desorption peak. Similarly, the desorption of hydrogen at 280, 300, and 375 K is probably triggered by reactions iii, iv, and v, respectively.[42] Indeed, the desorption of hydrogen at 300 K is a minor piece of evidence supporting the decomposition of η^1-(N)-NHCHO to η^1(N)-NCHO at this temperature. This scheme is also consistent with large amounts of HD in the 375 K peak (cf. Section III.A.3), since reaction v produces hydrogen adatoms that will recombine with adatoms on the surface, and the latter will be primarily deuterium rather than hydrogen. We emphasize once again that the problem of sample contamination (and, to a lesser degree, the difficulty of peak deconvolution) makes a more quantitative analysis of the amounts of H_2, HD, and D_2 desorbed in each peak impossible.

V. Conclusions

Following a saturation formamide exposure on the clean Ru(001) surface at 80 K, approximately 0.15 monolayer of formamide decomposes. This decomposition occurs via two distinct mechanisms. The major one, accounting for 0.10 monolayer of formamide that decomposes at saturation, is the only decomposition mechanism that occurs at low coverage and may be written as follows (the temperatures given are for the low coverage limit, and the second step is clearly not elementary):

$$NH_2CHO(g) \xrightarrow{80\ K} \eta^2(C,O)\text{-}NH_2CO + H$$

$$\eta^2(C,O)\text{-}NH_2CO \xrightarrow{230\text{-}250\ K} CO + NH + H + NH_3$$

$$NH_3 \xrightarrow{315\ K} NH_3(g)$$

$$CO \xrightarrow{480\ K} CO(g)$$

$$NH \xrightarrow{350\text{-}400\ K} N + H$$

$$2H \xrightarrow{420\ K} H_2(g)$$

$$2N \xrightarrow{770\ K} N_2(g)$$

(42) The fact that an H_2 thermal desorption peak occurs at 300 K following saturation exposures supports the idea of a dehydrogenation reaction occurring at this temperature and thus, indirectly, supports the presence of an N-bonded NHCHO species which converts to an N-bonded NCHO species.

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Table IV. Vibrational Frequencies (cm^{-1}) and Mode Assignments for NH and ND on Ru(001) (This Work) and Ni(111)[32]

	Ru(001)			Ni(111)		
mode	NH	ND	Shift	NH	ND	Shift
ν(NH)	3315	2460	1.35	3340	2480	1.35
δ(NH)	1350	1050	1.29	1270	950	1.34
ν(M-NH)	690	680	1.01	620	580	1.07

At saturation, approximately 0.08 monolayer of NH and 0.02 monolayer of NH_3 are produced.

For exposures greater than 2 L, where the amount of formamide that decomposes is greater than approximately 0.05 monolayer, a second decomposition mechanism occurs. In this mechanism, η^1(O)-bonded molecular formamide, formed from formamide adsorption at 80 K, converts near 225 K to an intermediate believed to be an N-bonded NHCHO species. At 300 K, this intermediate converts to an intermediate tentatively identified as η^1(N)-NCHO, a species analogous to NH but with the hydrogen atom replaced by a formyl group. This intermediate decomposes near 375 K to coadsorbed carbon monoxide, and nitrogen and hydrogen adatoms. For exposures greater than 4 L, where the amount of formamide that decomposes is greater than approximately 0.09 monolayer, some molecular desorption of η^1(O)-NH_2CHO occurs also at 225 K.

The reactions and surface intermediates observed in formamide decomposition on clean Ru(001) and on Ru(001)-p(1×2)-O are quite different. However, η^1(O)-NH_2CHO is formed on both surfaces under certain conditions of coverage and temperature. The amount of formamide that decomposes following a saturation exposure increases by a factor of 3, from 0.05 to 0.15 monolayer, in going from the oxygen covered to the clean surface.

Acknowledgment. The authors are grateful to Dr. Malina Hills for assistance in obtaining the ammonia thermal desorption spectra. This research was supported by the National Science Foundation (Grant No. CHE-8516615) and the donors of the Petroleum Research Fund, administered by the American Chemical Society.

Registry No. NH_2CHO, 75-12-7; Ru, 7440-18-8; NH_3, 7664-41-7; CO, 630-08-0.

Resonance Raman Spectroscopic Study of Bilirubin Hydrogen Bonding in Solutions and in the Albumin Complex

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Abstract: Resonance Raman spectra are reported for bilirubin in chloroform, dimethyl sulfoxide, and aqueous solutions, and for the 1:1 bilirubin/albumin complex and the bilirubin complexes of α- and β-cyclodextrin. From the known hydrogen-bonding patterns in the several free solutions, Raman markers for the presence or absence of internal hydrogen bonding are derived. From equilibrium and time-resolved deuteration, partial assignments of the spectra are proposed. The resonance Raman spectrum of the bilirubin/albumin complex demonstrates that the internal hydrogen bonds between propionate groups and the pyrromethenone rings are ruptured. Propionate hydrogen bonding is to amino acid residues of the protein only.

