

Spontaneous self-association of adenine and uracil in polycrystals from low temperature FTIR spectra in the range below 1000 cm^{-1}

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Abstract

FTIR spectra of solid samples of co-crystallized adenine and uracil were measured at 10 K in the range below 1000 cm^{-1} . New bands ascribable to the N3H (uracil) and NH₂ (adenine) out of plane vibrations, which disappear upon D-exchange, were revealed in comparison with the spectra of pure polycrystalline adenine and uracil obtained in the same conditions. The observed changes relate to the same groups that establish the H-bonds in base pairs of naturally occurring nucleic acids, despite the presence of an extra proton donor NH-group in both molecules. The well-established empirical correlation between the out of plane NH vibrational frequencies and H-bond energies was successfully applied for estimation of the latter in the mixed crystal.

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1. Introduction

Self-association of the nucleic acid bases has been the subject of many previous studies in gas, liquid and solid states, using both experimental and computational techniques. The infrared technique, which is one of the main sources of information about the H-bonding properties, has been applied repeatedly to the study of inter-base association in solid state [1,2] and solutions [3,4]. However, instead of dealing with the naturally occurring nucleic acid bases, almost all previously reported studies looked at the pairing of their methyl derivatives, such as 1-methyluracil (or 1-methylthymine) and 9-methyladenine, as models of nucleic acid components [1,2,5]. Such approach has been motivated essentially by the idea that the presence of an additional donor site in the unsubstituted bases should considerably complicate the interpretation of the experimental results. Indeed, these extra groups can, in principle, also be expected to participate in H-bonding between the two interacting

bases. The absence of detailed experimental data obtained for the naturally occurring bases is then a strong motivation to use a different approach to look at this problem.

Low temperature has been consistently demonstrated to be one of the most important conditions for successful investigation of H-bonding interactions by spectroscopic methods. Indeed, the proton mode bandwidths usually decrease from hundreds of cm^{-1} at 300 K to tens of cm^{-1} at 10 K, thus revealing fine spectral details not observable at higher temperatures (see for example [6] and references therein mentioned). However, to the best of our knowledge, there were not previous spectroscopic studies on base pairing where the advantages of working at low temperature were fully exploited. On the other hand, the utility of using the out of plane NH bending spectral region to address this problem was recognized long ago [1], but the full capabilities of this approach were also not realized at that time, because of lack of low temperature measurements. A very important property exhibited by the bands due to the out of plane NH modes is that, in contrast to those due to the NH stretching vibrations, they do not change their molar integral intensity with the H-bond energy and thus remain narrow and well resolved,

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independently of the strength of the H-bond interaction [7]. This spectral region can then be used, for example, for a direct quantitative estimation of the relative abundance of different H-bonds in a given crystal [8,9]. On the other hand, like in the case of the NH stretching region, the peak positions of the bands due to out of plane NH bending modes are sensitive to the H-bond energy (and, consequently, to the H-bond geometry) and can be used to estimate this property on the basis of simple empirical quantitative relationships [7,10–12].

In the present study, the out of plane NH bending spectral region (below 1000 cm^{-1}) and low temperature work conditions were used together, to look at the uracil/adenine H-bond interactions in the uracil/adenine mixed crystal. Low temperature FTIR spectra of co-crystallized samples of non-substituted uracil and adenine were compared with the spectra of the pure crystals of the two nucleic acid bases in the same experimental conditions, revealing new bands in the studied spectral region. These bands, which were found to be sensitive to deuteration, could be assigned to out of plane vibrations of the N3H (uracil) and NH_2 (adenine) groups, therefore demonstrating the involvement of these two groups (which are responsible for base pairing in nucleic acids) in the inter-base H-bond interactions in the studied co-crystal. Noteworthy, the extra imine proton donor groups present in the two nucleic acid bases were found not to be involved in inter-base H-bond interactions, occupying local environments in the co-crystal that should strongly resemble those existing in the corresponding pure crystals.

