Fluorescence of Cysteine and Cystine

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We interpret the UV absorption and fluorescence of cysteine and cystine from ab initio calculations of the ground states and lowest excited singlet states of the two molecules. We derive the optimized energies and geometry parameters from HF/6-31G computations on the ground state and CIS/6-31G computations of the excited state. We present vibrational frequencies of the ground and excited states and quantitative predictions for UV absorption and fluorescence. We also show experimental measurements of cystine fluorescence. Cystine is shown to fluoresce at 700 nm.

1. Introduction

Four of the common amino acids, namely the two aromatic amino acids phenylalanine and tyrosine, the heterocyclic tryptophan, and the disulfide cystine, exhibit characteristic ultraviolet absorption. The aromatic and heterocyclic amino acids re-emit the absorbed ultraviolet light in the form of fluorescence. It is useful to study the fluorescence properties of these amino acids both experimentally and theoretically since many studies on the properties of proteins and polypeptides are based on fluorescence measurements. The most recent review of this investigation field may be found in the book by Lakowicz.[1]

In a previous paper[2] we presented ab initio calculations of the ground and first excited singlet states of phenylalanine and tyrosine in order to gain a better insight in the fluorescence mechanism of these two molecules. Our theoretical results seem to be consistent with experimental information.

In the present paper we present the results of our ab initio calculations on the disulfide cystine and on the corresponding sulfhydril monomer cysteine. Both molecules, especially cystine, have been implicated in the fluorescence quenching of the aromatic and heterocyclic amino acids.[1] There are many other molecules that are capable of this type of fluorescence quenching, but the role of cysteine and cystine is of particular interest since the latter two molecules are naturally present in proteins and polypeptides. Our computational results generated an interest in investigating the long wavelength fluorescence spectra of cystine. The experimental data presented herein were found to support the theoretical predictions.

The crystal structure of L-cysteine was first reported by Kerr and Ashmore.[3] Kerr, Ashmore, and Koetzle[4] also reported a neutron diffraction study of the same crystal used in ref 3. A redetermination of the structure of L-cysteine was presented recently by Görbitz and Dalhus.[5] It should be noted that Kerr et al.[3,4] studied orthorhombic crystals, whereas Görbitz and Dalhus[5] measured a monoclinic form. In the latter crystal there are two molecules per unit cell, denoted by A and B, with slightly different geometrical parameters. All crystals contain zwitterions which are stabilized by hydrogen bond formation. Since our calculations were carried out on an isolated molecule rather than hydrogen-bonded zwitterions, we had some difficulty in comparing our theoretical results with the available experimental information in the literature.

The crystal and molecular structures of L-cystine were reported by Chaney and Steinrauf [6] in 1974. Their experimental data again corresponded to the zwitterion rather than the neutral molecule. They found that there are two different stable configurations of the zwitterion, namely a tetragonal and a hexagonal structure. Subsequent experimental papers deal with molecules related to cystine, namely tetramethyl-D-cystine[7] and L-cystine dihydrobromide.[8]

2. Methodology

We performed ground state and first excited singlet state calculations on cysteine and cystine using the Hartree−Fock (HF) and configuration interaction with single excitations (CIS) method, respectively. We performed HF computations on the ground states of cysteine and cystine and CIS computations on the first excited singlet states. All calculations were done using the 6-31G and 6-31G* bases in the Gaussian94 series of programs.[9] The Gaussian94 codes allowed for the calculation of vibrational frequencies for both ground and excited states, which was not possible in our earlier study.[2]

Quantitative predictions about the UV absorption and fluorescence of cysteine and cystine were derived from the computed energy values. We calculated four different energies under each basis set, namely the ground- and excited-state energies at the optimized ground-state geometry, and both energies at the optimized excited-state geometry. As a check the calculations were repeated using the 6-31G* basis set to include the effect of d orbitals where possible. The cystine molecule, however, proved exceedingly large for treatment with the larger basis set, despite the computational resources available at the Major Defense Department Supercomputing Center at which the calculations were performed.

Based on our calculated results that predicted a fluorescence peak at 700 nm for neutral cystine, we performed a detailed

