acid: Probing the Carbonyl Stretch Fundamental and the Effects of Water Complexation. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2605−2610.

(11) Perrin, D. D.; Dempsey, B. *Buffers for pH and Metal Ion Control*; Chapman and Hall: London, 1974.

(12) James, D. R.; Siemiarczuk, A.; Ware, W. R. Stroboscopic Optical Boxcar Technique for the Determination of Fluorescence Lifetimes. *Rev. Sci. Instrum.* **1992**, *63*, 1710−1716.

(13) Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S. J.; Windus, T. L.; Dupuis, M.; Montgomery, J. A. General Atomic and Molecular Electronic Structure System. *J. Comput. Chem.* **1993**, *14*, 1347−63.

(14) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A. Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; et al. *GAUSSIAN-03, Rev. B.01*; Gaussian Inc.: Pittsburgh, PA, 2003.

(15) Sobolewski, A. L.; Domcke, W. Ab Initio Study of Excited-State Intramolecular Proton Dislocation in Salicylic Acid. *Chem. Phys.* **1998**, *232*, 257−265.

(16) Leśniewski, S.; Kolek, P.; Pirowska, K.; Sobolewski, A. L.; Najbar, J. Franck−Condon Analysis of Laser-Induced Fluorescence Excitation Spectrum of Anthranilic Acid: Evaluation of Geometry Change upon $S_0 \rightarrow S_1$ Excitation. *J. Chem. Phys.* **2009**, *130* (054307), 1−14.

(17) From idealized theories, the solvent dielectric constant (i.e., the relative permittivity, ε) is often predicted to serve as a quantitative measure of solvent polarity. However, this approach is often inadequate since these theories regard solvents as a nonstructured isotropic continuum, not composed of individual solvent molecules with their own solvent/solvent interactions. They also do not take into account specific solute/solvent interactions such as hydrogen-bonding, which often play a dominant role in solute/solvent interactions. See ref 18 for more details.

(18) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 3rd ed.; VCH: Weinheim, Germany, 2003.

(19) Suppan, P. Solvatochromic shifts. The Influence of the Medium on the Energy of Electronic States. *J. Photochem. Photobiol., A* **1990**, *50*, 293−330.

(20) Suppan, P. Thermochromic Shifts of the Fluorescence Spectra of 4-N,N-Dimethylaminobenzonitrile in Solution. *J. Lumin.* **1985**, *33*, 29−32.

(21) Reichardt, C. Solvatochromic Dyes as Solvent Polarity Indicators. *Chem. Rev.* **1994**, *94*, 2319−2358.

