Infrared Spectral Hole Burning and Change of Conformation in Simple Amino Acid Salts

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We show that it is possible to produce infrared spectral holes in a new class of compounds, amino acid hydrochlorides (containing some deuterium). The holes form as the result of conformational changes in the crystals, and the ability to produce holes leads to assignments of the N−D and O−D stretching bands.

Introduction

Amino acids are important constituents of many systems of interest, particularly biological ones. The assignments of the major infrared bands of the acids and polypeptides (carbonyl bands and amide I, II, etc.) have long been available.[1,2] These appear below about 2000 cm^{-1}. The higher wavenumber regions of the spectrum, the region of the A and B bands, have been much less characterized, and indeed the region below about 3000 cm^{-1} is one of the "almost continuous bands,"[1] which are rather weak. Very detailed studies by Mizushima et al.[3] examine many of the possible isotopic combinations for the simplest amino acids, glycine and glycine hydrochloride, but this study, like most of the others, assigns only bands below 2000 cm^{-1}. An additional difficulty in assigning the spectrum is that many of the bands shift a great deal on changing the hydrogen-bond interactions; for example, by changing solvent. To make progress, at least some of the myriad of bands need to be definitely assigned. We show that we can burn spectral holes in amino acid hydrochlorides and use these to make assignments.

The early work on isotopic substitution and assignment has been extended by numerous workers—often with the goal of understanding the hydrogen bonding of the amino acids and their salts. Of most interest to us are the systematic studies of Dupuy and Garrigou-Lagrange[4,5] and of Khanna et al.[6] on the hydrochlorides of glycine and L-alanine. Both groups have investigated the hydrogen stretching bands. The number of peaks in the region of the stretching bands does not correspond to a simple assignment, and the likely conclusion is that the many bands seen are due to Fermi resonance and other types of combination.[4]

The crystal structures of both glycine and L-alanine hydrochloride are well-known.[7,8] The compounds crystallize in space group $P2_1/c$ and $P2_12_12_1$, respectively, with all the atoms in general positions. The neutron diffraction results for glycine hydrochloride and the X-ray results for both compounds are for room temperature. There is no evidence for a phase transition as the temperature is lowered, and so we assume that the low-temperature crystal structures are very similar to the room temperature ones. The hydrogen bonding parameters are shown in Table 1. There are three N−H hydrogen bonds, one of which is a weak bifurcated bond, an arrangement typical of amino acid crystals.[9,10]

In our previous work, we have studied ammonium compounds. These are prepared with about 4% deuterium to form some NH_3D^+.[11] Irradiation of one of the N−D stretching bands of a cold sample with a low power laser usually produces a conformational change and a concomitant spectral hole. This hole then identifies the position of an N−D stretching band of a definite conformation of the molecule. Recently, we have been using a difference frequency laser to produce the infrared radiation, with a spectral line width of about 1 cm^{-1}, and this laser allows us to explore a wider range of the infrared.[12] The power density at the sample is about 80 mW/cm^2. The spectrometer and sample arrangement have been described previously.[13]

TABLE 1: Hydrogen Bonding Geometry and Deuterium Stretching Frequencies

A−H···B	A···B[b] (Å)	∠A−H···B[b] (deg)	A−D stretch[c] (cm^{-1})
Glycine Hydrochloride			
Neutron Diffraction[a]			
$N-H^1\cdots Cl^-$	3.189 (1)	170.8 (1)	2238
$N-H^2\cdots Cl^-$	3.140 (1)	166.4 (2)	2187
$N-H^3\cdots Cl^-$ [d]	3.300 (1)	126.7 (2)	2368
$N-H^3\cdots O$[d]	2.992 (1)	131.6 (2)	
$O-H\cdots Cl^-$	3.004 (1)	178.5 (2)	2190
X-ray Diffraction[f]			
$N-H^1\cdots Cl^-$	3.183 (9)	162.8 (1)	
$N-H^2\cdots Cl^-$	3.164 (2)	105.8 (9)[e]	
$N-H^3\cdots Cl^-$ [d]	3.225 (9)	174.1 (7)[e]	
$N-H^3\cdots O$[d]	2.723 (4)	91.9 (2)[e]	
$O-H\cdots Cl^-$	3.068 (1)	175.2 (3)	
L-Alanine Hydrochloride			
X-ray Diffraction[f]			
$N-H^1\cdots Cl^-$	3.186 (4)	162.3 (5)	2229
$N-H^2\cdots Cl^-$	3.181 (1)	162.2 (7)	2201−2204 (?)
$N-H^3\cdots Cl^-$ [d]	3.258 (8)	114.0 (2)[e]	2362
$N-H^3\cdots O$[d]	2.964 (1)	148.2 (1)[e]	
$O-H\cdots Cl^-$	3.018 (3)	163.8 (4)	2175

[a] Reference 8. [b] The numbers in parentheses are the errors in the last digit quoted taken from refs 7 and 8. [c] This work. [d] The hydrogen atom participates in both of these bonds. [e] These angles are subject to large error due to uncertainty in the H-position. [f] Calculated from the data in ref 7.