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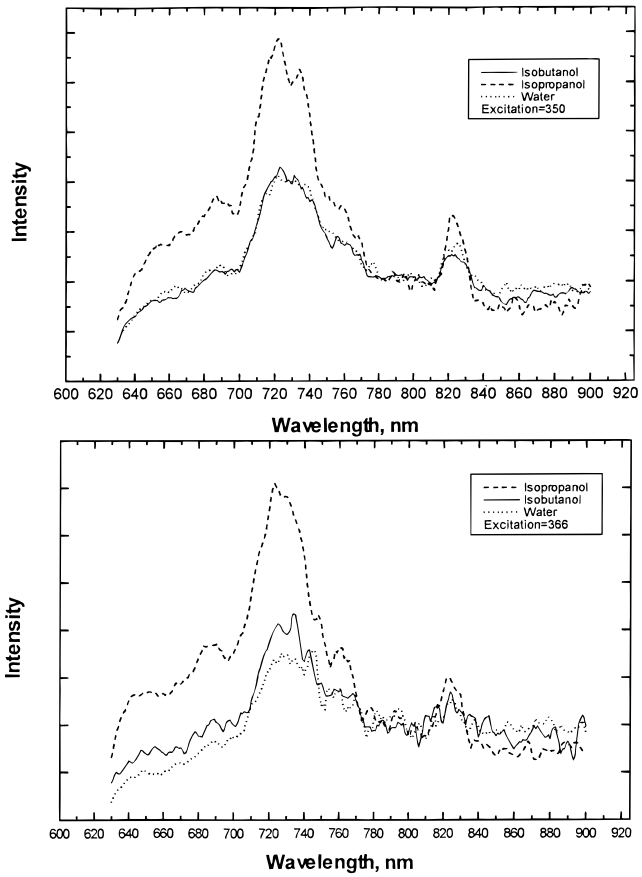

Figure 1. Fluorescence spectra of cystine.

experimental study of the fluorescence of this molecule. Previous observations were all done in aqueous solutions which favored the zwitterionic form of cystine. To examine the properties of the molecule, we examined the fluorescence of a suspension of cystine in water, 2-propanol, and isobutanol.

Approximately 0.5 mg of 99% DL-cystine (Sigma Chemical, St. Louis, MO) was suspended into 3 mL each of the following solvents: distilled water, 2-propanol (Fischer Scientific, Pittsburgh, PA), and isobutanol (Fluka Chemical, Ronkonkoma, NY). Samples were transferred into a 3.5 mL quartz cuvette with a micro-spinbar inside to maintain suspension.

Fluorescence measurements were performed using a LS-100 steady-state system (PTI, South Brunswick, NJ). The cuvette containing the sample was placed into a cuvette holder equipped with a magnetic stirrer and excited at 350 and 366 nm using a pulsed xenon flashlamp. Since the fluorescence of cystine was expected to be weak, all slit widths were set at 12 nm. To avoid potential second-order fluorescence, an RG630 optical filter (Newport, Fountain Valley, CA) was mounted between the sample and the emission monochromator. The fluorescence was measured from 630 to 900 nm in increments of 1 nm. An average of 10 fluorescence scans was used for off-line analysis. The resulting spectra are presented in Figure 1.

3. Results

a. Calculated Cysteine Fluorescence. We used four different theoretical sources of information to help us interpret the fluorescence mechanism: (1) changes in geometry on excitation, (2) changes in Mulliken atomic charge densities on excitation, (3) the molecular orbitals that are involved in the excitation, and (4) the vibrational frequencies for the ground and excited states.

TABLE 1: Bond Lengths of the Ground and First Excited Singlet States of Cysteine

	ground state		excited state		expt[5]	
	6-31G	6-31G*	6-31G	6-31G*	A	B
C1−C2	1.5316	1.5365	1.5361	1.5491	1.5312	1.5345
C1−C3	1.5390	1.5416	1.5311	1.5340	1.5280	1.5300
C1−N4	1.4435	1.4446	1.4441	1.4470	1.4839	1.4890
C1−H5	1.0840	1.0860	1.0862	1.0880		
C2−O6	1.2053	1.1840	1.2998	1.2756	1.2539	1.2650
C2−O7	1.3486	1.3265	1.4000	1.3778	1.2551	1.2435
C3−H11	1.0757	1.0789	1.0774	1.0806		
C3−H12	1.0797	1.0836	1.0798	1.0833		
C3−S13	1.8892	1.8254	1.8828	1.8226	1.8189	1.8070
N4−H9	0.9964	1.0022	0.9941	1.0010		
N4−H10	0.9976	1.0031	0.9950	1.0023		
O7−H8	0.9537	0.9513	0.9528	0.9497		
S13−H14	1.3535	1.3267	1.3544	1.3271	1.34	1.35
C3−H8	2.8439	2.7575	3.4830	3.1342		
S13−H8	2.5159	2.4875	3.0399	2.6783		

TABLE 2: Bond Angles of the Ground and First Excited Singlet States of Cysteine

	ground state		excited state		expt[5]	
	6-31G	6-31G*	6-31G	6-31G*		
C2−C1−C3	111.97	112.37	111.59	111.63	111.71	111.48
C2−C1−N4	112.00	112.30	115.10	115.51	111.32	108.49
C2−C1−H5	107.69	107.60	105.70	105.98		
C3−C1−N4	108.48	108.25	107.08	106.59	109.94	108.72
C3−C1− H5	108.63	108.34	109.24	109.23		
N4−C1−H5	107.93	107.80	107.99	107.74		
C1−C2−O6	122.50	122.09	118.26	115.37	117.98	118.26
C1−C2−O7	117.33	117.11	114.00	115.35	116.72	115.66
O6−C2−O7	120.17	120.80	113.17	112.72	125.30	126.06
C1−C3−H11	108.05		108.69			
C1−C3− H12	111.71	110.77	110.09	109.33		
C1−C3− S13	115.26	116.03	116.73	117.46	115.12	113.66
H11−C3−H12	108.53	107.48	108.87	107.74		
H11−C3−S13	107.94	108.80	108.36	109.16		
H12−C3−S13	105.14	105.71	103.81	104.68		
C1−N4−H9	114.95	109.85	117.05	111.31		
C1−N4−H10	114.47	109.64	117.30	111.40		
H9−N4−H10	111.21	105.27	113.98	107.44		
C2−O7−H8	117.68	112.81	112.79	107.99		
C3−S13−H14	98.11	97.52	98.52	98.03	98.1	90.6

