Solvent Effects on the Conformational Preferences of Serotonin: Serotonin−$(H_2O)_n$, $n = 1,2$

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Neutral serotonin−$(H_2O)_n$ clusters with $n = 1,2$ have been studied under jet-cooled conditions using a combination of resonant two-photon ionization (R2PI), UV−UV hole-burning (UVHB), and resonant ion-dip infrared (RIDIR) spectroscopy. Serotonin (5-hydroxytryptamine, SERO) is a close analogue of tryptamine, differing by the addition of an OH substituent in the 5-position on the indole ring, but sharing the same ethylamine side chain in the 3-position. Three conformational isomers of SERO−$(H_2O)_1$ were observed via UVHB, with S_0-S_1 origins at 32 671 (A), 32 454 (B), and 32 188 cm^{-1} (C). RIDIR spectroscopy provided infrared spectra in the hydride stretch region that reflected the hydrogen-bonding arrangement of each conformer. Two of the three SERO−$(H_2O)_1$ conformers have RIDIR spectra nearly identical to that of the only observed conformer of tryptamine−$(H_2O)_1$, differing only in the orientation of the 5-OH group (syn vs anti). In this structure, the H_2O molecule acts as H-bond donor to the NH_2 group on the ethylamine side chain, which is configured in the Gpy(out) conformation that is the global minimum in the absence of water. Comparison of the OH stretch RIDIR spectrum of the third SERO−$(H_2O)_1$ conformer with calculation leads to its assignment to a structure in which the water molecule forms a H-bonded bridge between the amino group and the 5-OH group of SERO, with the ethylamine side chain in the Gph(out) conformation that facilitates bridge formation, corresponding to the second most populated conformer in the isolated SERO monomer. The OH and CH stretch infrared absorptions for the single observed conformer of SERO−$(H_2O)_2$ indicate that it is also a bridge structure linking the NH_2 and OH groups of SERO, retaining the same Gph(out) ethylamine conformation as in conformer C of SERO−$(H_2O)_1$. The ultraviolet and infrared spectroscopy reflect the fact that the single-water bridge cannot optimally span the gap between the 5-OH and NH_2 groups, while the water dimer bridge forms a set of three strong H-bonds that lock in the Gph(out) ethylamine and anti 5-OH orientations in a near-optimal configuration.

I. Introduction

Serotonin (5-hydroxytryptamine) is a neurotransmitter that influences many different processes within the human body.[1−5] This is accomplished by interacting with more than 15 different receptors to carry out its diverse biological functions.[1,5] The ability of serotonin to bind to numerous different receptor sites is due, at least in part, to the flexibility of the ethylamine side chain and the 5-OH groups, which can reconfigure to adapt to each receptor. We have recently carried out a series of studies aimed at mapping out in significant detail the multidimensional potential energy surface for conformational isomerization in serotonin monomer (SERO).[6,7] Single-conformation ultraviolet and infrared spectra of SERO cooled in a supersonic jet revealed transitions assignable to the eight conformational isomers shown in Figure 1, five of which have an *anti*-OH and three with a *syn*-OH conformation for the 5-OH group.[6] Firm assignments for these eight conformations could be made on the basis of a comparison with the spectral signatures of tryptamine (TRA),[8] which possesses the same ethylamine side chain as SERO, but lacks the 5-OH group on the ring. Six of the SERO conformers are syn/anti OH pairs (Gpy(out) for A/A′, Gpy(up) for B/B′, and Gph(out) for C/C′). Not surprisingly, the most intense transition in SERO (conformer A) has a Gpy(out) ethylamine side chain conformation, the same conformation that is the global minimum in TRA. In this structure, the amino group is in the gauche position on the pyrrole side of indole, with the NH_2 lone pair oriented out away from the ring.

Because serotonin carries out its biological functions in aqueous solution, it is important to understand how the conformational preferences of serotonin change in the presence of water molecules. Many of the receptor sites for serotonin are nonpolar environments in which the NH_2 group may be neutral rather than protonated, as it would be in aqueous solution.[9−13] Despite the nonpolar environment, water molecules are likely to also be present in these binding pockets and may play a role in the way in which SERO interacts with the receptor. It is therefore of some interest to form and study SERO−$(H_2O)_n$ clusters using the same conformation-specific methods already employed on the monomer.[6] Our goal is to use these methods to probe the preferred binding sites for these water molecules, and determine how this binding affects the preferred conformations of serotonin itself.

There are reasons to anticipate the possibility that these water molecules could have a significant effect. First, previous studies of serotonin's close structural analogue, tryptamine, showed that complexation of a single water molecule to TRA collapses its seven observed monomer conformations to a single structure in which the water molecule is H-bonded to the amino group of the ethylamine side chain in the Gpy(out) orientation.[8,14−19]

Second, serotonin has both H-bond donor group (the 5-OH) and H-bond acceptor (NH_2) groups, spatially separated from one another. Under such circumstances in proteins,[20,21] water molecules often form H-bonded bridges that link these groups. It will be interesting to see whether such bridges are also formed

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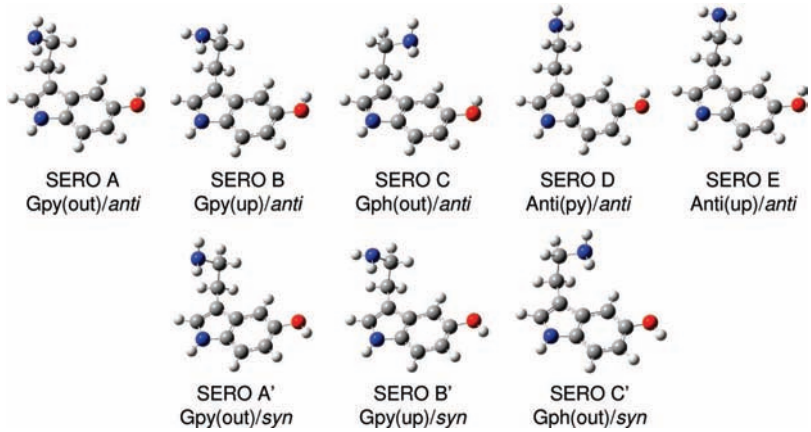

Figure 1. The eight conformations of serotonin monomer observed in the jet expansion (ref 6).

in SERO$-(H_2O)_n$ clusters, and what number of water molecules are most profitably involved. In fact, in tryptamine, it was found that two and three water molecules form a water bridge linking the amino group of the ethylamine side chain and the NH group of the indole ring.[19,22]

In this study, we report single-conformation infrared and ultraviolet spectra for SERO$-(H_2O)_n$, $n = 1,2$. These spectra provide evidence for three conformational isomers of SERO$-(H_2O)_1$ and one for SERO$-(H_2O)_2$. In SERO$-(H_2O)_1$, two types of bridges are formed, one that links the NH_2 group and the pyrrole CH, and the other that links the NH_2 and 5-OH groups. The bridge formed in the latter structure is "loose" in that a single H_2O molecule cannot fully bridge the gap between the NH_2 and 5-OH groups. In SERO$-(H_2O)_2$, the only structure observed is a water dimer bridge linking these same two groups, locking in an ethylamine conformation that was only one of many present in the absence of the water molecules.

II. Methods

A. Experimental Methods. The experimental methods used in the current study have been explained in detail elsewhere.[19] Serotonin was purchased commercially (Wako) and used without further purification. The jet-cooled supersonic expansion of the serotonin−water clusters was formed by flowing a 70% neon/30% helium/2.5% water gas mixture at a pressure of 1.0−1.5 bar over the sample, heated to 170 °C. The sample is picked up by the carrier gas and pulsed into the vacuum chamber at 20 Hz using a high-temperature pulsed valve (General Valve, Series 9, 0.4 mm diameter). The expansion is then skimmed and ionized by an ultraviolet laser in the ion source region of a linear time-of-flight mass spectrometer.

One-color resonant two-photon ionization (R2PI) spectra were acquired by using the doubled output of a Nd:YAG-pumped dye laser source. Sulforhodamine 640 and DCM laser dyes were used to acquire R2PI spectra in the wavelength region 303.5−315.5 nm.

R2PI spectra contain ion signals from all conformations present in the jet-cooled expansion. UV−UV hole-burning was used to determine the number of conformers that make up the R2PI spectra and their corresponding vibronic structure. UV−UV hole-burning fixes doubled output of one Nd:YAG-pumped dye laser, operated at 10 Hz, on a particular transition in the R2PI spectrum and scans a second UV source, spatially overlapped and pulsing at 20 Hz, over the R2PI spectral region, delayed by 200 ns from the hole-burn laser. Active baseline subtraction through a gated integrator is used to take the difference in ion signal from successive probe laser pulses, with and without the hole-burn laser present. Transitions that share the same ground-state level as the hole-burn laser appear as depletions in the UV−UV hole-burn spectra.

Infrared spectra of the individual conformers were taken using resonant ion-dip infrared spectroscopy. The infrared light generated by a seeded Nd:YAG-pumped optical parametric converter (LaserVision) operating at 10 Hz is spatially overlapped with the 20 Hz UV laser and temporally precedes it by 200 ns. The 20 Hz UV laser has its wavelength fixed on a particular $S_1 \leftarrow S_0$ origin or vibronic transition, providing a constant ion signal from only that conformer. As the infrared laser is tuned through the infrared region of interest, depletions in ion signal occur when the infrared laser scans across an infrared transition due to that particular conformer. This difference in ion signal intensity induced by the infrared laser is monitored by active baseline subtraction using a gated integrator.