(4*Z*,15*Z*)-Bilirubin IXα (structure **1a**), commonly called bilirubin, is the final product of hemoglobin metabolism.[1] The molecule is only sparingly soluble in aqueous solutions, although the dipropionate anion (structure **1b**) is soluble in alkaline solution. Bilirubin is carried through the bloodstream to the liver as an albumin complex. In the liver the water-soluble glucuronic acid ester is formed. Bilirubin diglucuronide is then transported to the kidneys and excreted in the urine.

Historically, bilirubin has been considered as merely a toxic waste product[2] with an interesting chemistry. If bilirubin is not

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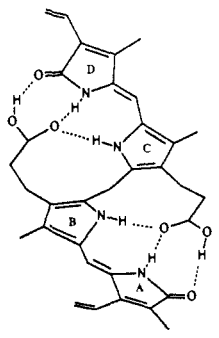
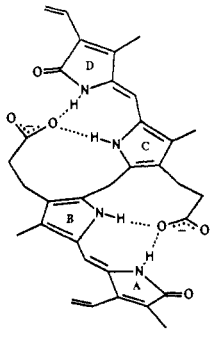


1a



1b

efficiently excreted, the excess bilirubin can cross the blood–brain barrier, causing irreversible brain damage. An important natural fail-safe mechanism for preventing this catastrophe is accumulation of bilirubin under the skin, where it undergoes a photoisomerization about one or both exo C=C double bonds to water-soluble isomers, which can be excreted.[2a] When used as a clinical procedure, the photoisomerization is called phototherapy.

The low solubility of bilirubin in water is a consequence of the strong internal hydrogen bonds, depicted in structure **1a**. Photoisomerization yields isomers in which these hydrogen bonds cannot form, so that the molecule becomes water soluble. Consequently, the details of the hydrogen bonding, the conformation of bilirubin in various solvents and complexes, and the details of photoisomerization have all been the objects of intensive study.

Stocker and co-workers have recently suggested that circulating bilirubin may have a protective role as a scavenger of peroxyl free radicals.[3] They found that at 2% oxygen, a micromolar concentration of bilirubin is a better inhibitor of free-radical-initiated lipid oxidation than α-tocopherol. This proposed new role may further increase interest in the chemistry of bilirubin and its complexes.

The internally hydrogen-bonded bilirubin structure, shown as **1a**, has been demonstrated by X-ray crystallography.[4] The molecule has two planar pyrromethenone systems, at an angle of about 96° to each other, and is maintained by six hydrogen bonds. A similar structure is maintained in crystals of the dipropionate salts of bilirubin.[5] However, only four internal hydrogen bonds remain, and the angle between the pyrromethenone systems increases to about 97°.

Extensive NMR studies demonstrate that bilirubin retains its internally hydrogen-bonded structure in chloroform[6a] and other nonpolar solvents. In chloroform, the lactam protons are exchanged with deuterium (CH_3OD) rapidly, while the less exposed pyrrole protons are exchanged at a slower rate.

In dimethyl sulfoxide, on the other hand, the internal hydrogen bonds are ruptured and replaced by hydrogen bonds to Me_2SO oxygen.[6b-d] However, insertion of solvent molecules into the solution complex perturbs but does not change the overall conformation of the molecule. The two pyrromethenone systems remain at a fixed angle, rather than freely rotating about the bridging methylene group.

The intramolecular hydrogen bonds are sufficiently strong that bilirubin is appreciably soluble in aqueous solution only at alkaline pH. The experimental facts of the acid–base chemistry of the propionic acid residues are not disputed, but the interpretation remains controversial.[7] Some authorities[7a] propose a propionate monoanion as the dominant species in the pH 7–9 region, while others[7b] argue that both propionic acid residues are deprotonated in this region. There is general agreement that the dianion is the major species in the pH 9–11 region and that deprotonation of the lactams is not important below pH 11.

Bilirubin is carried through the bloodstream as a complex between one or two bilirubin dianions and serum albumin.[2,8] The equilibrium properties of the complex are well known. Despite intensive study, how bilirubin dianion binds to albumin remains incompletely understood. Lamola and co-workers found that the quantum yield of fluorescence from the complex of bilirubin increases only moderately with solvent viscosity.[9] They calculated that the observed viscosity effect could be completely explained by the effects on the protein motion and concluded that the bilirubin must be completely enclosed in the albumin. In addition, Jacobsen and Brodersen observed that the absorption spectrum of bilirubin/albumin 1:1 underwent a blue shift, rather than the red shift predicted for an exposed chromophore.[10] Further evidence for this view was presented by Tran and Beddard whose circular dichroism studies have suggested that bilirubin is rigidly held in place.[11] Our own recent surface-enhanced Raman scattering (SERS) studies in the bilirubin system are consistent with bilirubin completely enclosed by albumin.[12]