We list the computed bond lengths and bond angles of the ground and excited states of cysteine in Tables 1 and 2. The molecular ground-state geometry and the numbering of the atoms are shown in Figure 2. It is observed that the only significant change in bond distances occurs in the COOH group, where the C═O bond distance increases by 0.1 Å and the C−O bond single-bond distance increases by 0.05 Å. This seems to indicate a change from sp^2 to sp^3 hybridization in C2 upon excitation. We also note a significant change in the nonbonding interatomic distances C3−H8 and S13−H8, which shows that the hydrogen atom in the COOH group rotates away from the sulfur atom and the rest of the molecule upon excitation.

The excited-state wave function of cysteine is obtained as

$$\Psi_{exc} = -0.27030\psi(28\rightarrow 33) + 0.59708\psi(30\rightarrow 33) + 0.10551\psi(30\rightarrow 34) \quad (1)$$

We show a pictorial representation of the four molecular orbitals 28, 30, 33, and 34 in Figure 3. It may be seen that the major contribution to the excited state is due to an excitation from molecular orbital 30 to molecular orbital 33, both of which are localized in the COOH group. A smaller contribution is due to an excitation from molecular orbital 28 to molecular orbital 33. Molecular orbital 28 is also localized in the COOH

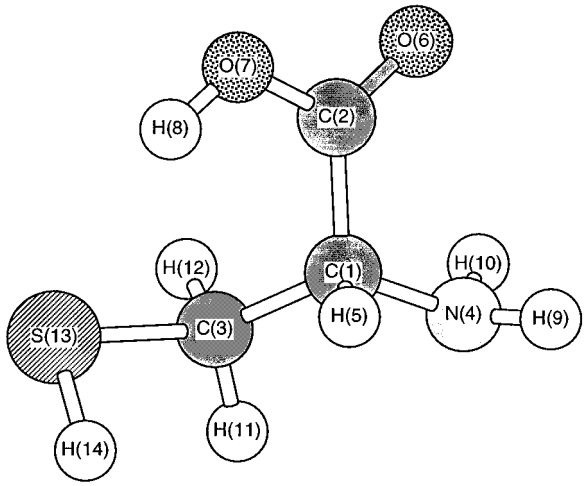

Figure 2. Geometry and atomic numbering of cysteine.

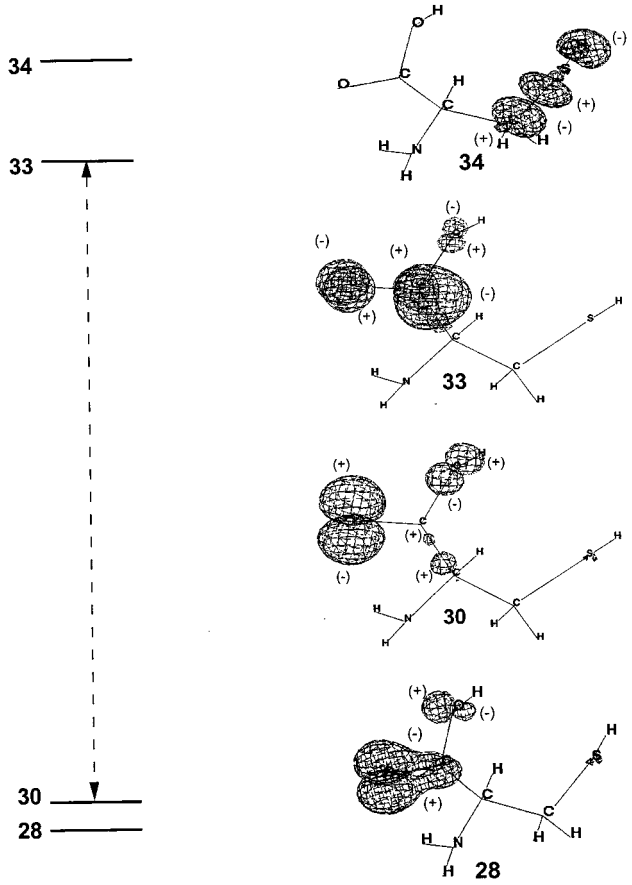

Figure 3. Pictorial representation of molecular orbitals involved in the fluorescence of cysteine.

group. Molecular orbital 34 involves the C−S−H group, but this orbital plays only a minor role in the excitation.