2. Experimental

Equimolar (1:1) amounts of adenine and uracil were solved in water (or heavy water) at heating, and refluxed during approximately 17 h. Water was removed at cooling with a flow of gaseous N_2 and the resulting powder was dried in a desiccator. Relatively fast precipitation resulted in a fine polycrystalline powder. Deuterated samples were obtained by dissolving the non-deuterated powder in D_2O , at $80\text{--}90^\circ\text{C}$, followed by boiling with excess of heavy water ($10^{-4}\text{:}2.5\text{ mol D}_2\text{O}$) during approximately 1 h.

The infrared spectra of polycrystalline samples in a KBr (1:200) disc, attached to the cold finger of an APD Cryogenics closed-cycle helium refrigeration system with a DE-202A expander, were recorded with a Mattson Infinity (60AR series) FTIR spectrometer, with spectral resolution of 1 cm^{-1} . The temperature (10–300 K) was measured directly at the sample holder by a silicon diode temperature sensor connected to a Scientific Instruments temperature controller (model 9650). The sample temperature during registration of spectra was stabilized to within 0.2 K. The temperature-induced spectral changes observed for all substances were found to be reversible and highly reproducible.

The assignment of the out of plane NH bending bands in the spectra of pure uracil crystal follows our previously pub-

lished low temperature FTIR measurements on this system [6]. The out of plane NH bending bands in adenine spectra were studied in [13,14]; our low temperature measurements confirmed the general assignments previously reported.

3. Results and discussion

The low temperature (10 K) IR spectra of polycrystalline samples of pure adenine, co-crystallized uracil/adenine and pure uracil are shown in Fig. 1 (frames 1, 2 and 3, respectively). The result of band deconvolution (using mixed Gaussian–Lorentzian functions) of the spectrum of the mixture is also presented in the figure. Thin vertical lines show the bands of each component, whose peak positions were found unchanged in the spectrum of the mixture. The

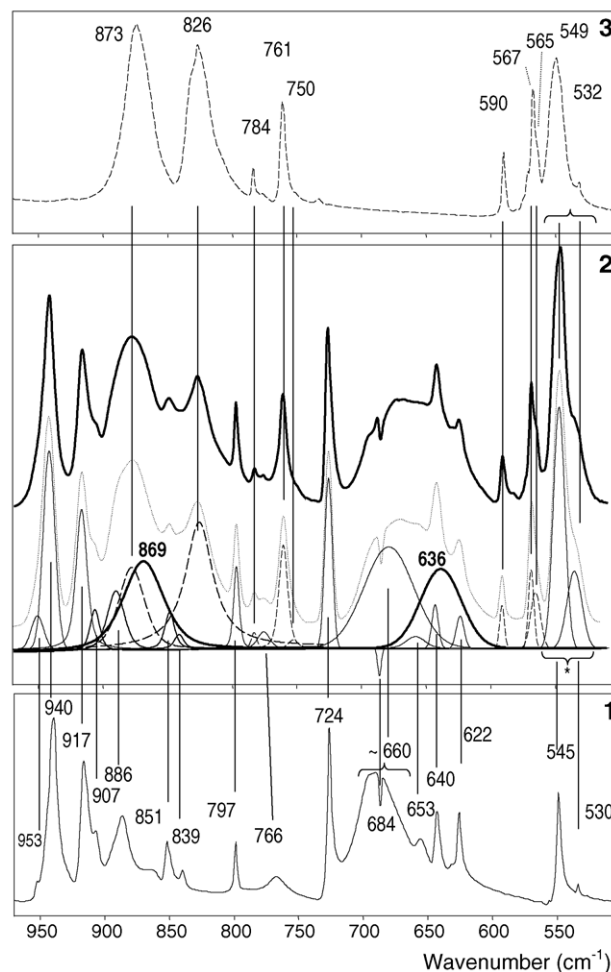


Fig. 1. FTIR spectra of polycrystalline adenine (frame 1), mixed uracil/adenine (1:1 molar) crystal (frame 2) and polycrystalline uracil (frame 3), at 10 K. In frame 2, the result of the spectral deconvolution (assuming mixed Gaussian–Lorentzian band profiles) is also shown. Thin vertical lines indicate band components with unchanged peak positions in both pure samples compared to the mixed crystal. The new band components observed uniquely in the mixed crystal are shown as solid thick lines in frame 2. (*) See text for discussion of this spectral region.