B. Computational Methods. A conformational search to find the conformational minima was carried out on both SERO$-(H_2O)_1$ and SERO$-(H_2O)_2$ clusters using the Amber force field in MACROMODEL.[23] The structures generated were then optimized using the Becke3LYP[24,25] and M0-52X[26] functionals with the 6-31+G* basis set[27] and ultrafine gradient on Gaussian 03[28] on a computer cluster.[29,30]

Binding energies were computed using the same sequence of calculations employed previously for melatonin−water clusters.[31] Because of the flexibility of the ethylamine side chain, the presence of a water molecule can result in a conformation of the monomer that is not a minimum on the monomer potential energy surface. As a result, we can compute different forms of the binding energy that emphasize different aspects of the binding. In the conventional calculation, the binding energies for the various SERO$-(H_2O)_1$ structures were calculated as

$$\mathrm{BE} = E_{\mathrm{cluster}} - E_{\mathrm{monomer}} - E_{\mathrm{water}} \quad (1)$$

where the energies of the cluster, monomer, and water are the fully optimized zero-point corrected energies from the DFT M05-2X/6-31+G* calculations. These results are given in the first column of numbers in Table 1, labeled BE (optimized monomer). In this case, the zero-point corrected binding energy used the zero-point energy of each optimized monomer structure. A second measure of the binding energies was computed using single-point energy calculations on the monomer structure after removing the water molecule (BE (SP monomer) in Table 1). In this case, the monomer energy employed a zero-point correction, that was the average of the zero-point vibrational energy correction of the serotonin monomers, 556.75 ± 0.51

TABLE 1: Calculated Binding Energies (kJ/mol) of SERO−$(H_2O)_n$, n = 1, 2[a]

structure	BE (optimized monomer)[b]	BE (single point monomer)[c]	difference (strain energy)[d]	BE relative to Gpy(out)/anti[e]
n=1, **I**	44.91	53.50	8.59	45.07 (0)
n=1, **II**	40.24	40.67	0.43	40.42 (4.83)
n=1, **III**	40.96	41.89	0.93	39.76 (5.31)
n=1, **IV**	41.24	43.78	2.55	36.65 (8.41)
n=1, **V**	36.81	38.49	1.68	32.76 (12.31)
n=1, **VI**	38.07	44.76	6.69	32.14 (12.93)
n=2, **I**	100.9	103.2	2.32	101.0

[a] All binding energies are zero-point corrected. [b] Binding energy relative to the optimized monomer minimum following removal of the H_2O molecule(s). [c] Binding energy relative to the monomer fixed at its geometry in the complex. [d] Difference between columns 1 and 2, equivalent to the monomer strain energy in the complex. [e] Binding energy relative to the Gpy(out)/*anti* structure of serotonin (conformer A). The numbers in parentheses are energies relative to structure **I**, reporting relative stabilities. These are the numbers displayed in Figure 2.

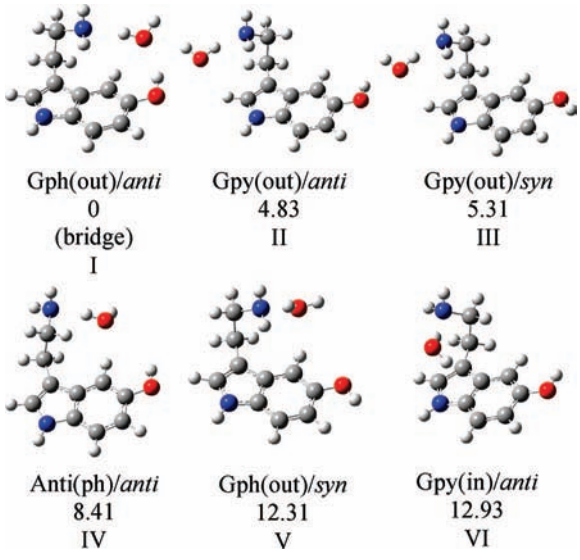

Figure 2. Six low-energy calculated structures of SERO−$(H_2O)_1$. The relative energies in kJ/mol (zero-point corrected) at the DFT M05-2X/6-31+G* level of theory are shown below the description of each structure.

kJ/mol. The difference between the binding energy calculated using the optimized monomer minimum and that of the single-point monomer is the strain energy imparted to the monomer by the presence of the water molecule (strain energy = BE (optimized monomer) − BE (SP monomer)). Finally, the binding energies were calculated using the lowest energy minimum of the monomer, Gpy(out)/anti or SERO(A),[6] as $E_{monomer}$ in eq 1. This measure emphasizes the relative energies of the various structures in the presence of the water molecule, labeled as BE(Gpy(out)/anti) in Table 1.

III. Results

A. Calculations. The six calculated lowest energy structures for SERO−$(H_2O)_1$ are shown in Figure 2 and are labeled **I**−**VI** in order of their relative energy at the DFT M05-2X/6-31+G* level of theory. Table 1 summarizes the binding energies calculated by each of the three methods just described in the previous section.

The lowest energy calculated structure (**I**) has SERO in the Gph(out)/anti conformation (conformer C of the monomer) with

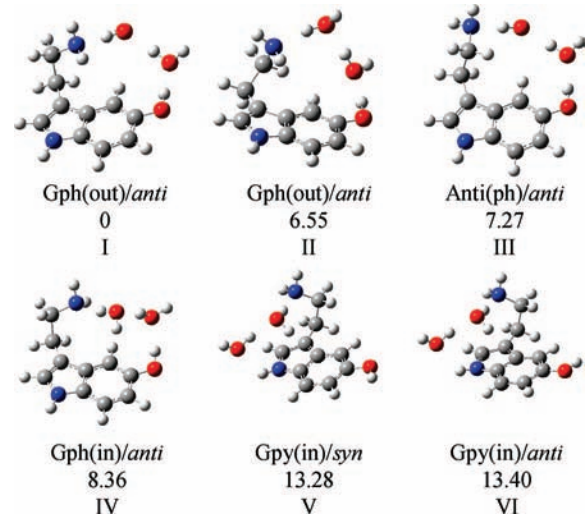

Figure 3. Optimized structures for six low-energy calculated structures of SERO−$(H_2O)_2$. The relative zero-point energies in kJ/mol at the DFT M05-2X/6-31+G* level of theory are shown below the description of each structure.

the water molecule forming a H-bonded bridge linking the lone pair of electrons of the amino group and the 5-hydroxy group on the indole ring, serving as donor to the former and acceptor to the latter. This is one of only two structures found that contain two traditional H-bonds, involving OH···N and OH···O linkages. On the other hand, the strain energy for this structure (Table 1) is relatively high (Table 1, 8.59 kJ/mol) due to the hydrogen atom of the 5-OH group angling slightly out-of-plane of the indole ring. This complex also has a stabilizing interaction between the water oxygen and the proximal CH on the indole ring (the 4-position). Interestingly, structure **V** has the same ethylamine side chain orientation (Gph(out)), with the 5-OH in the syn orientation. In this case, the water molecule is bound only to the amine group. This structure has a much smaller strain energy of 1.68 kJ/mol, which is only due to strain on the ethylamine side chain due to a ∼10° rotation about the C_β−C_α bond to allow the water molecule to interact with the proximal CH on the indole ring (the 4-position).

Structures **II** and **III** both possess a Gpy(out) ethylamine side chain conformation, differing in the 5-OH orientation (*syn* or *anti*), analogous to conformers A and A′ of SERO monomer. These structures are calculated to be 4.83 and 5.31 kJ/mol higher in energy than the lowest energy minimum. In both of these complexes, the water molecule is a hydrogen-bond donor to the lone pair of electrons on the amino group of the ethylamine side chain, but also gains small additional stabilization from a CH···O interaction with the proximal CH on the indole ring (the 2-position, indicated by a dotted line in Figure 2).

There are several other structures possible in which the ethylamine side chain takes up one of the other conformational minima of the SERO monomer, with the water molecule acting as H-bond donor to the nitrogen lone pair on the amino group. Of these, structure **IV** (anti(ph)/anti, 8.41 kJ/mol) is stabilized by a weak interaction with the proximal CH on the indole ring. Structure **VI**, with a relative energy of 12.93 kJ/mol, is of interest because this complex incorporates water as a double donor, with one OH in the usual configuration as donor to the amino group, and the other forming a π H-bond with the π cloud of indole.

The six lowest energy structures of the SERO−$(H_2O)_2$ clusters are shown in Figure 3. Four of the six lowest energy structures are bridge structures in which the two water molecules

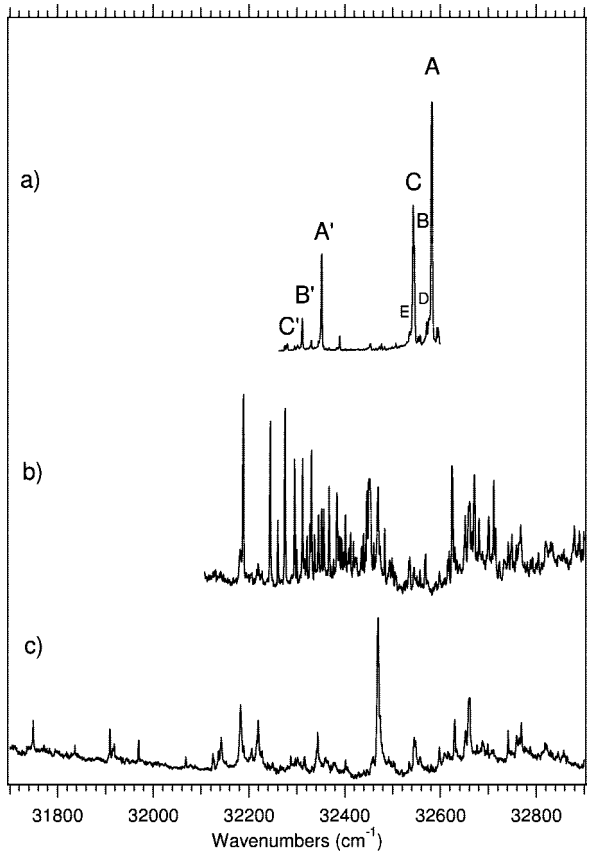

Figure 4. R2PI spectra in the S_0-S_1 origin region monitoring the (a) SERO monomer, (b) SERO$-(H_2O)_1$, and (c) SERO$-(H_2O)_2$ mass channels.