The evidence from the changes in geometry and from the pictorial representation of the molecular orbitals involved is consistent. It shows that the excitation is largely localized in the COOH group of the molecule. This conclusion is also consistent with the changes in vibrational frequencies upon excitation. The Mulliken charge densities presented in Table 3 are also consistent with these observations.

We mentioned already that the experimental bond distances and bond angles[3−5] are available only for the zwitterions. We report the most recent data[5] in Tables 1 and 2. Görbitz and Dalhus report two different types of molecules, A and B, in the elementary cell, and we report the parameters for both types.[5] The poor agreement between theory and experiment shown in the comparisons in Tables 1 and 2 is unusual and requires further study. The difference between the experimental and theoretical C−S bond distances is particularly large. The data for the C−S−H bond angle indicate that the theoretical results are in better agreement with experimental structure A than they are with structure B. However, it is important to emphasize that the theoretical results refer to an isolated molecule, while the experimental results refer to hydrogen-bonded zwitterions in a crystalline environment. The UV absorption of cysteine was predicted to occur near 200 nm and the fluorescence maximum near 300 nm.

TABLE 3: Atomic Charges in the Ground and Excited States of Cysteine

	ground state		excited state	
	6-31G	6-31G*	6-31G	6-31G*
C1	−0.101 320	−0.123 880	−0.041 130	−0.054 050
C2	0.777 124	0.759 074	0.722 078	0.682 180
C3	−0.546 404	−0.491 158	−0.550 507	−0.486 070
N4	−0.803 772	−0.814 236	−0.823 191	−0.820 371
H5	0.213 980	0.200 785	0.244 727	0.230 179
O6	−0.526 403	−0.537 155	−0.518 256	−0.528 851
O7	−0.736 52	−0.706 954	−0.719 700	−0.694 545
H8	0.463 092	0.495 242	0.443 807	0.479 678
H9	0.343 891	0.357 320	0.357 033	0.369 549
H10	0.349 935	0.366 619	0.345 096	0.352 124
H11	0.255 468	0.248 754	0.239 554	0.237 039
H12	0.230 053	0.221 522	0.212 833	0.206 591
S13	0.012 364	−0.079 014	0.024 017	−0.087 115
H14	0.068 514	0.133 083	0.063 639	0.113 642

The vibrational frequencies of the cysteine molecule in its ground and excited states are presented in Table 4. The relative intensities of the computed transitions are marked by a single asterisk when they exceeded a value of 25, two asterisks when they exceed a value of 100, and three asterisks when their value exceeded 300. The assignments are derived from the normal mode motions. The majority of the assignments are unambiguous, but in certain cases there is mixing of two different modes and then the assignments become less straightforward. In the latter situation it becomes helpful to make comparisons with different molecules.

The vibrational frequencies are more sensitive to changes in the electronic structure than the geometric parameters. The largest differences between ground- and excited-state frequencies are found in the C−O and C═O stretching modes, the O−C−O bending mode, and the O−H bending mode 27. This is consistent with our previous observation that the electronic excitation is mostly localized in the COOH group.

The infrared and Raman spectra of various complexes of cysteine and heavy metals were reported by Shindo and Brown[10] and by Sze, Davis, and Neville.[11] The available experimental data correspond to molecules that are slightly different from the system we calculated. The experimental results are also quite limited, and we were only able to identify the six experimental frequencies (listed in Table 4).

In previous theoretical studies[12,13] we were able to derive a set of semiempirical correction factors from a comparison of experimental and computed frequencies. In the present situation this is unfortunately not possible because of the scarcity of experimental information. The most accurate frequency predictions are thus obtained by multiplying the computed frequencies by the average correction factor of 0.905.[12]

TABLE 4: Computed Frequencies of the Ground and First Excited Singlet States of Cysteine and Their Assignments