It has usually been assumed that bilirubin is held in place in albumin complexes by some combination of hydrogen bonding and ring stacking.[8,13] Few studies provide unambiguous evidence for any particular structure. Recently, Lauffer and co-workers[14] have shown that *rac*-iron(III) *N,N'*-ethylenebis[(5-bromo-2-hydroxyphenyl)glycinate] forms an albumin complex at the high capacity bilirubin binding site. From the known conformation of this molecule, they proposed that bilirubin binds to albumin in a similar extended conformation, in which internal bilirubin hydrogen bonds must be broken. Presumably these would be replaced by bonds to amino acid residues on albumin, as first suggested by Jacobsen[13] on the basis of the large negative enthalpy of binding for the complex.

This proposal is quite different from the suggestion of Lightner and co-authors.[15] They advocate a positive chirality internally hydrogen-bonded isomer of bilirubin dipropionate on the basis of the circular dichroism of the complex.

Resonance Raman spectroscopy would appear to be a useful tool for resolving this question and, possibly, others about the bilirubin–albumin complex. Free bilirubin is nonfluorescent, but quite photosensitive. The albumin complex is sufficiently fluorescent that the measurement of resonance Raman spectroscopy is difficult. The photosensitivity of bilirubin further complicates the spectroscopy. Photoisomerization of bilirubin in the albumin complex occurs in less than 200 ps.[16] Photoisomerization is significant at any wavelength in the 400–500-nm region.[17] Acquisition of a spectrum of single photoisomer is difficult, if not impossible, with excitation in this region. Photoisomerization is

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Table I. Resonance Raman Shifts (cm^{-1}) of Bilirubin and Complexes in Different Environments

H_2O	D_2O	$CHCl_3$	$CHCl_3$[a] (CH_3OD)	Me_2SO	α-cyclodextrin[b]	β-cyclodextrin[c]	HSA[d]
961	950	951	951	963	963	963	961
993	988	990	986	994	993	994	993
1052	1052	1050	1048	1054	1049	1051	1055
1113	1115	1114	1114	1112	1114	1112	1112
1144	1144	1142	1142	1148	1145	1145	1140
1190	1191	1191	1191	1189	1189	1188	1189
1240 sh	1240	1247	1247	1244	1240	1240	1241
1271	1274	1268	1273	1266	1271	1271	1268
		1286	1286 sh				
1340	1336	1341	1338	1328	1340	1340	1348
1358 sh	1362	1360	1358	1344	1358	1358	1360
1441	1436	1436	1436	1440	1438	1438	1438
1460	1453	1450	1449	1455	1458	1457	1454
	1488	1498	1495	1492			1490
1560 sh	1560	1559	1559	1560	1560	1560	1562
1577	1568	1570	1571	1585	1579	1578	1586
1616	1613	1615	1614	1615	1615	1616	1615

[a] CH_3OC exchange in $CHCl_3$ solution. [b] Bilirubin/α-cyclodextrin complex. [c] Bilirubin/β-cyclodextrin complex. [d] Bilirubin/human serum albumin complex.

further complicated by photosensitized oxygenation[18] if air is not complete excluded from the system. This process is facile at wavelengths within the absorption band.

Margulies and Toporowiz[19] have reported the preresonant Raman spectra of bilirubin in $CHCl_3$ and of the bilirubin dianion in alkaline solution, using a flow cell. Ishitani and co-workers obtained the spectra of bilirubin in gallstones.[20] Similar spectra have been reported by Zheng and Tu.[21]

Our own preresonant inverse Raman spectra of free bilirubin in chloroform and dimethyl sulfoxide and bilirubin ditauride in aqueous solution[22] were the first investigations of the effects of differing hydrogen-bonding environments on the Raman spectrum. Several bands were proposed as markers for internal hydrogen bonding to various groups on the molecule. Preliminary empirical band assignments were made but have been partially revised on the basis of more recent studies of normal and surface-enhanced Raman spectra of the system.[12]

Infrared spectra of bilirubin and bilirubin salts, mostly as the solids, have been reported by several groups and summarized in the review literature.[1] Only fragmentary band assignments are available from this literature. Zheng and Tu have presented rather general assignments for bilirubin Raman spectra but make no attempt to distinguish various C=C environments, for example.[21] As part of an extended study of biliverdin,[23] Margulies and Toporowicz presented approximate normal coordinate analyses of biliverdin, its conjugate acids, and the two pyrromethenones which compose the molecule. The bands are in reasonable agreement with the experiment.