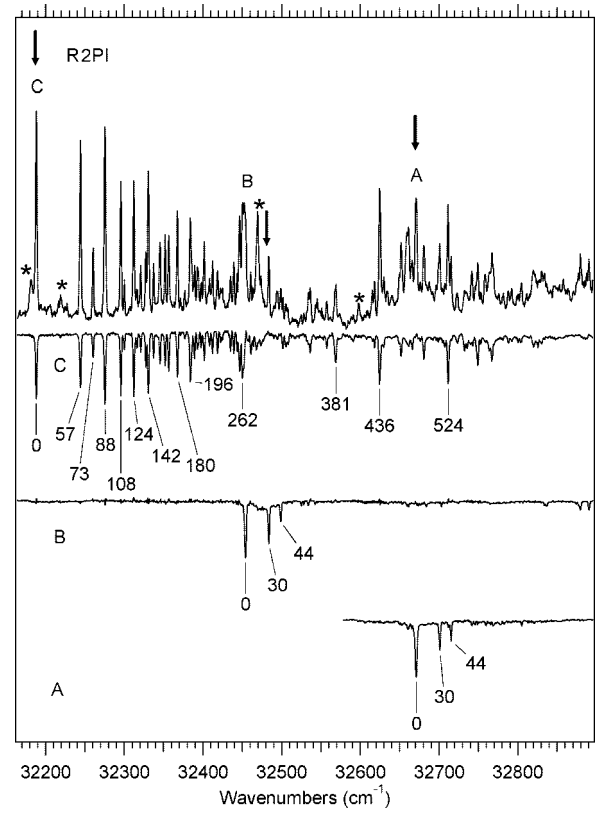

Figure 5. R2PI and UV−UV hole-burning spectra of the three conformations (C, B, A, respectively) of SERO$-(H_2O)_1$. Transitions used for UVHB are marked with arrows. Transitions in the R2PI spectrum due to SERO$-(H_2O)_2$ fragmenting into the SERO$-(H_2O)_1$ mass channel are shown by asterisks.

both act as donor and as acceptor in a water dimer bridge that links the NH_2 and OH groups of SERO. To incorporate this bridge, the OH group in these four structures is in the anti orientation that points the OH toward the ethylamine side chain. Both of the two lowest energy structures (structures **I** and **II**) incorporate the same Gph(out)/anti SERO structure that was the global minimum in SERO$-(H_2O)_1$. This orientation points the NH_2 group optimally toward the 5-OH. Structures **I** and **II** differ in the positions of the two free OH groups in the water dimer not involved in H-bonding, which in turn reconfigures the entire bridge structure to some extent. Structure **IV** is also a Gph structure with different amino group orientations, suggesting that a water dimer bridge can easily span the distance between NH_2 and OH groups. Structure **III** has the ethylamine side chain in the Anti(ph) orientation that also points the NH_2 group toward the 5-OH, but holds the water bridge further from the ring.

Two structures (**V** and **VI**) have the water dimer bridging the amino group of the ethylamine side chain and the indole NH, which was also observed experimentally for TRA$-(H_2O)_2$.[22] The structures form a syn/anti 5-OH pair, which in this case slightly favors the syn structure over anti by 0.12 kJ/mol, the opposite of that of other syn/anti pairs. These structures are both over 13 kJ/mol less stable than the global minimum, reflecting the better orientation and stronger H-bond that can be formed by the 5-OH than the indole NH.

B. Experimental. ***1. R2PI and UV−UV Hole-Burning Spectra.*** R2PI spectra monitoring the SERO, SERO$-(H_2O)_1$, and SERO$-(H_2O)_2$ mass channels are shown in Figure 4. The S_0-S_1 origins of the eight conformers of SERO monomer are labeled in Figure 4a.[6] The effect of the addition of the water molecule(s) is seen in the red-shifts of the UV spectra in Figure 4b,c.

The UV−UV hole-burning spectra for SERO$-(H_2O)_1$ shown in Figure 5 reveal that the R2PI spectrum is composed of contributions from three separate conformers with origins at 32 666 (A), 32 449 (B), and 32 183 cm^{-1} (C). The spectra of conformers A and B (Figure 5) have nearly identical vibronic structure. Furthermore, the shift between their two S_0-S_1 origins is 217 cm^{-1}, similar to the separation between *syn*- and *anti*-OH monomer pairs sharing the same ethylamine side chain conformation (∼230 cm^{-1}).[6] This suggests that A and B are a syn/anti 5-OH conformer pair. The S_0-S_1 origin of conformer C is 263 cm^{-1} further to the red than the other two conformers. This large red-shift is consistent with a structure in which the 5-OH group acts as H-bond donor to a water molecule, as has previously been seen in the cases of naphthol−water and phenol−water complexes.[32−49] Furthermore, unlike conformers A and B, the UVHB spectrum of conformer C (Figure 5) has extensive low-frequency vibronic structure. Most of this structure can be assigned to Franck−Condon activity in vibrations of frequency 57, 73, and 88 cm^{-1}. Vibrations in this frequency range are likely either intermolecular vibrations, or the low-frequency torsions of the ethylamine side chain, or both. Both the large red-shift and extensive vibronic structure are consistent with a bridge structure for conformer C; however, a more firm assignment must await the results of the RIDIR spectra.

The R2PI and UV−UV hole-burning spectra of SERO$-(H_2O)_2$ are shown in Figure 6a and b, respectively. When the lowest frequency transition is used as hole-burn transition (marked by an arrow in Figure 6a), all of the sharp

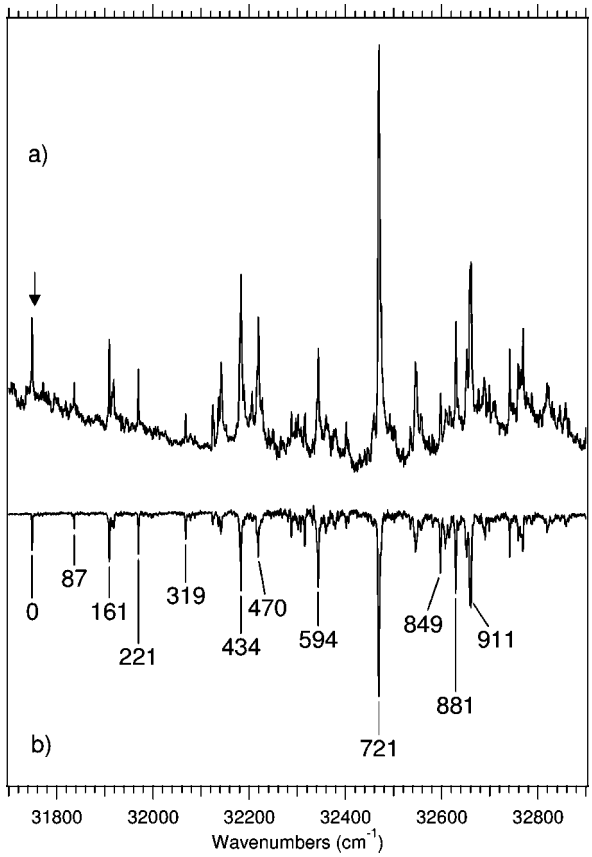

Figure 6. (a) R2PI and (b) UV−UV hole-burning spectra of SERO−$(H_2O)_2$ with relative frequencies labeled. The transition used for UVHB is marked with an arrow.

vibronic structure present in the SERO−$(H_2O)_2^+$ mass channel appears in the UVHB difference spectrum, indicating that all of these transitions can be ascribed to a single conformer. Therefore, the addition of a second water molecule to SERO reduces the number of conformations from three in SERO−$(H_2O)_1$ to one in SERO−$(H_2O)_2$. The broad background in the R2PI spectrum is due to higher order clusters fragmenting into the SERO−$(H_2O)_2^+$ mass channel, as confirmed by their absence in the UVHB spectrum.

The S_0-S_1 origin of SERO−$(H_2O)_2$ is red-shifted by 440 cm^{-1} relative to the red-most SERO−$(H_2O)_1$ origin (conformer C), indicating that the 5-OH group is involved as a strengthened H-bond donor.

The vibronic activity in this SERO−$(H_2O)_2$ cluster is unusual in two respects. First, the S_0-S_1 origin is small relative to many of the vibronic bands in the first 1000 cm^{-1} of the spectrum. In contrast, in SERO monomer and in SERO−$(H_2O)_1$ conformers A and B, the S_1 origin dominates the spectrum. In particular, in Figure 6b, the transition 721 cm^{-1} above the origin is several times the size of the 0_0^0 transition. Much of this size difference is due to a drop-off in laser power in the origin region. Nevertheless, after correction for laser power, the origin is still a factor of 2 smaller than the transition at 721 cm^{-1}, indicating a larger geometry change upon electronic excitation in SERO−$(H_2O)_2$ in the indole-like vibrations. Second, in contrast to conformer C of SERO−$(H_2O)_1$, there is much less low-frequency vibronic activity in the spectrum. We will return to discuss these aspects of the UV spectroscopy of this cluster after we understand its structure better.