		ground state		excited state		
	assignment	6-31G	6-31G*	6-31G	6-31G*	expt[11]
1	O−H stretch	3977.54**	4041.25**	3994.19**	4073.57**	
2	N−H stretch	3886.73	3808.65	3924.32	3823.11	
3	N−H stretch	3777.16	3731.32	3801.13	3734.08	
4	C−H stretch	3362.05	3335.13	3341.29	3317.64	
5	C−H stretch	3278.13	3258.81	3267.54	3253.98	2996
6	C−H stretch	3226.26*	3226.58	3194.69	3196.88*	2949
7	S−H stretch	2692.33	2911.20	2685.15*	2907.57	2569
8	C═O stretch	1946.01***	2051.31***	1587.30	1664.39	1740
9	N−H bend	1864.24*	1851.84*	1843.21*	1840.42*	
10	C−H bend	1628.39	1621.21	1618.51	1607.19	
11	C−H bend	1547.91	1471.11**	1516.70	1472.12	
12	C−H bend	1485.01	1344.77	1465.77	1300.52	
13	C−C stretch	1445.32*	1563.26*	1281.70	1532.43	
14	C−O stretch	1360.37***	1520.78**	1170.36*	1225.15*	1225
15	C−H bend	1334.22	1320.80	1434.45*	1432.65*	
16	O−H bend	1306.85	1430.64**	1362.75*	1355.72*	
17	O−H bend	1273.26	1298.65	1324.99*	1331.96	
18	C−N stretch	1243.26*	1248.00	1199.21	1201.19	
19	C−C stretch	1109.50*	1140.32*	1080.03*	1100.78*	
20	C−C stretch	1079.48	1090.04*	1075.37	1088.82*	
21	S−H bend	904.88*	933.43	884.47	898.00*	
22	NH_2 wag	841.10**	1035.46*	643.81***	985.15**	
23	C−C stretch	825.83	842.17	802.31	798.72	
24	S−C stretch	807.87	845.01	771.17	811.32	682
25	C−C stretch	731.65	759.35	561.10	585.03	
26	O−C−O bend	696.23	700.48	574.63	453.01	
27	O−H bend	596.47**	624.17**	317.32*	385.41	
28	C−N bend	523.82	534.42	438.03	545.00	
29	C−S bend	380.10	326.95	362.78	275.54	
30	C−N bend	334.81*	385.33	340.88*	372.48*	
31	C−C−S bend	312.92	344.91*	266.28	345.14	
32	S−H bend	259.48	281.32	189.38*	223.51**	
33	C−S bend	212.65	197.66	156.15**	195.21	
34	NH_2 wag	166.22*	235.47*	293.95*	328.66*	
35	torsion	123.91	125.57	110.62	119.90	
36	torsion	57.14	57.46	60.60	60.52	

b. Calculated Cystine Fluorescence. Both cysteine and cystine are optically active, but cysteine possesses a single asymmetric center, whereas cystine possesses two centers. Our computations do not differentiate between the D and L forms of cysteine. In the case of cystine it is necessary to differentiate between the two situations where the two asymmetric centers enhance each other's optical activity to produce either a D or L molecule or where the two centers cancel each other's optical activity to give an optically inactive molecule. We found that the optically active form of cystine has the lower energy, and all our theoretical results in this section refer to the optically active form of cystine. It is not necessary to differentiate between the D or L configurations since they have identical energies. We obtained the lowest energy by first imposing symmetry restrictions (C_2 symmetry) during the geometry optimization. We removed the symmetry restriction during a subsequent geometry optimization, which led to a slightly lower energy and slightly nonsymmetric geometry. The optimized geometry and the atomic numbering of cystine are presented in Figure 4. The optimized bond distances and bond angles of the ground and first excited states of cystine are presented in Tables 5 and 6, respectively, together with the experimental results of Chaney and Steinrauf.[6] It was found that there are two stable configurations of cystine, a tetragonal and a hexagonal form. Our computed bond angles predict a tetragonal structure, and we report therefore the tetragonal bond distances and bond angles in Tables 5 and 6.

The experimental data in Tables 5 and 6 refer to the zwitterion, as was the case for cysteine, whereas the theoretical results again refer to the isolated molecule. It may be seen from

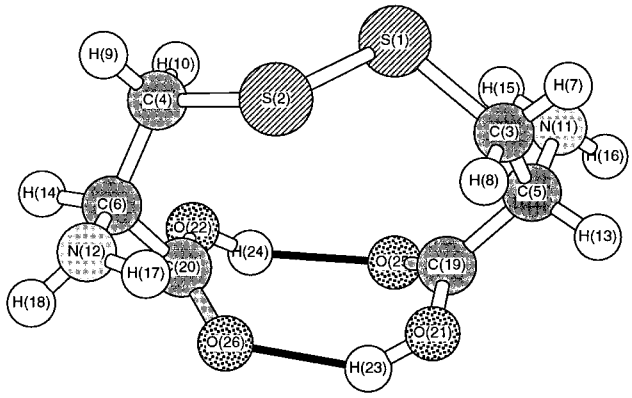

Figure 4. Geometry and atomic numbering of cystine.

TABLE 5: Bond Lengths of the Ground and First Excited Singlet States of Cystine

	ground state	excited state	expt[6]
S1−S2	2.2407	2.6554	2.04
S1−C3	1.8976	1.8853	1.83
S2−C4	1.8977	1.8853	1.80
C3−C5	1.5347	1.5431	1.53
C3−H7	1.0766	1.0772	
C3−H8	1.0772	1.0775	
C4−C6	1.5348	1.5431	1.53
C4−H9	1.0765	1.0772	
C4−H10	1.0772	1.0775	
C5−N11	1.4362	1.4355	1.48
C5−H13	1.0844	1.0833	
C5−C19	1.5127	1.5163	1.50
C6−N12	1.4363	1.4355	1.44
C6−H14	1.0844	1.0833	
C6−C20	1.5127	1.5163	1.53
N11−H15	0.9962	0.9969	
N11−H16	0.9947	0.9949	
N12−N17	0.9963	0.9969	
N12−H18	0.9947	0.9949	
C19−021	1.3328	1.3315	1.23
C19−025	1.2197	1.2205	1.21
C20−022	1.3328	1.3315	1.24
C20−026	1.2197	1.2205	1.24
021−H23	0.9642	0.9653	
022−H24	0.9642	0.9653	

the data in Table 5 that there is no doubt about the nature of the excitation since the S−S bond distance increases by 0.4 Å upon excitation, whereas the changes in all other bond distances are insignificant. Similarly, it may be seen that the only noticeable change in bond angle upon excitation occurs in the S−S−C bond angles. The same trend is observed in the atomic charge densities of the ground and excited states of cystine in Table 7, where only the two sulfur atoms and the adjacent carbon atoms show meaningful changes upon excitation.