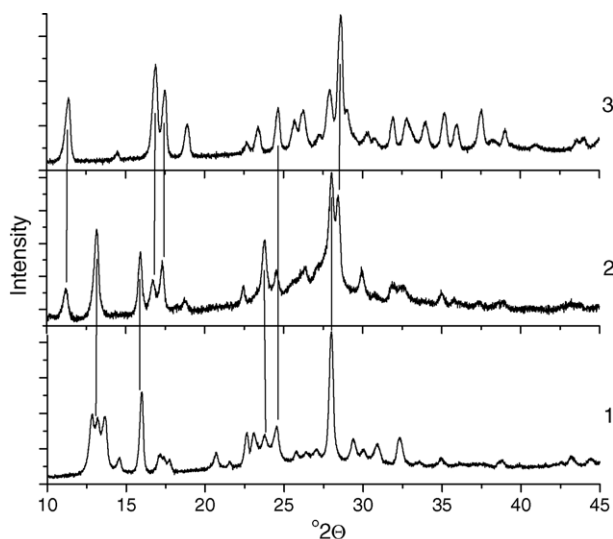


Fig. 2. X-ray powder pictures at 300 K of pure adenine (trace 1), mixed uracil/adenine crystal (trace 2) and pure uracil (trace 3). The vertical lines show peaks with different relative intensities in the mixed crystal relatively to the pure adenine and pure uracil powders.

frequencies of the bands that are only present in the spectrum of the uracil/adenine co-crystal are given in bold in frame 2.

It is clearly seen that the spectrum of mixture differs significantly from those of the pure components in the 900–800 and 700–600 cm^{-1} spectral regions. Despite it was not possible to obtain samples where the concentration of the mixed crystal was 100%, the X-ray powder diffraction data shown in Fig. 2 unequivocally reveal significant changes in both positions and intensities of several lines of the X-ray spectrum of the mixture (frame 2 of Fig. 2) relatively to pure adenine (frame 1) and uracil (frame 3) powders, thus confirming the formation of a new phase.

The changes observed in the IR spectra upon co-crystallization, in comparison with pure base crystals, reflect the specific uracil/adenine H-bonding interactions.

In the spectrum of pure uracil (frame 3 of Fig. 1), the bands at 873 and 826 cm^{-1} were assigned to out of plane bending modes of the N3H and N1H groups, respectively [6]. All the remaining bands observed in this spectrum have major contributions from skeletal modes ($\nu\text{C4-C5}$: 784 cm^{-1} ; $\gamma\text{C2=O}$: 761 cm^{-1} ; $\gamma\text{C4=O}$: 750 cm^{-1} ; δ_{ring} : 590 cm^{-1} ; $\delta\text{C2=O}$: 567/565 cm^{-1} ; δ_{ring} : 549 cm^{-1} and γ_{ring} : 532 cm^{-1} [15,16]). In the spectrum of the mixture (trace 2), it can be seen that the component of deconvolution at ca. 877 cm^{-1} , which relates to N3H group of uracil, considerably decreased its intensity relatively to the component at 827 cm^{-1} , which relates to N1H group. As most of the bands with major contributions from skeletal modes, the band due to the out of plane N1H vibration has its intensity practically unchanged relatively to the spectrum of pure uracil, indicating that the N1H group is not involved in inter-base H-bond interactions and must occupy a local environment in the co-crystal that shall strongly resemble that existing in the pure uracil crystal.

On the other hand, in this spectral range, a new band emerged at 869 cm^{-1} in the spectrum of the mixture (see frame 2 of Fig. 1). This band must be ascribed to the N3H group of uracil participating in an H-bond structure that differs from that of pure uracil, and is related with the uracil/adenine base-pair interaction.