2. RIDIR Spectra. RIDIR spectra of the three conformers of SERO−$(H_2O)_1$ in the 2800−3800 cm^{-1} region are shown in Figure 7. This wavenumber range spans the OH, NH, and CH stretch fundamentals. These spectra reveal much about the H-bonding arrangement present in these conformers. First, the RIDIR spectra of conformers A and B are nearly identical to one another, apart from a small shift in the OH stretch fundamental of the 5-OH group (3665 in A and 3670 cm^{-1} in B), as shown in more detail in the expanded view of this region to the right of the main figure. The frequency in conformer A is identical to that in the anti conformers of SERO monomer, while that in conformer B is identical to the syn conformer, further confirming the notion that conformers A and B form a syn/anti pair, which is otherwise nearly identical. Since these transitions are not shifted from their value in the monomer, we surmise that the 5-OH group is not involved in H-bonding with water in conformers A and B. Second, in all three conformers, the indole NH stretch fundamental is unperturbed from its value in SERO monomer (3530 cm^{-1}), also eliminating its involvement in any of the H-bonding arrangements. The symmetric and antisymmetric stretch fundamentals of the NH_2 group are very weak and are not observed in this study. Third, in all three spectra, one of the OH stretch fundamentals of the water molecule appears as a single, sharp transition near 3720 cm^{-1}, consistent with its assignment to a free OH stretch fundamental of a water molecule that is involved in a single H-bond to another acceptor group. The intense, substructured absorptions in the 3300−3400 cm^{-1} region of all three conformers can be assigned to the H-bonded OH stretch fundamental of the water molecule. The large shift to lower frequency, increase in intensity, and broadening are all characteristic features of a H-bonded OH stretch.[19,50] Finally, the RIDIR spectra of all three conformations possess a weak transition near 3200 cm^{-1} ascribable to the first overtone of the OH bend of the water molecule.

The RIDIR spectrum of conformer C is unique in that the 5-OH stretch fundamental is shifted down in frequency from its free position by just over 100 cm^{-1} to 3551 cm^{-1}, indicating that the 5-OH group is involved as a hydrogen-bond donor to the water molecule in conformer C. When combined with the H-bonded OH stretch transition at ∼3400 cm^{-1}, one can immediately conclude that the water molecule forms a H-bonded bridge in conformer C involving the 5-OH group. The alkyl CH stretch region of conformer C is also noticably different from that of conformers A and B, indicating a different orientation of the ethylamine side chain, a point to which we will return shortly.

Figure 8 takes a further look at conformers A and B of SERO−$(H_2O)_1$ (b,c) by comparing their infrared spectra to that previously found for TRA−$(H_2O)_1$ (a).[14,19] The close similarity between these spectra is immediately apparent. In fact, all of the spectral features in the RIDIR spectra of A and B are essentially identical to those in the spectrum of TRA−$(H_2O)_1$, with the exception of the 5-OH stretch that is necessarily present in the former and lacking in the latter. Because the structure of the TRA−$(H_2O)_1$ complex has already been firmly assigned[14] to a structure in which the water molecule H-bonds to the NH_2 group of the ethylamine side chain in a Gpy(out) conformation, we surmise on this basis that conformers A and B of SERO−$(H_2O)_1$ share this same Gpy(out) structure. The lone difference is the orientation of the 5-OH group (anti in A and syn in B), which is not involved in H-bonding because the Gpy(out) ethylamine side chain conformation points the nitrogen lone pair in a direction opposite to the 5-OH group.

To this point, we have deduced that conformer C of SERO−$(H_2O)_1$ is a water bridge structure involving the 5-OH

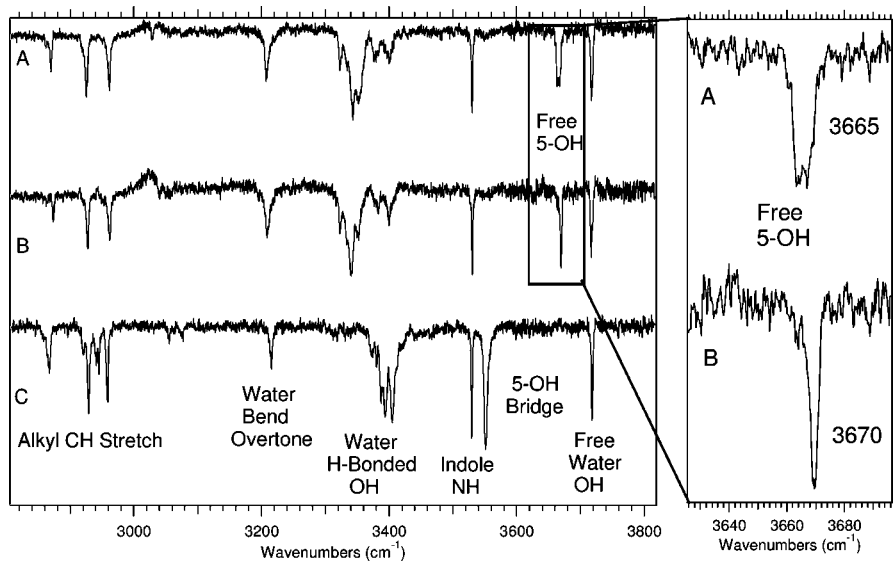

Figure 7. RIDIR spectra of the three conformations (A, B, C, respectively) of SERO−$(H_2O)_1$. The section of the upper two spectra enclosed in a rectangle is shown in expanded scale to the right to see the shift in the 5-OH stretch fundamental between the two scans.

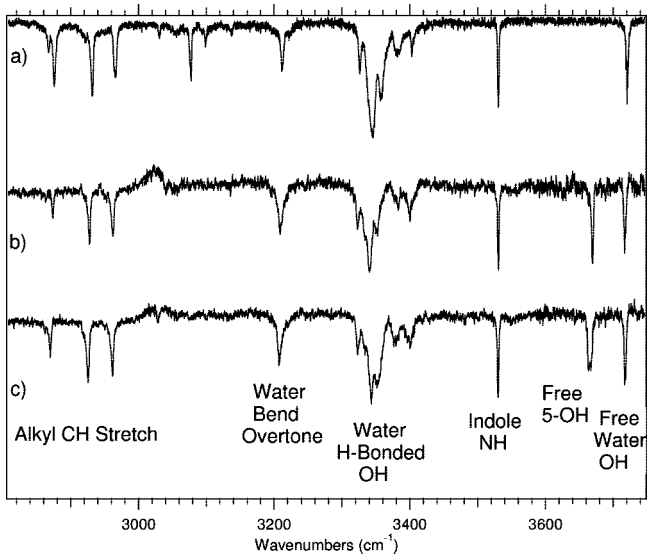

Figure 8. RIDIR spectra of (a) TRA−$(H_2O)_1$[14,19] and (b,c) conformers B and A of SERO−$(H_2O)_1$ in the 2750−3740 cm^{-1} region.

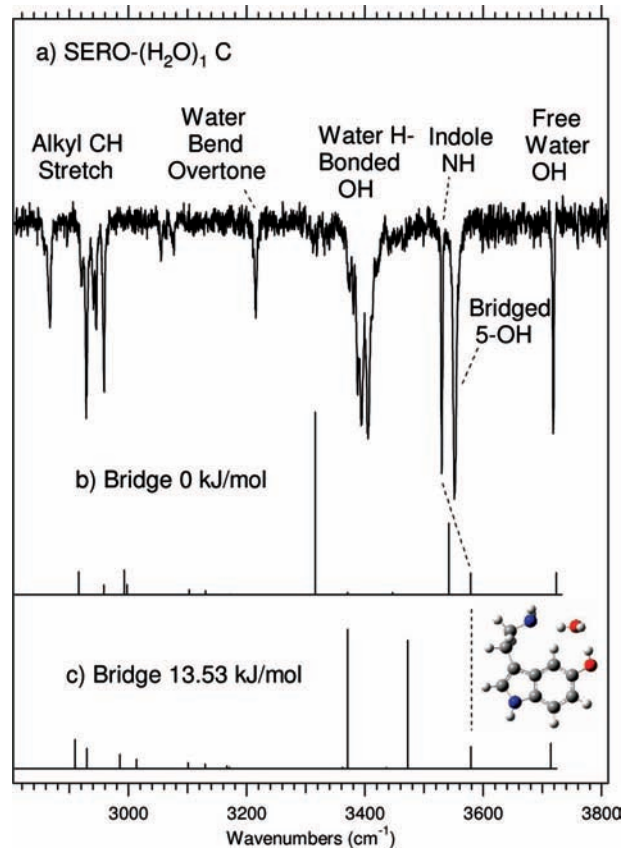

Figure 9. (a) RIDIR spectra of conformer C of SERO−$(H_2O)_1$. Calculated DFT B3LYP/6-31+G* IR frequencies of (b) structure **I** from Figure 2, and (c) the H-bonded structure shown. IR frequencies are scaled by 0.975. This scale factor brings the calculated free OH stretch transitions into agreement with experiment, but fails to correctly reproduce the wavenumber position of the indole NH stretch fundamental (3530 cm^{-1}), whose assignment is known from previous work on serotonin monomer and indole.

group as donor, but have not yet identified either the terminal acceptor site in the bridge or the ethylamine side chain conformation involved. To do so requires comparison with calculated vibrational frequencies and infrared intensities for possible bridge structures. Figure 9 shows the calculated frequencies (DFT B3LYP/6-31+G*) of the two bridged water complexes corresponding to structure **I** and a Gph(in)/anti bridged structure found by the force field conformational search (section IIIA). A single scale factor for the indole NH and OH stretch transitions cannot simultaneously reproduce both well. Because the assignment of the indole NH stretch fundamental is not in question (its frequency is unshifted from its position in serotonin monomer),[6,7] we choose a scale factor that brings the experimental and calculated OH stretch frequencies into agreement with one another (0.975). Taking this systematic error in the indole NH stretch into account, one can see that the spacing and relative intensity predicted for structure **I** better matches experiment. This structure is also calculated to be the global minimum (Figure 2). Furthermore, the pattern of transitions in the alkyl CH stretch region of Figure 9b is a better match with experiment than is Figure 9c. As a result, we assign conformer C of SERO−$(H_2O)_1$ to structure **I** in Figure 2, a Gph(out)/anti structure with the water molecule serving as a bridge between the amino group and the 5-OH.