In the present paper, we report the preresonant Raman spectrum of the bilirubin/albumin complex. We interpret our results in light of refined Raman spectra and band assignments for bilirubin in several environments. We use these measurements to describe the hydrogen bonding and conformation of bilirubin in the human serum albumin complex.

Experimental Section

Bilirubin, human serum albumin (fatty-acid free, from globulin-free albumin), α-cyclodextrin, and β-cyclodextrin were obtained from Sigma.

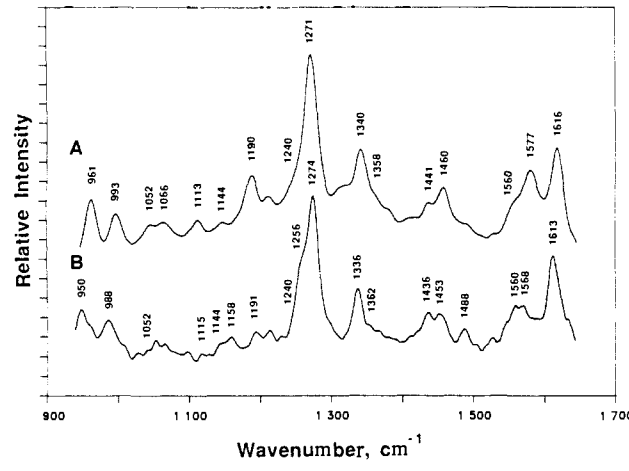

Figure 1. Resonance Raman spectra of 5×10^{-4} M (A) bilirubin dicarboxylate, pH 10; (B) bilirubin dicarboxylate, pD 10 (excitation wavelength, 514.5 nm).

Deuterated methanol, CH_3OD, was spectral grade from Sigma. Deuterium oxide, D_2O, was spectral grade from Cambridge Isotope Laboratories. All other reagents were ACS Reagent Grade and were used as received. Type I water was used to prepare all solutions.

Most spectra were obtained at bilirubin concentrations of 5×10^{-4} M in each solvent or solution. Aqueous solution spectra of free bilirubin were obtained in solutions at pH 10 or pD 10 obtained by adjusting the solution pH with sodium hydroxide. Bilirubin/albumin complexes were 1:1 in each component and were used in phosphate buffer, pH 7.4. Bilirubin/cyclodextrin spectra were obtained at pH 10 from solutions containing 5×10^{-4} M bilirubin and 5×10^{-2} M cyclodextrin.

The Raman spectrograph has been previously described.[12] The monochromator was operated with spectrograph entrance slit width 150 μm, approximately 5 cm^{-1} resolution. Argon ion laser (Lexel 85-1) excitation at 514.5 nm was used in all experiments. The instrument was calibrated at least once during each sequence of measurements, using the Raman spectrum of indene[24] to establish wavenumber shifts. Where necessary, fluorescence background was removed from spectrum files numerically. The fluorescence signal was subtracted by fitting several points on the Raman/fluorescence spectrum to a cubic and using the least-squares polynomial to define a background for subtraction.

An optical fiber system was used to irradiate the sample and collect scattered light. Laser power was restricted to 20–30 mW at the sample. Samples were deaerated with nitrogen gas. Most spectra were acquired with 100-s integration time. The samples were continuously stirred during exposure to laser light.

Deuterium exchange experiments, using CH_3OD in $CHCl_3$, followed the protocol of Navon and Kaplan.[6a] In these experiments, spectra were

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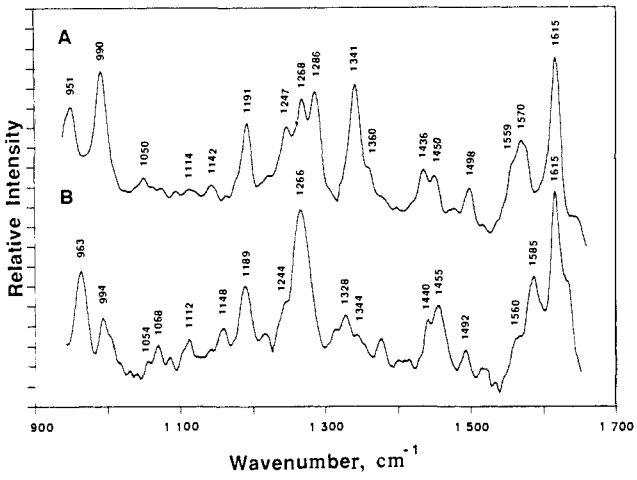

Figure 2. Resonance Raman spectra of 5×10^{-4} M (A) bilirubin in chloroform; (B) bilirubin in Me_2SO (excitation wavelength, 514.5 nm).