In the studied range, the infrared spectrum of the pure crystal of adenine is considerably more complex than that of uracil. The assignments proposed by Hirakawa et al., which were based on extensive isotopic substitution spectroscopic studies [13] of this compound identified the bands corresponding to those observed at low temperature at 886 and ca. 660 cm^{-1} as being due to the out of plane bending modes of the N9H and NH_2 groups, respectively. This assignment is confirmed also with low temperature (100 K) measurements by Majoube [14]. All the remaining bands appearing in the spectral range shown in Fig. 1 (trace 1) were assigned by Hirakawa et al. to vibrations having dominant contributions of skeletal modes [13]. The band observed at 684 cm^{-1} shall deserve here some comments, since it is an interesting example of an Evans hole [17,18], that was not previously identified as that. Evans holes result from the interaction between certain vibrational energy levels, this interaction being appreciable in only a fraction of the molecules. Such a restricted perturbation is possible because one of the energy levels involved, which should be very sensitive to intermolecular interactions, may assume any of a wide range of energy values. At any instant, some of the molecules are in a suitable condition for a relatively strong perturbation to occur, while in most of the molecules the two levels are too far apart for appreciable interaction. Most of times, the sensitive mode to intermolecular interactions is associated with a group which participates in H-bonding, such the NH_2 group, while the second vibration is usually a skeletal mode with relatively low cross section in infrared and low sensitivity to intermolecular interactions [18]. This is exactly the situation occurring in adenine crystal, where the out of plane NH_2 vibration (absorbing at ca. 660 cm^{-1}) may interact by the above described mechanism with the skeletal twisting vibration of the five-membered ring of the molecule, which absorbs at 684 cm^{-1} [13]. Evans holes are easily recognizable since they appear as an inverse band superimposed on a broad intense band [17,18], and this is exactly what is observed—especially well at 10 K—in the case of the spectrum of the adenine crystal (see frame 1 in Fig. 1).

Comparing now the spectrum of pure adenine and mixed crystals, it can be seen that the band due to the out of plane mode of the N9H group (observed at 886 cm^{-1} [13,14]) has a relative intensity to the skeletal bands that is nearly the same in both crystals (this is clearly visible by comparing the results of the deconvolution of the spectrum of the mixed crystal shown in Fig. 1 with the spectrum of pure adenine crystal). Such result suggests that the N9H group of adenine is not influenced by the base-pairing interaction. On the contrary, the relative intensity of the band at ca. 660 cm^{-1} , (which, as already mentioned, is related to the out of plane NH_2 wagging mode), is appreciably different in the two crystals, as revealed

by the deconvolution of the broad complex band found in this spectral region in the spectrum of the uracil/adenine mixed crystal (see frame 2 of Fig. 1). This result is consistent with the participation of the NH₂ group of adenine in the base pairing. Accordingly, a new band is observed in the spectrum of the mixed crystal in this spectral region (with maximum at 636 cm⁻¹; see frame 2 of Fig. 1) that can be assigned to the out of plane NH₂ vibration of adenine molecules involved in a H-bonding structure which differs from that found in the pure adenine crystal.

Note that all above discussed bands assigned as NH-proton modes are strongly influenced by deuteration and their assignments are doubtless.

The relative intensities of the bands appearing near 550–530 cm⁻¹, in the spectra of the three crystals, shall also deserve here further comments. In fact, it is clear that the intensities of the two bands observed in this spectral region increase considerably in the mixed crystal relatively to the pure uracil and pure adenine crystals (see Fig. 1). For both molecules, the bands appearing at these frequencies have been assigned to vibrations with dominant contributions from skeletal coordinates [6,13–16] and are expected not to change appreciably their intensity upon local environment changes due to the base pairing. A possible explanation for this result is that these modes have effectively appreciable contributions either from the N3H (in uracil) or the NH₂ coordinates (in adenine).

In summary, when compared with the spectra of the pure crystals, two new bands were found in the spectrum of polycrystalline uracil/adenine co-crystallized mixture. These bands are due to the N3H proton out of plane bending mode of uracil (869 cm⁻¹) and NH₂ out of plane wagging vibration of adenine (636 cm⁻¹) molecules, and demonstrate that inter-base H-bonding in the mixed crystal involves directly these two groups as H-bond donors; on the other hand, the H-bonds established by both N1H of uracil and N9H of adenine should strongly resemble those existing in the corresponding pure crystals and not be involved in base pairing.