The infrared spectrum of the single observed conformer of SERO−$(H_2O)_2$ is shown in Figure 10a. Three intense, broad

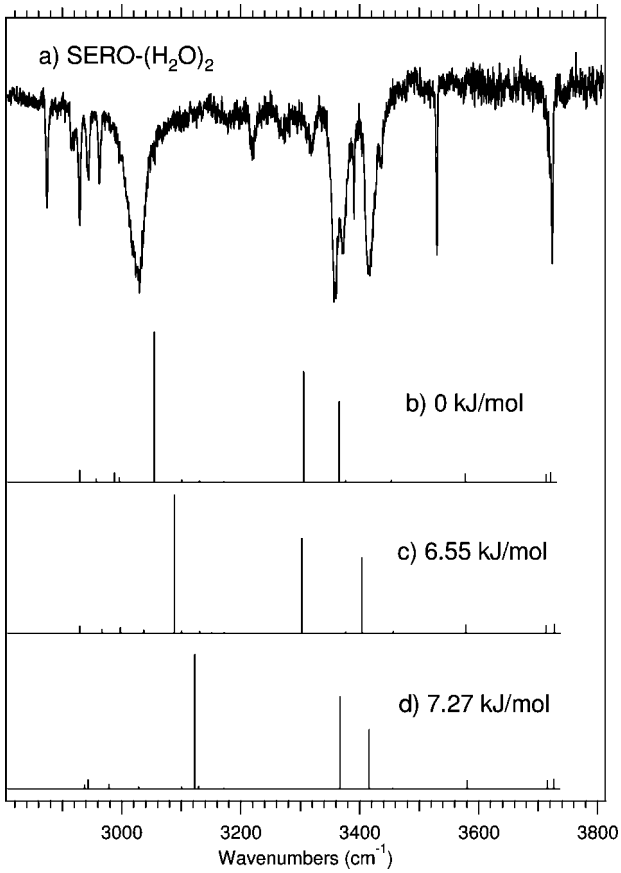

Figure 10. (a) RIDIR spectra of the single conformer of SERO−$(H_2O)_2$. (b−d) Calculated harmonic vibrational frequencies and infrared intensities for structures I−III from Figure 3, at the DFT B3LYP/6-31+G* level of theory. IR frequencies have been scaled by 0.975.

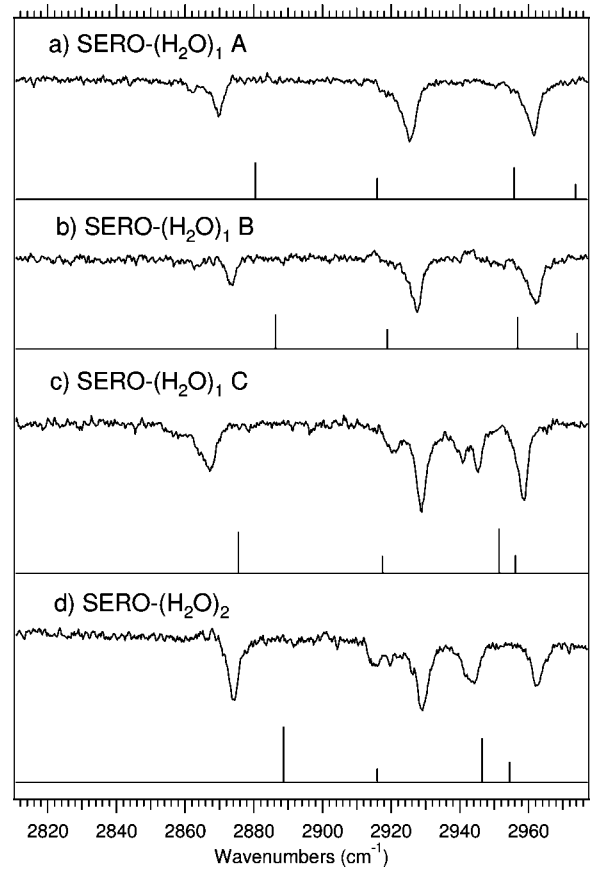

Figure 11. (a−d) RIDIR spectra of all observed conformers of SERO−$(H_2O)_n$ with the corresponding calculated IR frequencies shown under each, calculated at the DFT B3LYP/6-31+G* level of theory.

absorptions appear with frequency maxima at 3029, 3357, and 3417 cm^{-1}. These reflect the presence of three H-bonded OH stretch transitions. As anticipated already on the basis of the UV spectrum, the 5-OH stretch fundamental is shifted from its free value, indicating that it is responsible for one of the H-bonds as a donor group. There are two free water OH stretches (one from each of the water molecules) seen as an asymmetrically broadened peak from 3720–3724 cm^{-1}. Once again, the indole NH stretch appears at its unperturbed frequency (3530 cm^{-1}) and therefore is not involved in hydrogen bonding with either water molecule.

Figure 10b−d presents calculated vibrational frequencies and infrared intensities for the three lowest-energy conformers (structures **I**, **II**, and **III** in Figure 3) of SERO−$(H_2O)_2$, for comparison with experiment. The calculated infrared frequencies are again scaled based by 0.975. The three calculated spectra are all reasonably close to experiment, not surprising, given that all three structures are water dimer bridges that differ either in the positions of the free OH groups (structure **II**) or in the ethylamine side chain conformation (**III**).

Up to this point, we have used the RIDIR spectra in the alkyl CH stretch region so far only as a qualitative diagnostic in assigning the particular bridge structure responsible for conformer C of SERO−$(H_2O)_1$. However, this region is a particularly sensitive diagnostic of the ethylamine side chain conformation, which is composed of four CH stretch fundamentals associated with the C_α and C_β methylene groups. Figure 11a−c presents an expanded view of the 2810−2980 cm^{-1} region of SERO−$(H_2O)_1$ A−C and the calculated vibrational frequencies and infrared intensities for the structures to which they were assigned. For SERO−$(H_2O)_1$ A and B, the near-identical experimental spectra reflect the fact that these two structures share the same ethylamine side chain conformation, Gpy(out). The calculated pattern of transitions is in reasonable agreement with experiment, although not in quantitative detail.

What is most striking, however, is that the experimental spectra of SERO−$(H_2O)_1$ C (Figure 11c) and the only observed conformer of SERO−$(H_2O)_2$ (Figure 11d) are also nearly identical to one another in essentially every detail. This implies strongly that the ethylamine side chains present in the two clusters are the same. Because we have already assigned SERO−$(H_2O)_1$ C to the Gph(out)/anti bridge structure, we surmise that SERO−$(H_2O)_2$ is also the Gph(out)/anti water dimer bridge structure **I**, the calculated global minimum. In these cases, the calculated spectra are also nearly identical to one another and reproduce the experimental patterns reasonably well, although not in quantitative detail.

IV. Discussion

A. Preferred Binding Sites for Water and Their Influence on the Conformational Preferences of Serotonin. While in the serotonin monomer eight different conformations with five different ethylamine side chain orientations were found,[6] the addition of a water molecule locks in just two ethylamine side chain orientations, Gpy(out) and Gph(out). In tryptamine−water clusters, only the Gpy(out) orientation was observed.[14,19] This ethylamine side chain configuration allows for a bridge to form between the amino group and the proximal CH on the indole ring (at position 2). In serotonin−water complexes, there is an

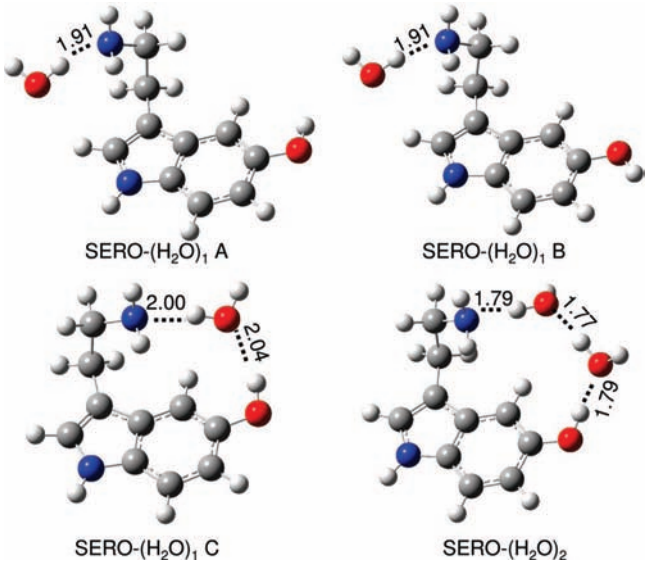

Figure 12. Structural assignments for the observed SERO−$(H_2O)_n$ clusters, with hydrogen-bond distances in angstroms taken from the DFT M05-2X/6-31+G* calculations.

additional site of binding, the 5-OH group. Nevertheless, two of the three observed structures (A and B) involve water binding to the amino group in the same ethylamine conformation as in tryptamine (Figure 12, top row). Furthermore, conformers A and B form a syn/anti 5-OH pair, which are present in the expansion with similar intensity, indicating that the strength of the interaction of the water molecule with the amino group is influenced very little by the 5-OH group. This preference for the NH_2 site was predicted by Delchev and Mikosch in a theoretical study, where they calculated the relative energies of water binding to serotonin at each of its H-bonding sites (5-OH donor/acceptor, indole NH donor, amino group acceptor) and found that the water bound to the amino group was the most stable of the four H-bonding possibilities.[51] However, this study only looked at an Anti(py) ethylamine side chain orientation, which was not observed in this study. The SERO–$(H_2O)_1$ complexes A and B benefit from the same additional stabilization from the O···HC(2) group as in TRA–$(H_2O)_1$.