The detailed mechanisms that result in hole burning are not well enough understood at the moment to allow predictions, and so the possibilities for hole burning are identified empirically. We now find that we can hole burn the N−D bands of some amino acid hydrochlorides.

Experiments

The salts were crystallized by dissolving the amino acid in slightly warm concentrated HCl containing a bit of D_2O. The crystals formed on cooling were mulled in mineral oil and then put between KBr plates. The spectra were checked using crystals prepared using different amounts of D_2O to make sure that only molecules containing a single deuterium atom were present. In the limit of low deuterium concentration, the crystals

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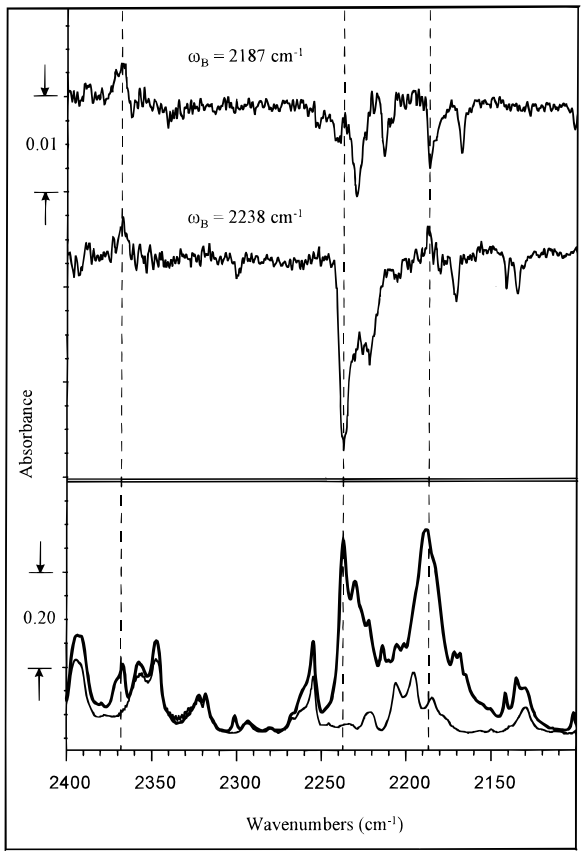

Figure 1. Infrared spectral hole burning results of ~4% deuterated glycine hydrochloride in the N−D stretching frequency region at 7 K. The absorption spectra of ~4% deuterated glycine hydrochloride (dark line) and of the undeuterated species (light line) are shown in the bottom panel. The upper panel shows the difference spectra from hole-burning experiments with laser frequencies set at 2187 and 2238 cm^{-1}. Obvious antiholes appear at 2368 and 2187 cm^{-1}. A smaller antihole is thought to be at 2238 cm^{-1}. The laser irradiation time was $^1/_2$ to 1 h. Note the differences in the vertical scales between the absorption spectra and the difference spectra. Note also the presence of additional holes.

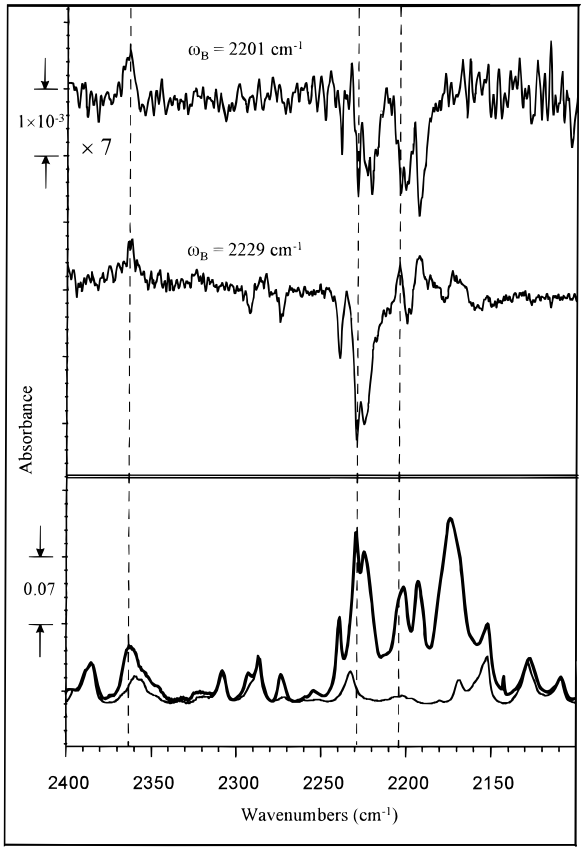

Figure 2. Infrared hole burning in L-alanine hydrochloride. Compare to Figure 1. The laser frequencies were set at 2201 and 2229 cm^{-1}. The fiducial line near 2201 cm^{-1} is at 2204 cm^{-1} to put it through the center of the apparent hole and antihole. The top spectrum is of seven coadded spectra, and so the effective scale is 1.4×10^{-4} absorbance unit, rather than the 1×10^{-3} abs of the middle set of difference spectra. Additional holes appear in these spectra as well.

should show three N−D stretching bands due to the NH_2D group, one for each of its three possible orientations, and one OD band. The final deuterium ratio was estimated at about 4%.