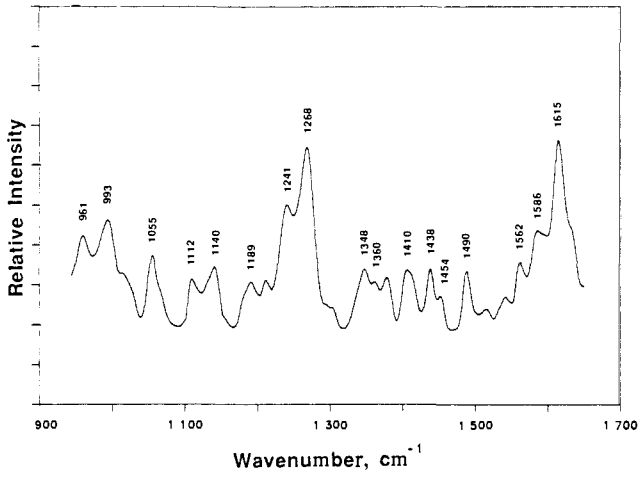

Figure 3. Resonance Raman spectrum of bilirubin/albumin complex at 5×10^{-4} M (excitation wavelength, 514.5 nm).

integrated for 20-s intervals. The integration was continuously repeated for the duration of each experiment, about 7 min.

Results

The Raman spectra of bilirubin in aqueous solution (pH 10), deuterium oxide (pD 10), chloroform, and dimethyl sulfoxide are shown in Figures 1 and 2. In addition to the bands in the 900–1700-cm^{-1} region shown here, there are broad bands (not shown) around 690 cm^{-1}. In general, the spectra are in good agreement (± 2 cm^{-1}) with spectra obtained under similar conditions in our own or other laboratories. Band positions are summarized in Table I.

We were able to obtain good Raman spectra with 514.5-nm excitation, using the fiber optic probe in stirred solutions. At 514.5 nm, photoisomerization is inefficient,[17] so that our spectra are of the *Z,Z* isomer with little or no contamination from the photoisomers.

However, with excitation at 514.5 nm, only the strongest bands were visible in static solutions. Even with rapid stirring, excitation at 496 or 488 nm produced spectra which contained additional bands from photoisomerization and/or decomposition products in addition to the bilirubin spectrum.

The bilirubin chromophore has about 120 normal modes. In our spectra there are shoulders on many bands, as well as several bands which are sufficiently broad that they are clearly composed of two or more unresolved components. We have listed the strongest bands, and noted the presence of shoulders, where appropriate.

The resonance Raman spectrum of the bilirubin/albumin complex is shown as Figure 3. The background fluorescence which is approximately 100-fold more intense than the Raman

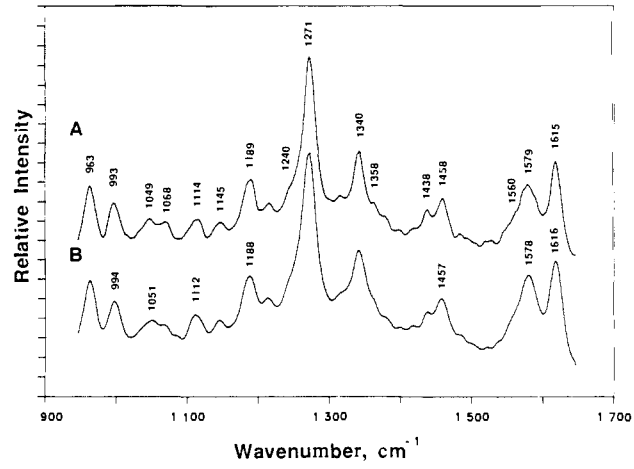

Figure 4. Resonance Raman spectra of 5×10^{-4} M bilirubin in 5×10^{-2} M (A) α-cyclodextrin; (B) β-cyclodextrin solution (excitation wavelength, 514.5 nm).

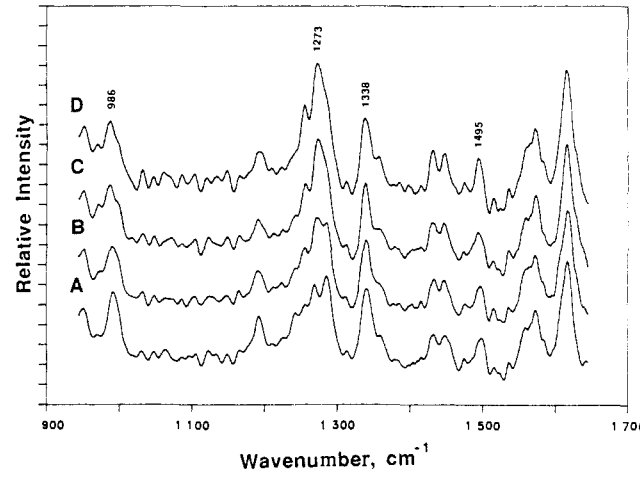

Figure 5. Time-resolved resonance Raman spectra of deuterium exchange in 5×10^{-4} M bilirubin in chloroform, following addition of 100 μL of CH_3OD to 6 mL of solution: (A) $t = 0$ s, (B) $t = 20$ s, (C) $t = 60$ s, (D) $t = 360$ s.

signal, has been subtracted from the spectrum of Figure 3. The bilirubin/albumin Raman spectrum differs from the spectrum of the bilirubin dianion in solution. In particular, the bands at 1268, 1348, 1454, 1490, and 1586 cm^{-1} are shifted from their frequencies in the free anion spectrum. Relative intensities are also quite different.