The cystine molecule was predicted to absorb UV light at around 349 nm and to fluoresce at around 707 nm. The most interesting feature of our computations is the prediction of fluorescence at around 700 nm for the isolated cystine molecules. Previous experimental observations for the cystine molecule in aqueous solution all show photodissociation for the hydrated ion. Fluorescence in the infrared region by an amino acid has not to our knowledge been observed before.

The photophysics and photochemistry of cystine were reviewed by Creed.[14] It seems that all experimental results refer to aqueous solutions of cystine either in acid or basic environments. It is found that cystine dissociates into a variety of products upon absorption of UV radiation. All experimental results indicate that the UV radiation causes photodissociation by breaking the S−S bond.

TABLE 6: Bond Angles of the Ground and First Excited Singlet States of Cystine

	ground state	excited state	expt[6]
S2−S1−C3	102.87	96.84	105.7
S1−S2−C4	102.86	96.84	104.1
S1−C3−C5	113.87	112.33	115.7
S1−C3−H7	104.61	107.07	
S1−C3− H8	107.86	107.69	
C5−C3−H7	108.48	108.14	
C5−C3−H8	111.66	110.92	
H7−C3−H8	110.10	110.64	
S2−C4−C6	113.85	108.14	116.8
S2−C4−H9	104.63	107.07	
S2−C4−H10	107.87	107.69	
C6−C4−H9	108.48	108.14	
C6−C4−H10	111.67	110.92	
H9−C4−H10	110.11	110.64	
C3−C5−N11	111.61	111.36	108.8
C3−C5−H13	105.81	106.08	
C3−C5−C19	109.64	109.10	114.5
N11−C5−H13	108.81	108.92	
N11−C5−C19	113.76	113.86	111.4
H13−C5 −C19	106.78	107.15	
C4−C6−N12	111.62	111.36	110.6
C4−C6−H14	105.81	106.08	
C4−C6−C20	109.63	109.10	114.8
N12−C6−H14	108.81	108.91	
N12−C6−C20	113.76	113.86	109.7
H14−C6−C20	106.79	107.15	
C5−N11−H15	115.63	115.88	
C5−N11−H16	116.03	115.93	
H15−N11−H16	112.68	112.79	
C6−N12−H17	115.63	115.88	
C6−N12−H18	116.03	115.93	
H17−N12−H18	112.68	112.79	
C5−C19−021	112.36	112.62	116.7
C5−C19−025	124.51	124.29	117.6
021−C19−025	123.10	123.04	125.7
C6−C20−022	112.36	112.62	115.6
C6−C20−026	124.51	124.29	117.3
022−C20−026	123.10	123.04	126.9
C19−021−H23	115.25	115.34	
C20−022−H24	115.26	115.34	

The excited-state wave function of the cystine molecule is obtained in the form

$$\Psi_{exc} = -0.12095\psi(60\rightarrow 64) + 0.68748\psi(63\rightarrow 64) \quad (2)$$

The major contribution to the excitation is due to molecular orbitals 63 and 64, which are both localized on the S−S bond. A minor contribution is due to a charge transfer from the NH_2 groups (molecular orbital 60) to molecular orbital 64.

It was proposed by Kosower and Kosower[15] that the interaction between the two filled S lone pair orbitals causes a splitting of their degenerate energy levels, which leads to the creation of a low-lying excited state. They also suggested that the excitation is due to a forbidden n $\rightarrow \sigma^*$ transition. From our pictorial representation of molecular orbitals 60, 63, and 64 in Figure 5 it may be seen that our results are consistent with the suggestions by Kosower and Kosower.[15] However, we find that the excitation is primarily due to a $\pi \rightarrow \sigma^*$ transition (orbital 63 → orbital 64). There is some n $\rightarrow \sigma^*$ character in this excitation also since the p atomic orbitals that combine to make orbital 63 are slightly tilted relative to each other. Also there is some contribution from orbital 60 → orbital 64. It should also be noted that our calculations predict an increase in S−S bond distance, but not a bond breakage. This variance with experimental results may again be due to the fact that experimental observations involve hydrated zwitterions.