The spectra of the glycine hydrochloride and the L-alanine hydrochloride were taken under somewhat different conditions: the glycine hydrochloride spectra were taken with a MCT detector, and the L-alanine hydrochloride spectra were taken with an InSb detector. The two detectors have different spectral ranges and different amounts of detector noise. The spectra taken with the MCT detector were therefore averaged over longer times to compensate.

The infrared bands due to deuterium can be identified from the spectrum of a deuterium-containing sample by subtracting the spectrum of a sample without deuterium. The difference spectra show fewer bands but still far too many to assign (Figures 1 and 2). We irradiated a number of the deuterium-related bands and found that as with the ammonium compounds, spectral holes were produced (Figures 1 and 2). For example, irradiating the deuterium-sensitive band at 2238 cm^{-1} in glycine hydrochloride (for $^1/_2$ hr) yields a hole at that position (Figure 1). Small antiholes are produced at 2368 and 2187 cm^{-1}. On the other hand, irradiating at 2187 cm^{-1} yields an antihole at 2368 cm^{-1} and a complicated pattern at about 2238 cm^{-1}. A number of other holes are also formed, for example at about 2140 cm^{-1}.

The L-alanine hydrochloride gives rather similar results. In particular, irradiation of the L-alanine at about 2229 cm^{-1} gives similar spectra to irradiating the glycine at 2238 cm^{-1}. However, irradiating the alanine at a lower frequency (2201 cm^{-1}) gave much smaller holes than the comparable irradiation for the glycine. Here we coadded the results of seven irradiation experiments. For each one, the sample was irradiated for about $^1/_2$ hr and then scanned for 80 s. By redoing the experiments and adding, we avoided very long irradiation times that might produce second-order effects or otherwise change the sample. The result of adding the spectra is in effect to expand the scale by another factor of 7; i.e., the spectral holes in the top panel of Figure 2 are a factor of 7 smaller than they would appear on the scale of the second set of difference spectra. The holes produced by the irradiation at 2201 cm^{-1} are particularly noisy. We picked 2201 cm^{-1} to match the peak in the absorption (see the figure). However, the minimum in the hole spectrum and the maximum in the antihole are at about 2204 cm^{-1} and that is where we drew the fiducial line. We cannot determine the exact position of the hole because of the noise.

For the compounds containing NH_3D^+, the hole burning involves the rotation of the ammonium group, with the D occupying a new position. For the amino acid salts, we expect each of the three major NH_2D bands to burn by a similar mechanism; that is, the NH_2D rotates upon irradiation. We do not expect the O−D band to burn in the same way.

Assignments

Hole burning at the various positions near 2200 cm^{-1} results in an antihole near 2370 cm^{-1} in both compounds. The antihole marks the "weak" hydrogen-bonded position (Table 1). Note that assigning this band from the absorption spectrum alone would be nearly impossible because of the many closely spaced bands—which in turn appear to be made up of a number of components. The low intensity of this band is consistent with the weakness of the hydrogen bond.[14]

In the glycine, the absorption peak at 2238 cm^{-1}, which matches the hole produced by the laser at the same frequency, is clearly due to one of the two hydrogens participating in the medium-strength hydrogen bonds. Again, this corresponds nicely to the peaks and holes at 2229 cm^{-1} in the L-alanine. The second medium-hydrogen-bonded position is more difficult to identify. For the glycine compound, a hole and an antihole at 2187 cm^{-1} are clear but do not correspond to the maximum of a simple absorption peak. The appearance of this compound peak changes on substitution of N^{15} for N^{14} with one component shifting and the other not,[15] and so we assign the nonshifting component of the peak to the O−D stretch. For the L-alanine, a burnable peak is at 2201 cm^{-1}, and it is this we assign to the third N−D stretch. The large absorption at 2175 cm^{-1} is the O−D stretch.

The many additional small holes produced on burning one of the putative N−D stretches (Figures 1 and 2) confirms that the N−D stretch character is mixed into many bands by Fermi resonance or other such mechanism. The multiple bands also reduce the intensity of individual bands, and this and perhaps the mixing of simple motions account for the difficulty in burning the amino acid salts. Comparison may be made to the Tutton salts for which substantial holes are produced within a few minutes with the same apparatus.[16] For the Tutton salts, even burning of the combination bands is much easier (requires shorter times) than burning the fundamental bands for the amino acid salts.

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