3.1. Estimation of H-bond energies in the mixed crystal

An approximate estimation of the energy of the inter-base hydrogen bonds in the mixed uracil/adenine crystal can be done using the empirical relationship between the H-bond energy (kJ mol⁻¹) and the blue shift of the out of plane mode involving the H-bond donor group (ν_4 : in cm⁻¹) relatively to the frequency of the same mode in the non hydrogen bonded species [11]

$$-\Delta H = 0.67\Delta\nu_4^2, \quad (1)$$

where $\Delta\nu_4^2 \equiv (10^{-2}\nu_4^H)^2 - (10^{-2}\nu_4^0)^2$, the superscripts H and 0 pertaining to H-bonded and free molecules, respectively. The calculation of the shifts in the position of ν_4 bands in the crystal state can be made relatively to their frequency (ν_4^0) in the monomers obtained from matrix isolation spectroscopy. In the case of the out of plane N3H vibration of uracil, the ν_4^0 reference value can be taken as 664 cm⁻¹ [19]. On the other hand, the mean value of the peak positions of the out of plane NH₂ proton modes of the matrix isolated adenine is ca. 530 cm⁻¹ [20]; this value is here accepted as ν_4^0 (NH₂). From equation (1), the energies of inter-base H-bonds in the uracil/adenine co-crystal can then be estimated as 21.0 and 8.3 kJ mol⁻¹ for N3H imine and amine NH₂ protons, respectively. As a result, the H-bond energy per mole of base pairs is estimated as ca. 29 kJ mol⁻¹, in good consonance with the experimentally determined value for uracil/adenine complex formation in solution (ca. 26 and 30 kJ mol⁻¹, in chloroform and tetrachloromethane solutions, respectively [3]). This result stresses once again the power of the method here presented to energetically characterize the H-bonding schemes occurring in complex molecular systems with biological importance, like those involving formed spontaneously by molecular self-assembly of nucleic acid bases.

The precise characterization of the nature of the inter-base arrangement in the mixed crystal is not possible to undertake from our experiments. According to ab initio calculations [21], the Watson–Crick (W–C) uracil/adenine pair is more stable than the Hoogsteen (HG) pair in vacuum (see Fig. 3 for

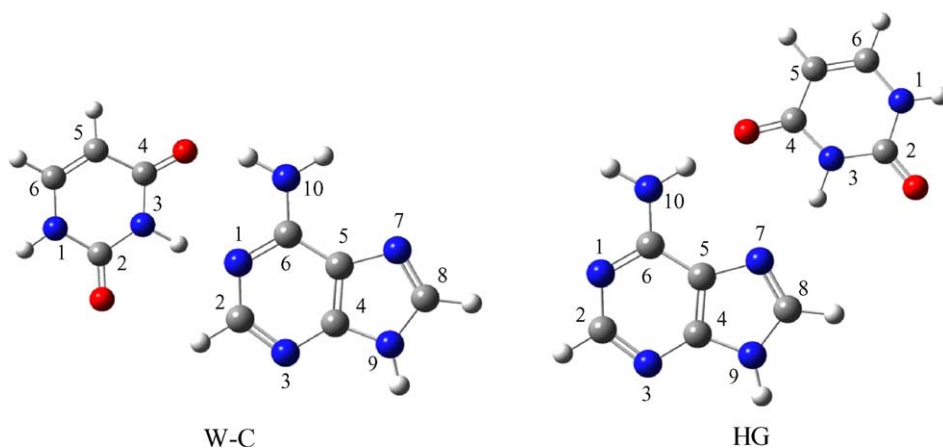


Fig. 3. Schematic representation of the Watson–Crick (W–C) and Hoogsteen (HG) uracil/adenine pairs, with atom numbering.

schematic structures of both W–C and HG pairs). However, the dipole moment of the HG pair is seven times as large as that of the W–C pair. When the effect of dipole–dipole interaction between solute and solvent is taken into consideration, the HG pair becomes preferable in the solution because of the stabilization based on this interaction. We can expect the same to happen in the solid (as was observed previously for 1-methylthymine/9-methyladenine mixed crystal [1]). However, it must be stressed that Sowerby et al. [22] were recently able to construct mixed uracil/adenine monolayers using a methodology similar to that used in the present study to obtain the mixed crystal, and observed that the resultant structures exhibited aperiodic structures; small crystalline domains within these monolayers were suggested to represent undetermined single phase configurations of the molecules, while the remaining aperiodic structures should be interpreted, geometrically, in terms of the 21 theoretically possible adenine/adenine (6), uracil/uracil (6) and uracil/adenine (9) hydrogen bonding interactions. In this case, the energy values now obtained shall represent an average value for nine putative inter-base pairing H-bonding interactions, weighted by their relative abundance in the co-crystal.