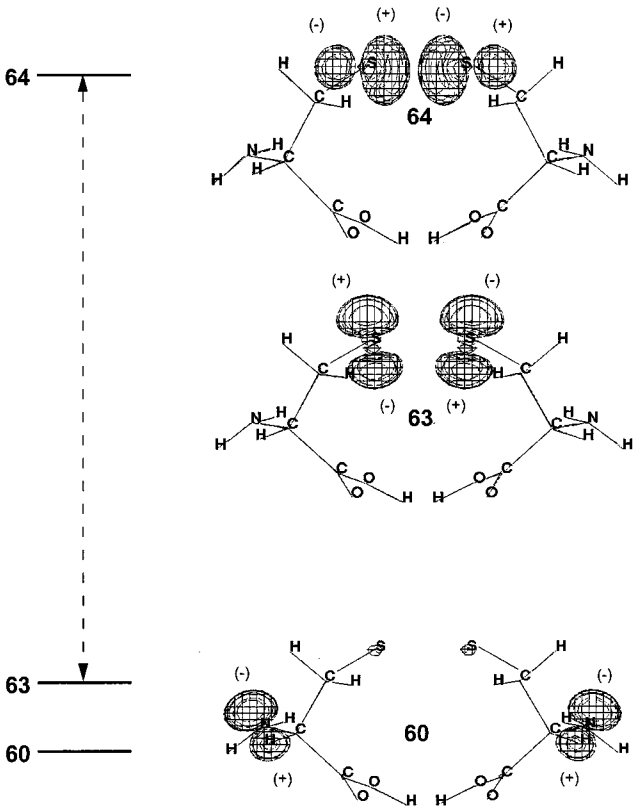

Figure 5. Pictorial representation of molecular orbitals involved in the fluorescence of cystine.

TABLE 7: Atomic Charges in the Ground and Excited States of Cystine

	ground state	excited state
S1	0.057 362	0.074 428
S2	0.057 626	0.074 440
C3	−0.545 445	−0.570 930
C4	−0.545 296	−0.570 931
C5	−0.050 921	−0.048 759
C6	−0.050 920	−0.048 752
H7	0.243 978	0.247 482
H8	0.255 431	0.259 463
H9	0.243 913	0.247 482
H10	0.255 367	0.259 462
N11	−0.820 245	−0.819 703
N12	−0.820 368	−0.819 700
H13	0.235 851	0.234 259
H14	0.235 836	0.234 256
H15	0.360 344	0.354 035
H16	0.342 132	0.343 253
H17	0.360 440	0.354 031
H18	0.342 145	0.343 251
C19	0.802 956	0.806 207
C20	0.803 024	0.806 202
O21	−0.752 857	−0.752 506
O22	−0.752 773	−0.752 512
H23	0.504 477	0.506 312
H24	0.504 421	0.506 317
O25	−0.633 228	−0.633 546
O26	−0.633 253	−0.633 541

We report the vibrational frequencies of the ground and excited states of the cystine molecule in Table 8. The relative intensities of the calculated modes are annotated by the same notation described for Table 4. The vibrational assignments were again derived by visual inspection of the normal modes. Because of the molecular symmetry, we identify pairs of vibrational modes, and the difference between the frequencies is an indication of the magnitude of the interaction between

TABLE 8: Computed Frequencies of the Ground and First Excited Singlet States of Cystine

	assignment	ground state	excited state
1	N−H stretch	3910.88	3910.71
2	N−H stretch	3910.71	3910.71
3	O−H stretch	3827.25***	3806.95**
4	O−H stretch	3803.02*	3782.35*
5	N−H stretch	3795.45	3794.94*
6	N−H stretch	3795.28	3794.76
7	C−H stretch	3368.63	3358.94
8	C−H stretch	3368.04	3358.37
9	C−H stretch	3293.09	3283.00
10	C−H stretch	3292.93	3282.87
11	C−H stretch	3230.52	3242.02
12	C−H stretch	3230.25	3241.99
13	C═O stretch	1884.93***	1878.68***
14	C═O stretch	1872.26**	1865.71**
15	N−H bend	1857.68	1856.07
16	N−H bend	1856.70**	1854.36**
17	C−H bend	1609.71	1605.01
18	C−H bend	1607.55	1599.20**
19	C−C stretch	1567.01*	1562.78
20	C−C stretch	1559.60	1559.79
21	C−H bend	1511.65	1513.56
22	C−H bend	1510.70*	1510.59**
23	C−H bend	1467.91	1460.47
24	C−H bend	1459.14	1445.57**
25	C−C stretch	1425.97	1426.24
26	C−C stretch	1423.41	1423.94*
27	C−H bend	1365.94	1350.12
28	C−N bend	1362.26	1345.49
29	C−O stretch	1314.96*	1310.14*
30	C−O stretch	1307.74**	1299.60**
31	C−H bend	1278.50**	1269.53
32	C−H bend	1276.26	1267.08*
33	C−N stretch	1242.67	1234.52
34	C−N stretch	1242.50*	1234.08*
35	C−C stretch	1094.79*	1081.40*
36	C−C stretch	1087.83*	1075.68*
37	C−C stretch	997.12*	979.39*
38	C−C stretch	988.40	975.82
39	C−C stretch	907.88*	916.19*
40	C−C stretch	899.31	908.84*
41	C−COOH bend	864.55**	800.60*
42	C−COOH bend	850.04**	788.51*
43	O−H bend	786.23*	878.97**
44	O−H bend	785.43*	857.91**
45	NH_2 wag	769.08***	761.80***
46	NH_2 wag	748.77***	747.72***
47	O−C−O bend	696.82	702.15*
48	O−C−O bend	693.20	698.21
49	S−C stretch	647.61	655.18
50	S−C stretch	645.63*	652.89
51	C−O bend	560.82	563.82
52	C−O bend	559.05	561.94
53	C−N stretch	479.02	468.76*
54	C−N stretch	475.48*	462.68
55	S−S−stretch	451.96	333.93
56	C−C bend	378.55	376.59
57	C−C bend	371.67*	364.58*
58	C−N bend	303.01*	296.22*
59	C−N bend	297.84	294.36
60	N−H bend	231.91	233.19*
61	N−H bend	219.46**	220.69**
62	C−S−S−C bend	198.09	197.56
63	C−S−S−C bend	196.96	187.23
64	C−C−N bend	187.60	166.33
65	C−C−N bend	183.75	157.71
66	NH_2 wag	162.56	130.42
67	COOH wag	149.34	126.76
68	COOH wag	121.26	122.46
69	torsion	90.69	104.99
70	torsion	78.54	83.22
71	torsion	64.77	65.25
72	torsion	41.21	61.59