(22) Takara, M.; Ito, A. S. General and Specific Solvent Effects in Optical Spectra of *ortho*-Aminobenzoic Acid. *J. Fluoresc.* **2005**, *15*, 171−177.
(23) Stalin, T.; Rajendiran, N. Intramolecular Charge Transfer Associated with Hydrogen Bonding Effects on 2-Aminobenzoic Acid. *J. Photochem. Photobiol., A* **2006**, *182*, 137−150.
(24) Pozdnyakov, I. P.; Plyusnin, V. F.; Grivin, V. P. Photophysics and Photochemistry of 2-Aminobenzoic Acid Anion in Aqueous Solution. *J. Phys. Chem. A* **2009**, *113*, 14109−14114.
(25) Sillen, L. G.; Martell, A. E. *Stability Constants of Metal-Ion Complexes*; The Society: London, 1964.
(26) Abou-Zied, O. K. Revealing the Ionization Ability of Binding Site I of Human Serum Albumin Using 2-(2′-Hydroxyphenyl)-Benzoxazole as a pH Sensitive Probe. *Phys. Chem. Chem. Phys.* **2012**, *14*, 2832−2839.
(27) Abou-Zied, O. K. Spectroscopy of Hydroxyphenyl Benzazoles in Solution and Human Serum Albumin: Detecting Flexibility, Specificity and High Affinity of the Warfarin Drug Binding Site. *RSC Adv.* **2013**, *3*, 8747−8755.
(28) Marquezin, C. A.; Hirata, I. Y.; Juliano, L.; Ito, A. S. Spectroscopic Characterization of 2-Amino-N-hexadecyl-benzamide (AHBA), A New Fluorescence Probe for Membranes. *Biophys. Chem.* **2006**, *124*, 125−133.
(29) Markova, N.; Enchev, V.; Timtcheva, I. Oxo-Hydroxy Tautomerism of 5-Fluorouracil: Water-Assisted Proton Transfer. *J. Phys. Chem. A* **2005**, *109*, 1981−1988.
(30) Kyrychenko, A.; Waluk, J. Excited-State Proton Transfer through Water Bridges and Structure of Hydrogen-Bonded Complexes in 1H-Pyrrolo[3,2-h]quinoline: Adiabatic Time-Dependent Density Functional Theory Study. *J. Phys. Chem. A* **2006**, *110*, 11958−11967.
(31) Carballeira, L.; Perez-Juste, I. Influence of Water in the Tautomerism of 2,2′-Bipyridine-3,3′-Diol. *J. Mol. Struc. (THEOCHEM)* **1996**, *368*, 17−25.
(32) Tian, Y. -C.; Fang, W. -H. Solvent Effects on the Photodissociation of Formic Acid: A Theoretical Study. *J. Phys. Chem. A* **2006**, *110*, 11704−11710.
(33) Liao, R. -Z.; Ding, W. -J.; Yu, J. -G.; Fang, W. -H.; Liu, R. -Z. Water-Assisted Transamination of Glycine and Formaldehyde. *J. Phys. Chem. A.* **2007**, *111*, 3184−3190.
(34) Abou-Zied, O. K. Examining [2,2′-bipyridyl]-3,3′-Diol as a Possible DNA Model Base Pair. *J. Photochem. Photobiol., A* **2006**, *182*, 192−201.
(35) Abou-Zied, O. K. The Role of Water in Solvating the Hydrogen-Bonding Center of 2-(2′-Hydroxyphenyl)Benzoxazole. *Chem. Phys.* **2007**, *337*, 1−10.
(36) Abou-Zied, O. K.; Al-Shihi, O. I. K. Caging and Solvent Effects on the Tautomeric Equilibrium of 3-Pyridone/3-Hydroxypyridine in the Ground State: A Study in Cyclodextrins and Binary Solvents. *Phys. Chem. Chem. Phys.* **2009**, *11*, 5377−5383.
(37) Abou-Zied, O. K. Effect of NH_2 Rotation on the Fluorescence of 2-Aminopurine in solution. *J. Photochem. Photobiol., A* **2013**, *261*, 1−6.
(38) Melo, E. C. C.; Costa, S. M.; Maçanita, A. L.; Santos, H. The use of the n-(9-Anthroyloxy)Stearic Acids to Probe the Water Content of Sodium Dodecyl Sulfate, Dodecyltrimethylammonium Chloride, and Triton X-100 Micelles. *J. Colloid Interface Sci.* **1991**, *141*, 439−453.
(39) Belletête, M.; Lachapelle, M.; Durocher, G. Polarity of AOT Micellar Interfaces: Use of the Preferential Solvation Concepts in the Evaluation of the Effective Dielectric Constants. *J. Phys. Chem.* **1990**, *94*, 5337−5341.
(40) Belletête, M.; Lessard, G.; Durocher, G. Electronic Spectroscopy of Aromatic Schiff bases. X. Specific Interactions between 2-(p-dimethylaminophenyl)-3,3-dimethyl-3H-indole and Water in *p*-Dioxane-Water Mixtures. Polarity Surrounding the Fluorescence Probe. *J. Lumin.* **1989**, *42*, 337−347.
(41) Connors, K. A. *Binding Constants. The Measurements of Molecular Complex Stability*; Wiley: New York, 1987.
(42) Pachêco, V. B.; Chaudhuri, P. Effects of Microhydration on the Electronic Properties of *ortho*-Aminobenzoic Acid. *J. Phys. Chem. A* **2013**, *117*, 5675−5684.
(43) El-Hakam, E. A.; El-Nasr, A.; Fujii, A.; Yahagi, T.; Ebata, T.; Mikami, N. Laser Spectroscopic Investigation of Salicylic Acids Hydrogen Bonded with Water in Supersonic Jets: Microsolvation Effects for Excited State Proton Dislocation. *J. Phys. Chem. A* **2005**, *109*, 2498−2504.