Conformer C of SERO−$(H_2O)_1$ holds the ethylamine side chain in the Gph(out) position, with the water molecule acting as H-bond donor to the amino group and acceptor to the 5-OH group, thereby forming a bridge that links the two (structure **I**, Figure 2). The spectroscopic evidence for this assignment is immediately evident in the shift of the 5-OH stretch fundamental toward lower frequency, indicative of it being involved in a H-bond to water.

While the Gph(out) water complex is not seen in TRA−$(H_2O)_1$, we noted in the serotonin monomer paper that the Gph(out) structure was somewhat more stable in serotonin than in tryptamine, as reflected in the increase in the ratio of intensities of the Gph(out)/anti:Gpy(out)/anti S_0-S_1 origin transitions in the UV spectrum of serotonin monomer by about a factor of 4 relative to what it is in tryptamine monomer.[6] We believe this added stabilization is due to a slight interaction of the 5-OH and NH_2 groups, whether through space or through bond, even in the monomer. With the addition of a single water molecule, this conformation is stabilized strongly by virtue of forming a H-bonded bridge linking the two groups. One could imagine such conformational locking as a common consequence of H-bonded bridge formation, whether facilitated by the presence of water or in its absence in specific H-bonding interactions with its receptor sites.

The Gph(out) ethylamine conformation is the only one capable of bringing 5-OH and NH_2 groups into close enough proximity to form a H-bonded bridge with a single water molecule. Even so, there are several pieces of evidence that this single water bridge is straining to span the gap. First, despite having two H-bonds formed rather than one, the net stabilization is 45.1 kJ/mol, much less than the sum of isolated 5-OH···OH and OH···NH_2 H-bonds. For example, the sum of the net stabilization energy for structure with a water molecule hydrogen bonded to the 5-OH in a syn position and structure **II** is 63.47 kJ/mol, almost 20 kJ/mol more than for the observed conformer C. Second, the calculated structure for conformer C has considerable strain in it relative to the other structures (Table 1, 8.6 kJ/mol). The SERO structure in conformer C is shifted from the Gph(out) monomer structure by a small adjustment of the ethylamine side chain and a hindered rotation of the 5-OH group out of plane by ∼20°. The contributions to the strain energy from the ethylamine side chain and 5-OH groups are similar in size. Finally, both H-bonds in the bridge have heavy-atom distances that are elongated relative to their values in optimal single H-bonds, and the H-bond angles are bent away from linear.

Addition of a second water molecule to serotonin completes the conformational locking process for both the 5-OH and the ethylamine side chains, producing a single observed structure involving the Gph(out)/anti conformation with a water dimer bridge connecting the two groups. It would be interesting to theoretically model the cluster formation process in the supersonic expansion that leads to this single downstream structure, because the set of eight monomer conformations must anneal out in the process of binding to the water molecule(s).

There is much evidence that this water dimer bridge better spans the gap between the amino and 5-OH groups, forming three strong H-bonds in so doing. As shown in Table 3, this strengthening of the H-bonds is reflected in a dramatic reduction in the heavy-atom bond distances of the 5-OH···O (−0.19 Å) and OH···N (−0.18 Å) H-bonds relative to their values in the bridge SERO−$(H_2O)_1$ structure **I** (conformer C). At the same time, the H-bond angles are now nearly linear, as one would expect for optimal H-bonds. Furthermore, the central water−water H-bond has a heavy atom separation of 2.75 Å, a value close to that found in water trimer (2.76 Å).[52]

One of the principal attributes of a H-bonded bridge is the cooperative strengthening of the H-bonds involved in such a structure. The total binding energy associated with the water dimer bridge is 101 kJ/mol, more than double that of the single water molecule bridge (structure **I**, 45.1 kJ/mol). The three hydrogen bonds thus have an average energy of ∼34 kJ/mol, as compared to 22 kJ/mol in the SERO−$(H_2O)_1$ bridge. The strain energy is also relatively small (2.3 kJ/mol), indicating that the water dimer bridge is a very stable, cooperative network of hydrogen bonds.

B. Spectroscopic Consequences of Water Complexation to Serotonin. Having made firm assignments for the three conformers of SERO−$(H_2O)_1$ and one of SERO−$(H_2O)_2$, it is worthwhile to briefly return to the spectroscopic signatures accompanying these structures.

The UVHB spectra provide ultraviolet spectra of the individual conformations, and thus reflect the perturbations imposed by the water molecule(s) on the S_0-S_1 transition of serotonin, which is a $\pi-\pi^*$ transition of the indole ring. The S_0-S_1 origin transitions for conformers A and B of SERO−$(H_2O)_1$ are blue-

TABLE 2: Ultraviolet and Infrared Transitions of SERO−$(H_2O)_n$ Clusters[a]

	electronic		infrared			
conformer	S_0-S_1 origin	frequency shift[b]	indole NH	5-OH	water OH[c]	structural assignment
			SERO Monomer			
A	32 584		3530	3667		Gpy(out)/anti
B	32 548		3531	3668		Gpy(up)/anti
C	32 545		3530	3668		Gph(out)/anti
D	32 560		3531	3668		anti(py)/anti
E	32 537		3530	3667		anti(up)/anti
A′	32 353		3531	3671		Gpy(out)/syn
B′	32 313		3530	3672		Gpy(up)/syn
C′	32 282		3530	3671		Gph(out)/syn
			SERO−$(H_2O)_1$			
A	32 671	+87(SERO(A))	3530	3665	3344(OH···NH_2), 3717(F)	**III** (NH_2 and CH bound to SERO A)
B	32 454	+101(SERO(A′))	3531	3669	3341(OH···NH_2), 3717(F)	**IV** (NH_2 and CH bound to SERO A′)
C	32 188	−357(SERO(C))	3530	3551	3394(B), 3719(F)	**II** (bridge from NH_2 to 5-OH in SERO C)
			SERO−$(H_2O)_2$			
A	31 749	−439(SERO−$(H_2O)_1$(C))	3530	3357/3417[d]	3029(OH···NH_2), 3417/3357(OH···O)[d], 3720-3724(2F).	**I** (5OH···OH···OH···NH_2)

[a] Transitions reported in wavenumbers (cm^{-1}). [b] The relative shift in the S_0–S_1 origin transition from the origin of the indicated conformer. [c] Water OH stretches with OH···NH_2 refer to water bound to the amino group of the ethylamine side chain, B indicates a water bridge, and F indicates a free water OH. The bridged water is between the amino group of the ethylamine side chain and the 5-OH. [d] The 3357/3417 doublet are mixed 5-OH/water OH···O stretches in the bridge.

TABLE 3: Key Heavy Atom Bond Distances and Angles for the Observed Conformers of SERO−$(H_2O)_1$ and SERO−$(H_2O)_2$ Clusters

	bond lengths (Å)			angles (deg)		
conformer	N−O	O−O	O−O-5	N···HO	O···HO	O···HO-5
			SERO−$(H_2O)_1$			
A	2.87			165		
B	2.87			165		
C	2.97		2.96	167		157
			SERO−$(H_2O)_2$			
A	2.79	2.75	2.77	179	176	176

shifted from their corresponding monomer transitions (Gpy(out)/anti and Gpy(out)/syn, respectively) by 87 and 101 cm^{-1}, respectively (Table 2). The direction of this shift is the same one present in TRA−$(H_2O)_1$, which shares the same binding site, although its magnitude in SERO−$(H_2O)_1$ is larger. The electronic frequency shift measures the difference in binding energies of the complex in the S_0 and S_1 states. Thus, the blue shift in SERO−$(H_2O)_1$ A and B reflects a decrease in the binding energy upon electronic excitation by 87 and 101 cm^{-1}, respectively. The magnitude of the blue shift is only about 3% of the total binding energy of the complex (Table 1), consistent with the electronic excitation being localized on the indole π cloud, to which the water molecule is not bound in any direct way. Furthermore, because neither the ethylamine side chain nor the water molecule are perturbed by the electronic excitation significantly, Franck−Condon factors involving the ethylamine torsions or intermolecular vibrations are principally $\Delta v = 0$, leading to uncongested spectra in the low-frequency region of the UV spectrum (Figure 5). Not surprisingly, the resulting vibronic spectrum looks very similar to that found for TRA−$(H_2O)_1$.[14−19]

In conformer C, on the other hand, the S_0–S_1 origin is red-shifted by 355 cm^{-1} relative to its corresponding monomer conformation (Gph(out)/anti). This red-shift is similar in sign and magnitude to that found in the phenol−H_2O (−356 cm^{-1})[36] and 2-naphthol−H_2O (−371 cm^{-1} for cis and −332 cm^{-1} for trans)[42] complexes, both of which have the aromatic OH group acting as H-bond donor to the water molecule. In this case, the $\pi-\pi^*$ electronic excitation increases the strengths of the H-bond(s) to water, because the aromatic O is directly involved in the electronic excitation. In the condensed phase, aromatic alcohols reflect this increased H-bonding capacity in a lowering of the excited-state pK_a.[53−55]

Beyond the shift in the S_0-S_1 origin, the ultraviolet spectrum of conformer C also reflects changes in the structure of the complex upon electronic excitation in the extensive low-frequency vibronic structure present (Figure 5, C). We have already ascribed this structure in section III.B.1 to intermolecular vibrations of the complex and/or torsions of the ethylamine side chain, which are expected to be in this frequency range.