TABLE 9: Computed Fluorescence and Excitation Energies

	cysteine		cystine
	6-31G	6-31G*	6-31G
ground-state energy (ground-state geometry)	−719.174 887	−719.363 500	−1437.233 537
ground-state energy (excited-state geometry)	−719.127 200	−719.308 797	−1437.206 064
excited-state energy (ground-state geometry)	−718.947 065	−719.118 219	−1437.103 107
excited-state energy (excited-state geometry)	−718.975 262	−719.141 969	−1437.141 671
excitation energy (cm^{-1})	50 001	53 811	28 624
flourescence energy (cm^{-1})	33 347	36 601	14 133

the modes. We were unable to find experimental information on the IR and Raman spectra of cystine and were unable to derive a set of semiempirical correction factors for the frequencies. We recommend therefore that the computed frequencies are multiplied by an average correction factor of 0.905 in order to optimize the accuracy of the frequency predictions.

c. Experimental Cystine Flourescence. The fluorescence centered around 725 nm is presented in Figure 1 for excitation at 350 and 366 nm for the three different solvents. The relative intensities of the three spectra presented in Figure 1 are a function of the completeness of the suspension. Thus, DL-cystine was more easily suspended in 2-propanol than in either isobutanol or water. Since no buffer was employed, the cystine was insoluble in the aqueous solution, which presumably retards the hydrolysis of the disulfide bond. The spectra are reproducible in all three solvents regardless of excitation wavelength. None of the solvents exhibited any fluorescence in the reported spectral region.

4. Discussion

The electronic absorption and fluorescence spectra of cysteine and cystine were calculated using quantum chemical techniques. The calculated absorption and fluorescence frequencies for cysteine match experimental observations. However, the calculations predicted a fluorescence peak for cystine at around 700 nm that had not been observed before. On the basis of our calculations we performed a detailed experimental study of the fluorescence of cystine. We acquired spectra from a suspension of cystine in three different solvents and observed fluorescence at around 700 nm.

The fluorescence spectra of cystine presented in Figure 1 show considerable structure. The larger features of these spectra are reproducible. It is plausible that these features are vibrational structure. One would expect that the structure in these spectra would be the result of transitions from the excited state $\nu = 0$ to a ground state $\nu = 1$. Thus the spectra should be directly related to the ground-state vibrational manifold. There appears to be some evidence that this is in fact the case. The peak at 820 nm is approximately 1700 cm^{-1} from the main peak at 720 nm. This is approximately the vibrational frequency corresponding to the C═O stretching mode, which has a fairly large vibrational intensity in the ground state. The peak at 745 nm is also very reproducible. This corresponds to a shift of approximately 460 cm^{-1}, which is quite close to the frequency of the S−S stretching vibrational motion in the ground state. The peak at 760 nm corresponds to a frequency shift of 730 cm^{-1}, which may correspond to NH_2 wagging motions, which also have large calculated IR intensities.

According to Creed,[14] the UV absorption of cystine in aqueous solution has been observed at a variety of wavelengths in the vicinity of 300 nm. Our calculated value for the isolated

molecule is 349 nm. However, since the experimental and the theoretical results pertain to different molecular environments, a direct relationship between the calculated and observed absorption maxima may not be meaningful. The fluorescence peak of tyrosine and phenylalanine is also located near 300 nm. This would explain why the fluorescence of these latter molecules is quenched by cystine.

The lack of experimental data for the cysteine and cystine molecules prevents a direct comparison between our computed geometric parameters and corresponding experimental quantities. However, we present the computed geometrical parameters for the two molecules in order to provide a useful estimate of the structure of the isolated species.

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