The resonance Raman spectra of bilirubin complexes with α-cyclodextrin and β-cyclodextrin are shown as Figure 4. The Raman spectra of these complexes are quite similar to the Raman spectra of free bilirubin dianion.

Two sets of experiments were performed to further identify bands sensitive to hydrogen bonding. First, the spectrum of bilirubin in deuterium oxide was measured. The protons on the lactam and pyrrole nitrogens are exchanged for deuterium, shifting most bands in the spectrum. Only the bands at 1052, 1113, 1190, 1560, and 1616 cm^{-1} are unaffected, or shifted less than 3 cm^{-1}.

Because these experiments do not distinguish pyrrole protons from lactam protons, we employed time-resolved exchange in chloroform,[6a] as shown in Figure 5. Within 1 min, frequency shifts in the bands at 990, 1268, and 1498 cm^{-1} and intensity increases in the band at 1268 cm^{-1} were completed. By the criteria of Navon and Kaplan,[6a] we identify these as lactam bands. As discussed below, the 1268-cm^{-1} band is composed of two or three incompletely resolved bands, identified by its most prominent component. The band at 1341 cm^{-1} continues to increase for about 6 min, identifying it as a pyrrole mode. Our measurements do not unambiguously define other bands as containing major contributions from a pyrrole or lactam N–H.

Discussion

Bilirubin Raman Spectrum Band Assignments. We define enironment-sensitive bands as the bands which vary over a range of at least 4 cm^{-1} among water, chloroform, and dimethyl sulfoxide. Identifying the bands by their frequencies in water, we find strong or medium intensity bands at 961, 993, 1271, 1340, 1441, 1460, and 1577 cm^{-1}. There is an environment-sensitive band at ca. 1490–1498 cm^{-1} which does not appear in the spectra of aqueous solutions. There are weaker or unresolved environment-sensitive bands at 1052, 1144, 1240 (sh), and 1358 cm^{-1} (sh). The bands at 1113, 1190, 1560 (sh), and 1616 cm^{-1} vary by 3 cm^{-1} or less. These are considered environment-insensitive.

The 961-cm^{-1} band is strongly environment-sensitive. In chloroform the band appears at 951 cm^{-1}, but in aqueous solution it appears at 961 cm^{-1}. The band serves as a marker for presence or absence of a hydrogen bond from a carboxylic acid to the opposite pyrromethenone ring. In dimethyl sulfoxide the band appears at a similar frequency, 963 cm^{-1}. Because in both Me_2SO and aqueous solution there is no hydrogen bond from an internal proton to the lactam oxygen, the band must be a lactam mode which involves the C=O moiety. In deuterium oxide, a new band appears at 950 cm^{-1}, while the 960-cm^{-1} band appears as a shoulder. This resolution into two bands suggests that the 961-cm^{-1} band has two unresolved components, from the dissimilar A and D lactam rings of the chromophore.

The 993-cm^{-1} band is also a lactam mode, with some environment sensitivity. The deuterium exchange rate in $CHCl_3$ confirms the lactam ring character. The deuterium dependence in aqueous solution is also consistent with lactam ring participation, involving the C–N bonds. This band is a marker for the presence or absence of hydrogen bonds to lactam N–H, although it is less sensitive than the 961-cm^{-1} band.

We are not yet able to assign the weak 1052-cm^{-1} band. It is only slightly sensitive to the hydrogen-bonding environment and is too weak for us to follow its deuterium exchange rate confidently.

The weak 1113-cm^{-1} appears to be largely insensitive to hydrogen bonding.

There are several overlapping weak bands in the 1140–1160-cm^{-1} region. The most prominent of these is the 1144-cm^{-1} band. However, in D_2O buffers, the most prominent band is at 1158 cm^{-1}, with the lower frequency band appearing only as a shoulder.

The 1190-cm^{-1} band does not change in different hydrogen-bonding environments, and may be ring modes with large components of C–C stretches.

The 1271-cm^{-1} band is several incompletely resolved bands, identified by the major component in aqueous solution. We include the shoulders at 1240 and 1256 cm^{-1} in this band. In $CHCl_3$ the first two bands are unresolved, leading to a band at 1247 cm^{-1} as well as the main band at 1268 cm^{-1}. A second intense band 1286 cm^{-1} in chloroform suggests that there are further unresolved bands around 1270 cm^{-1} in aqueous solution. This system is identified as lactam ring modes, from its rate of deuterium exchange in chloroform, and from the different relative intensities in aqueous solution, chloroform and Me_2SO.