As Figure 5 shows, the extensive low-frequency Franck−Condon activity can be ascribed to progressions and combination bands involving four low-frequency modes at 57, 73, 88, and 108 cm^{-1}. Table 4 lists the low frequency modes of the bridge structure of SERO−$(H_2O)_1$ (structure **I**) in the ground electronic state, calculated at the DFT B3LYP/6-31+G* level of theory. Given the nature of the modes, one would not anticipate large changes in frequency upon electronic excitation. There is a reasonable match in frequency between the lowest four observed and calculated frequencies, which are a mix of ethylamine torsion and intermolecular vibrations involving translational and rocking motions of the water molecule in the binding pocket. We anticipate that a major consequence of electronic excitation is the strengthening of the 5-OH···water H-bond, which should shift the water molecule toward the 5-OH group. It is not unreasonable that this shift would also produce a torque in the NH_2 group of the ethylamine side chain. Thus, this low-frequency vibronic structure also reflects the fact that a single water molecule cannot span the gap between 5-OH and NH_2 groups in SERO well, and so has two non-optimal H-bonds, whose strength is changed upon electronic excitation.

To see whether these qualitative arguments are captured by calculations, excited-state calculations were carried out at the CIS/6-31+G* level of theory and compared to ground-state calculations at the HF/6-31+G* level of theory. These showed a decrease in the 5-OH···O heavy atom distance of 0.32 Å,

TABLE 4: DFT B3LYP/6-31+G* Calculated Low-Frequency Vibrations for the Two Bridge Structures SERO−$(H_2O)_1$ Conformer C and SERO−$(H_2O)_2$

SERO−$(H_2O)_1$ conformer C		SERO−$(H_2O)_2$	
low-frequency mode (cm^{-1}) (expt. (S_1))[a]	molecular vibration	low-frequency mode (cm^{-1}) (expt. (S_1))[a]	molecular vibration
46 (57)	water along-bridge translation, $C_\alpha-C_\beta$ torsion	36	free OH flip
67 (73)	water along-bridge translation, $C_\alpha-C_\beta$ torsion	43	free OH flip
80 (88)	ethylamine rock	67	water along-bridge translation, $C_\alpha-C_\beta$ torsion
123 (108)	water rock	81	eater rock
138	water−amine stretch	90 (87)	ethylamine rock
150	free OH flip	138	water−torsion mix
162	water−torsion mix	156 (161)	water−torsion mix
171	water−amine stretch	172	water rock

[a] The numbers in parentheses are the experimental frequencies of the low-frequency vibrations in the ultraviolet spectrum, placed in the table next to their tentative assignments. See text for further discussion.

and a minor lengthening of the OH···N distance by 0.02 Å in the excited state, consistent with the arguments just made.

Despite the fact that the only observed structure for SERO−$(H_2O)_2$ is a H-bonded bridge structure much like conformer C of SERO−$(H_2O)_1$, its ultraviolet spectrum (Figure 6c) exhibits much less low-frequency vibronic activity. This change is an indication that the water dimer bridge locks much more tightly into the "pocket" between the amino and 5-OH groups, resulting in a smaller geometry change upon electronic excitation. As seen in Table 4, there are several calculated low-frequency vibrations involving motion of the water molecules and ethylamine torsional modes in the same frequency range as in SERO−$(H_2O)_1$. Of these, only two appear in the spectrum. On the basis of the present data, firm assignments are not possible.

At the same time, the addition of a second water molecule perturbs the spectrum in other ways. First, the S_0-S_1 origin of SERO−$(H_2O)_2$ is shifted another 439 cm^{-1} to the red with respect to conformer C of SERO−$(H_2O)_1$ or 794 cm^{-1} relative to the monomer. Thus, in this water dimer bridge that is well-matched to the gap between 5-OH and NH_2 groups, the strength of the H-bond to the 5-OH group is even more dramatically enhanced upon electronic excitation.

Second, the higher-frequency vibronic structure in SERO−$(H_2O)_2$ is quite different from that in SERO monomer or in SERO−$(H_2O)_1$. Whereas the S_0-S_1 origin dominates the spectrum in these other cases, in SERO−$(H_2O)_2$, the S_0-S_1 origin is quite small relative to other vibronic bands, most notably the very intense vibronic band 720 cm^{-1} above the origin, which is an in-plane ring-breathing mode, that is largely a phenyl ring localized vibration. This mode typically has strong Franck−Condon factors in indole and its derivatives,[56−60] but it may be enhanced here even further due to the strong interaction of the 5-OH group with the water bridge.

The strengths and types of hydrogen bonds present in SERO−$(H_2O)_{1,2}$ have their most direct spectroscopic manifestation in the hydride stretch RIDIR spectra, which have so far been used primarily to make assignments to particular structures (section III.B.2). Table 2 summarizes the frequencies of these absorptions. Because SERO−$(H_2O)_1$ A and B have spectra nearly identical to that of TRA−$(H_2O)_1$, which has been discussed previously,[51] we focus attention here primarily on the two bridge structures. Our contention is that the single water bridge is not optimal, because it cannot span the distance between the two groups without giving up H-bonding strength and/or inducing strain in the flexible groups to which it is attached. The calculated spectrum for conformer C predicts that the two bridging H-bonded OH stretch modes are largely localized (∼90%) on the OH···NH_2 and 5-OH···O groups, with the former lower in frequency than the latter by ∼225 cm^{-1}. The OH···NH_2 stretch appears experimentally at 3394 cm^{-1}, about 50 cm^{-1} higher in frequency than in conformers A and B, where water is singly bound to the NH_2 group without any constraints imposed by the bridge. Furthermore, the 5-OH···O H-bonded stretch fundamental has a frequency of 3551 cm^{-1}, which is shifted to lower frequency by 117 cm^{-1} relative to the free anti 5-OH (Table 2). This is to be compared with shifts of 142 and 138 cm^{-1} for cis and trans 2-naphthol−(H_2O), respectively, and 133 cm^{-1} for phenol−water.[35,42,46,47] This confirms the general notion that both the OH···NH_2 and the 5-OH···OH-bonds are not optimal in conformer C of SERO−$(H_2O)_1$, consistent with the hypothesis that a single water molecule does not span the gap between the 5-OH and NH_2 groups, and giving up strength in either H-bond to gain the other in forming a bridge.

In SERO−$(H_2O)_2$, the water dimer bridge has greatly strengthened H-bonds, due to a better size match and to the cooperative strengthening that is anticipated in H-bonded bridges such as this. The lowest frequency OH stretch, which calculations indicate is largely localized on the OH group bound to NH_2, is now shifted from 3394 all of the way down to 3029 cm^{-1}, nearly 700 cm^{-1} below its free value. According to the calculations, the other two OH groups in the bridge (the 5-OH and first interior water OH) have OH stretch modes that are strongly coupled to one another, producing in-phase and out-of-phase combinations of the two local modes. These appear as a pair of transitions at 3357 and 3417 cm^{-1}. One is thus led to a picture of the SERO−$(H_2O)_2$ bridge as having a strong anchor to the NH_2 group and two equal links to the 5-OH. The calculated structure for SERO−$(H_2O)_2$ reflects this, with similar heavy-atom distances in the two OH···O bonds (∼2.75 Å, Table 3).

Finally, it is worth stressing once again the role played by the alkyl CH stretch region in the assignments made. This region of the spectrum is a notoriously difficult one in which to make definitive assignments to specific normal modes. This is the case because of the large numbers of C−H groups with similar frequencies, the sensitivity of the form of the normal modes to the position and orientation of each alkyl group, and the ubiquitous presence of Fermi resonances between the CH stretch fundamentals and overtones of the CH bends. Nevertheless, there are now a growing number of examples in which the patterns present in the alkyl CH stretch region are diagnostic of the conformation taken up by the alkyl group. Here, we have used this region to confirm that conformers A and B of SERO−$(H_2O)_1$ share the same Gpy(out) ethylamine side chain

conformation as one another and as TRA−$(H_2O)_1$. The near-identical alkyl CH stretch spectra of SERO−$(H_2O)_1$ C and SERO−$(H_2O)_2$ played a similar role in assigning both structures to Gph(out) structures. It may be possible in the future to use this growing body of single-conformation spectra in this region to better understand the spectroscopic signatures of various alkyl conformations for use in other contexts.

V. Conclusions

This study explored the conformational preferences of serotonin−water clusters in a supersonic jet. This work builds directly on earlier studies[8,14–19] of tryptamine−water clusters, where the principal attachment point for water was at the NH_2 group of the ethylamine side chain. Serotonin differs from tryptamine by virtue of its 5-OH group on the indole ring, providing a second strong H-bonding site for water. This has ramifications for all aspects of the observed spectroscopy. In SERO−$(H_2O)_1$, three conformational isomers were observed, which employed one of two ethylamine side chain conformations, Gpy(out) and Gph(out). In the former case, the ethylamine side chain pointed away from the 5-OH group, with water binding to the ethylamine nitrogen lone pair, as it does in TRA−$(H_2O)_1$. Two such structures are thus observed, with very similar ultraviolet and infrared spectra, differing only in the orientation of the 5-OH group remote from the attachment point of the water molecule. Conformer C of SERO−$(H_2O)_1$ takes up the Gph(out) ethylamine side chain conformation, which points the NH_2 group toward the 5-OH group. In this case, a single water molecule can act as a bridge joining these two groups. We have presented multiple pieces of evidence that this bridge is not optimal, with both H-bonds weakened, and strain induced in the 5-OH and ethylamine side chains in forming a bridge. In SERO−$(H_2O)_2$, this same Gph(out) conformation is used to form a strong water dimer bridge to the 5-OH group. Because this is the only observed conformer of SERO−$(H_2O)_2$, one is left with the impression that this structure is near-optimal, locking the ethylamine and 5-OH groups into single conformations that enable its formation.