Despite that the precise characterization of the inter-base arrangement in the mixed crystal is not possible from infrared spectra, some new suggestions can be made on the experimentally observed fact that the intensities of both new bands (see Fig. 1) in the mixed crystal are approximately equal. In pure adenine [13,14] and cytosine crystal [23] the NH₂ band is essentially (twice) more intensive than the NH band, in line with the number of protons in each group, i.e. the molar intensities of NH proton modes per one proton in both groups of both base crystals are approximately equal. In isolated molecules of uracil and thymine in matrices the intensity of the N3H band is higher than that of N1H not more than ca. 35% (from data of experimental and computational works – ref. [18–20,24] in [6]). Because of the frequencies of the N1H (in uracil) and imine's out of plane proton mode bands are very close in all three crystals, it is reasonable to suggest that the molar intensities of these bands in uracil, adenine and cytosine are also close in magnitude. On this base, it can be expected that the intensities of the new bands of N3H of uracil and NH₂ of adenine in the mixed solid also must be essentially different in magnitude—in any arrangement there are one imine and two amine protons, which are forming the base pair. But this obviously contradicts the experimental spectrum shown on Fig. 1—the intensities of both new bands (see Fig. 1) in the mixed crystal are approximately equal. It means that the intensity per one proton mode of the NH₂ group is approximately twice less than that in N3H imine group. The possible reason for this might be involvement of both NH₂ protons in additional H-bonds, as it is shown in Fig. 4. The formation of one additional (bifurcated) H-bond increases the frequency (ν) of the bending vibration of the proton and lessens its amplitude (x) [in a first approximation $x \propto (\nu)^{-1/2}$] and, consequently, the corresponding band intensity. This arrangement can correlate with the “aperiodic” structures observed

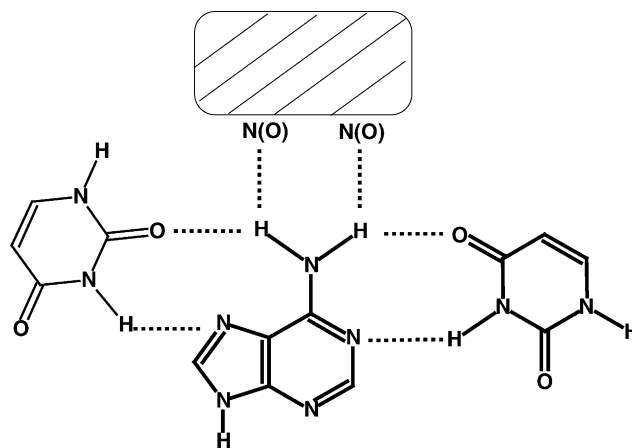


Fig. 4. Possible arrangement of H-bonding network with bifurcated H-bonds of the amine group of adenine in the uracil/adenine mixed solid.

by Sowerby et al [22] in the mixed adenine–uracil monolayers. It should also be noted that the involvement of the imine proton in “bifurcated” H-bonds was previously suggested in order to explain the different intensities of the ν_4 bands of N1H and N3H groups in crystalline uracil spectra [6].

4. Conclusion

The use of low temperature together with spectroscopic analysis of the out of plane NH bending region of the infrared spectra has been shown to constitute an easily accessible and extremely powerful methodology to energetically characterize the H-bonding schemes occurring in complex molecular systems with biological importance, like those resulting from molecular self-assembly of nucleic acid bases, such as the uracil/adenine co-crystal. Using the simple well-established empirical correlation between the out of plane NH vibrational frequencies and H-bond energies, the base pairing H-bond energy in the mixed uracil/adenine solid was estimated from the spectroscopic data as being ca. 29 kJ mol⁻¹, which is comparable to the previously obtained experimental values obtained in chloroform and tetrachloromethane solutions (ca. 26 and 30 kJ mol⁻¹, respectively [3]).

Acknowledgments

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