Because deuterium exchange rate dependence in chloroform is slow, the 1340-cm^{-1} band is a pyrrole mode. This band shifts to 1328 cm^{-1} in Me_2SO, but in $CHCl_3$ it is 1341 cm^{-1}. The band is sensitive to the change from internal to external hydrogen bonds. It is probably closely related to the ν_4 mode in pyrrole, which is largely a symmetric C–N stretch. However, Margulies and Toporowicz[23b] calculate that this mode should occur at 1405 cm^{-1}. We can find no band in this region.

The 1441-cm^{-1} band is observed at 1440 cm^{-1} in Me_2SO, but at 1436 cm^{-1} in $CHCl_3$, thus identifying it as a mode involving the lactam C=O moiety.

The 1460-cm^{-1} band also is sensitive to lactam carboxyl hydrogen bonding, shifting to 1455 cm^{-1} in Me_2SO and 1450 cm^{-1} in chloroform. This band is composed of lactam C–N and C–C stretches.

We cannot find a 1490-cm^{-1} band in aqueous solution. However, in D_2O there is a band at 1488 cm^{-1}, and corresponding bands in Me_2SO (1492 cm^{-1}) and chloroform (1498 cm^{-1}). In chloroform, deuterium exchange leads to a −3-cm^{-1} shift, which is complete in 1 min. Therefore, this band is primarily localized on the lactam moiety.

The C=C stretching region, 1550–1620 cm^{-1}, contains several closely spaced and incompletely resolved bands. Following Margulies,[23b] we tentatively assign the lower frequency bands to C=C stretches of the pyrrole moieties. The frequency of the 1560-cm^{-1} band is independent of hydrogen-bonding environment, consistent with this assignment.

The 1577-cm^{-1} band is extremely sensitive to the environmental changes. It is shifted −7 cm^{-1} in chloroform, but to 8 cm^{-1} higher in Me_2SO. Although the environment sensitivity makes it unlikely that this band is a pure C=C stretch, a ring mode containing coupled C=C and C=O stretches is plausible. In any event, the pattern is consistent with localization on the lactam moieties.

The 1616-cm^{-1} band is not sensitive to environment or deuteration. We early[22] assigned it as an exo C=C stretch. That assignment is quite reasonable and in good agreement with Margulies and Toporowicz.[23b] There is a shoulder visible at 1630 cm^{-1}, which may be the other exo C=C stretch.

Hydrogen Bonding in Bilirubin Complexes. Even though definitive band assignments are not yet possible, it is possible to use the resonance Raman spectra to monitor changes in the bilirubin environments. The differences in the spectra among chloroform, dimethyl sulfoxide, and aqueous solutions provide empirical markers for different hydrogen-bonding environments. Independent of the details of the assignments, the bands at 961, 1328–1360, and 1577 cm^{-1} provide useful guides to the state of hydrogen bonding around the chromophore.

From a circular dichroism study, Lightner and co-workers[25] propose that in the cyclodextrin complexes, internal hydrogen bonding is retained. We have previously reached a similar conclusion from the surface-enhanced Raman spectra of the complexes. Our resonance Raman data support retention of internal hydrogen bonding in the complexes. The spectra are quite similar to those of the free anion, with band shifts of no more than ±3 cm^{-1}. However, the equilibrium constants of the bilirubin/cyclodextrin complexes are unknown. Although we work with a 100-fold excess of cyclodextrin and observe the absorption spectra characteristic of these complexes, rather than of free bilirubin, we cannot be certain that our solutions do not contain some free bilirubin dianion.

By contrast, the spectrum of the bilirubin/albumin complex is quite different from the spectrum of bilirubin anion or either bilirubin/cyclodextrin complex. In fact, the spectrum bears a strong resemblance to the spectrum of bilirubin in dimethyl sulfoxide. In particular, the 1577-cm^{-1} band is shifted to 1586 cm^{-1} in the complex. As in Me_2SO, but not in aqueous solution, there is a band around 1490 cm^{-1}. The major lactam bands at 961 and 993 cm^{-1} are at frequencies appropriate to bilirubin lacking proton hydrogen bonds to the lactam carbonyls.

It is risky to draw conclusions from intensities in the absence of an excitation profile. We note that the relative intensities in the spectrum of the albumin complex are closer to the pattern for the free molecule in Me_2SO than to the pattern in aqueous buffers. For example, in Me_2SO and in the albumin complex, the 1615-cm^{-1} band is more intense than the 1268-cm^{-1} band. Whether this reflects changes in the origin of the band, the damping constant or the excited-state geometry remains uncertain. However, the overall appearance of the spectrum is clearly not that of the free anion.