It would be very interesting to explore the potential energy surfaces for SERO−$(H_2O)_1$ and SERO−$(H_2O)_2$ in more detail, to better understand the way in which one or two strongly bound water molecules have affected the overall potential energy landscape. From an experimental standpoint, it may be possible to use population transfer methods[7,61,62] to obtain barrier heights separating the three conformers of SERO−$(H_2O)_1$, shuttling the water molecule between the various structures via laser excitation. From a computational standpoint, accurate disconnectivity diagrams[63] for serotonin monomer and its single and double-water complexes could shed additional light on the effects of these water molecules on the conformational preferences of serotonin.

It seems obvious that serotonin's diverse biological activity is due in part to the flexibility of the ethylamine and 5-OH groups and the spatial separation between them, which enable it to bind well to a number of different receptor sites. Whether water molecules play some role in preshaping the molecule as it enters the binding sites, or in forming H-bonds between sites on serotonin or between serotonin and the receptors themselves, is an open question worth further exploration.

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References and Notes

(1) Adayev, T.; Ranasinghe, B.; Banerjee, P. *Biosci. Rep.* **2005**, *25*, 363.
(2) Chaouloff, F.; Berton, O.; Mormede, P. *Neuropsychopharmacology* **1999**, *21*, S28.
(3) Hariri, A. R.; Holmes, A. *Trends Cognitive Sci.* **2006**, *10*, 182.
(4) Jouvet, M. *Neuropsychopharmacology* **1999**, *21*, S24.
(5) Pucadyil, T. J.; Kalipatnapu, S.; Chattopadhyay, A. *Cell. Mol. Neurobiol.* **2005**, *25*, 553.
(6) LeGreve, T. A.; Baquero, E. E.; Zwier, T. S. *J. Am. Chem. Soc.* **2007**, *129*, 4028.
(7) LeGreve, T. A.; Clarkson, J. R.; Zwier, T. S. *J. Phys. Chem. A* **2008**, *112*, 3911.
(8) Carney, J. R.; Zwier, T. S. *J. Phys. Chem. A* **2000**, *104*, 8677.
(9) Chanda, P. K.; Minchin, M. C. W.; Davis, A. R.; Greenberg, L.; Reilly, Y.; McGregor, W. H.; Bhat, R.; Lubeck, M. D.; Mizutani, S.; Hung, P. P. *Mol. Pharmacol.* **1993**, *43*, 516.
(10) Chattopadhyay, A.; Rukmini, R.; Mukherjee, S. *Biophys. J.* **1996**, *71*, 1952.
(11) Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. A. *Pharmacol. Rev.* **1994**, *46*, 157.
(12) Hoyer, D.; Hannon, J. P.; Martin, G. R. *Pharmacol., Biochem. Behav.* **2002**, *71*, 533.
(13) Wang, C. D.; Gallaher, T. K.; Shih, J. C. *Mol. Pharmacol.* **1993**, *43*, 931.
(14) Clarkson, J. R.; Herbert, J. M.; Zwier, T. S. *J. Chem. Phys.* **2007**, 126.
(15) Connell, L. L.; Corcoran, T. C.; Joireman, P. W.; Felker, P. M. *Chem. Phys. Lett.* **1990**, *166*, 510.
(16) Peteanu, L. A.; Levy, D. H. *J. Phys. Chem.* **1988**, *92*, 6554.
(17) Schmitt, M.; Bohm, M.; Ratzer, C.; Vu, C.; Kalkman, L.; Meerts, W. L. *J. Am. Chem. Soc.* **2005**, *127*, 10356.
(18) Sipior, J.; Sulkes, M. *J. Chem. Phys.* **1988**, *88*, 6146.
(19) Zwier, T. S. *J. Phys. Chem. A* **2001**, *105*, 8827.
(20) Kandori, H.; Shichida, Y. *J. Am. Chem. Soc.* **2000**, *122*, 11745.
(21) Sumii, M.; Furutani, Y.; Waschuk, S. A.; Brown, L. S.; Kandori, H. *Biochemistry* **2005**, *44*, 15159.
(22) Carney, J. R.; Dian, B. C.; Florio, G. M.; Zwier, T. S. *J. Am. Chem. Soc.* **2001**, *123*, 5596.
(23) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
(24) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
(25) Lee, C. T.; Yang, W. T.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(26) Zhao, Y.; Truhlar, D. G. *J. Chem. Theory Comput.* **2006**, *2*, 1009.
(27) Frisch, M. J.; Pople, J. A.; Binkley, J. S. *J. Chem. Phys.* **1984**, *80*, 3265.
(28) 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.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; 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, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision E.01; Gaussian, Inc.: Wallingford, CT, 2004.
(29) Dooley, R. A.; Pamidighantam, S. Computational Chemistry Grid: Production Cyberinfrastructure for Computational Chemistry; Proceedings of the 13th Annual Mardi Gras Conference, Baton Rouge, LA, Feb. 2005.
(30) Milfield. K. G., C.; Pamidighantam, S.; Giulani, J. Cluster Computing through an Application-oriented Computational Chemistry Grid; Proceedings of the 2005 Linux Clusters, The HPC Revolution, April 2005.
(31) Florio, G. M.; Zwier, T. S. *J. Phys. Chem. A* **2003**, *107*, 974.
(32) Abe, H.; Mikami, N.; Ito, M. *J. Phys. Chem.* **1982**, *86*, 1768.
(33) Berden, G.; Meerts, W. L.; Schmitt, M.; Kleinermanns, K. *J. Chem. Phys.* **1996**, *104*, 972.
(34) Burgi, T.; Droz, T.; Leutwyler, S. *Chem. Phys. Lett.* **1995**, *246*, 291.
(35) Ebata, T.; Mizuochi, N.; Watanabe, T.; Mikami, N. *J. Phys. Chem.* **1996**, *100*, 546.
(36) Fuke, K.; Kaya, K. *Chem. Phys. Lett.* **1983**, *94*, 97.
(37) Gerhards, M.; Schmitt, M.; Kleinermanns, K.; Stahl, W. *J. Chem. Phys.* **1996**, *104*, 967.

(38) Lipert, R. J.; Bermudez, G.; Colson, S. D. *J. Phys. Chem.* **1988**, *92*, 3801.
(39) Lipert, R. J.; Colson, S. D. *J. Chem. Phys.* **1988**, *89*, 4579.
(40) Lipert, R. J.; Colson, S. D. *Chem. Phys. Lett.* **1989**, *161*, 303.
(41) Lipert, R. J.; Colson, S. D. *J. Phys. Chem.* **1990**, *94*, 2358.
(42) Matsumoto, Y.; Ebata, T.; Mikami, N. *J. Chem. Phys.* **1998**, *109*, 6303.
(43) Schmitt, M.; Jacoby, C.; Kleinermanns, K. *J. Chem. Phys.* **1998**, *108*, 4486.
(44) Schutz, M.; Burgi, T.; Leutwyler, S.; Fischer, T. *J. Chem. Phys.* **1993**, *99*, 1469.
(45) Stanley, R. J.; Castleman, A. W. *J. Chem. Phys.* **1991**, *94*, 7744.
(46) Tanabe, S.; Ebata, T.; Fujii, M.; Mikami, N. *Chem. Phys. Lett.* **1993**, *215*, 347.
(47) Watanabe, T.; Ebata, T.; Tanabe, S.; Mikami, N. *J. Chem. Phys.* **1996**, *105*, 408.
(48) Zierhut, M.; Dummler, S.; Roth, W.; Fischer, I. *Chem. Phys. Lett.* **2003**, *381*, 346.
(49) Zierhut, M.; Roth, W.; Dummler, S.; Fischer, I. *Chem. Phys.* **2004**, *305*, 123.
(50) Pimentel, G. C.; McClellan, A. L. *The Hydrogen Bond*; W. H. Freeman: San Francisco, CA, 1960.
(51) Delchev, V. B.; Mikosch, H. *J. Mol. Model.* **2006**, *12*, 272.
(52) Liu, K.; Loeser, J. G.; Elrod, M. J.; Host, B. C.; Rzepiela, J. A.; Pugliano, N.; Saykally, R. J. *J. Am. Chem. Soc.* **1994**, *116*, 3507.
(53) Krishnan, R.; Fillingim, T. G.; Lee, J.; Robinson, G. W. *J. Am. Chem. Soc.* **1990**, *112*, 1353.
(54) Lee, J.; Robinson, G. W.; Webb, S. P.; Philips, L. A.; Clark, J. H. *J. Am. Chem. Soc.* **1986**, *108*, 6538.
(55) Robinson, G. W.; Thistlethwaite, P. J.; Lee, J. *J. Phys. Chem.* **1986**, *90*, 4224.
(56) Anderson, B. E.; Jones, R. D.; Rehms, A. A.; Ilich, P.; Callis, P. R. *Chem. Phys. Lett.* **1986**, *125*, 106.
(57) Bickel, G. A.; Demmer, D. R.; Outhouse, E. A.; Wallace, S. C. *J. Chem. Phys.* **1989**, *91*, 6013.
(58) Fender, B.; Callis, P. R. *Chem. Phys. Lett.* **1996**, *262*, 343.
(59) Fender, B. J.; Sammeth, D. M.; Callis, P. R. *Chem. Phys. Lett.* **1995**, *239*, 31.
(60) Sammeth, D. M.; Siewert, S. S.; Spangler, L. H.; Callis, P. R. *Chem. Phys. Lett.* **1992**, *193*, 532.
(61) Clarkson, J. R.; Dian, B. C.; Moriggi, L.; DeFusco, A.; McCarthy, V.; Jordan, K. D.; Zwier, T. S. *J. Chem. Phys.* **2005**, 122.
(62) Dian, B. C.; Clarkson, J. R.; Zwier, T. S. *Science* **2004**, *303*, 1169.
(63) Becker, O. M.; Karplus, M. *J. Chem. Phys.* **1997**, *106*, 1495.

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