Conclusions

From the Raman spectra, we conclude that internal hydrogen bonds in bilirubin are broken. This conclusion rests on the similarities between the spectrum of the bilirubin/albumin complex and the spectrum of free bilirubin in dimethyl sulfoxide. In Me_2SO the NMR evidence[6b,c] is that one or more solvent molecules is bound to the propionic acid and to the lactam or the pyrrole

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residues of the opposite pyrromethenone system. The solvent bridges the propionic acid and the pyrromethenone to form a structure which is perturbed from the usual intramolecular hydrogen-bonded conformation.

However, the resonance Raman spectra provide information about the chromophores only. The spectra contain no propionate or central bridge methylene bands or bands directly attributable to bound water or amino acid residues, if any. While Raman spectroscopy demonstrates that the intramolecular hydrogen bonds of the aqueous dianion are broken in the albumin complex, it does not directly reveal where the propionates are.

It is known that the primary bilirubin binding site is in the region of residues 190–240.[8b] This region contains several lysines and arginines. It has previously been proposed that bilirubin propionates bind to these sites.[8,13] Our Raman spectra provide further evidence that this binding occurs, and that there are no new hydrogen bonds to the bilirubin lactam or pyrrole groups.

The recent circular dichroism studies of Lightner and coworkers[15] may be interpreted as measuring the angle between extended pyrromethenone groups, rather than demonstrating retention of internal hydrogen bonds. With this interpretation, the circular dichroism can be reconciled with the model compound studies of Lauffer[14] and with our own results.

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Mn(I)-Induced 1,6-Demethanation across the CC Triple Bond of Linear Alkynes in the Gas Phase. A Case for the Generation of Manganese Cycloalkynes?†

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Abstract: Complexes of Mn(alkynes)$^+$ were generated in the gas phase and found to exhibit a reactivity which is even richer than that of the analogous Fe(alkyne)$^+$ species. Among the many unimolecular dissociations, the Mn$^+$-induced demethanation of 4-octyne is of particular interest. The study of isotopomers and the effects of alkyl chain lengths reveals the operation of an unprecedented 1,6-elimination mode across the CC triple bond, and the experimental results may be explained by invoking the intermediate generation of the as yet unknown metallacycloalkynes. The implications of the unexpected, rich gas-phase ion chemistry of Mn$^+$ with regard to theoretical models are discussed.

The gas-phase chemistry of naked transition metal ions with organic substrates remains a major focus in quite different areas encompassing catalysis, organic, organometallic, and theoretical chemistry.[1] While most transition metal ions M$^+$ readily undergo oxidative insertion (eq 1) in CX bonds (X = hydrogen, carbon,

$$M^+ + \underset{X}{\overset{C}{|}} \underset{\text{reductive elimination}}{\overset{\text{oxidative addition}}{\rightleftharpoons}} \overset{+}{M}\overset{C}{\underset{X}{\diagdown}} \qquad (1)$$

halogen, nitrogen, oxygen, etc.), Mn$^+$ shows a distinctly lower reactivity toward these substrates.[1d,2,3] The decreased reactivity of Mn$^+$ in comparison with, for example, Fe$^+$, Co$^+$, or Ni$^+$ has been explained by using several qualitative theoretical concepts. Allison and Ridge[1g,2c] ascribe the reduced reactivity of Mn$^+$ to the quite high promotion energies to generate from the $3d^54s^1$ ground-state configuration of Mn$^+$ either an $3d^44s^2$ or an $3d^44s^14p^1$ excited state, which may then be used to form two covalent bonds. Based on this concept and taking into account results from a generalized valence bond approach,[4] it was indeed possible to establish an inverse relationship between the transition metal ion reactivity and the promotion energies of M$^+$. Armentrout[1f,2f] and Weisshaar[2e] explain the low reactivity of Mn$^+$ in terms of a frontier orbital MO concept, developed earlier by Saillard and Hoffmann.[5] According to this, the metal insertion of a $3d^n s^0$ configuration is favored over that with a $3d^{n-1}s^1$ because of *two* favorable donor–acceptor interactions in the former. These are the interaction of the doubly occupied σ_{CX} orbital (donor) with the *empty* M^+_{4s} (acceptor) and that of a *doubly occupied* d_{xz}, say, (donor) with the empty σ^*_{CX} (acceptor). Spin conservation also requires double occupancy of the d_{xz} which can only occur for metal ions ($3d^n$, high spin) with $n > 6$, i.e., for Fe$^+$, Co$^+$, and Ni, but not for the ground-state Mn$^+$ ion.

† Dedicated to Professor Klaus Hafner, Darmstadt, on the Occasion of his 60th Birthday.

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