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Solid State NMR of a Molecular Self-Assembly: Multinuclear Approach to the Cyanuric Acid-Melamine System

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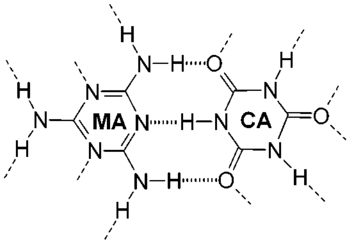
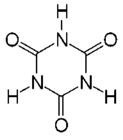
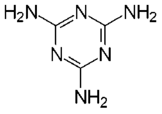
ABSTRACT



Trinuclear MAS NMR, involving naturally abundant (^{13}C, ^{15}N) and easily deuterated (^{2}H) nuclei, is shown to offer newer opportunities to study molecular self-assembly in noncrystalline supramolecular systems.

Supramolecular hydrogen-bonded assemblies are fascinating systems for understanding the science of self-assembly of structurally complement molecules and hold considerable potential for development of molecular devices.[1,2] Structural characterization of supramolecular structures is key to unravel the principles of molecular self-assembly. X-ray crystallographic analysis has provided considerable insights into the structural organization of self-assembled systems.[3,4] However, this technique becomes restrictive when the self-assembled species fail to form single crystals or are noncrystalline with poor solubilities. In view of this, exploration of alternative experimental approaches for characterization and derivation of structural information on molecular self-assembly is in order. Though the capability of solid-state NMR for such analysis is well recognized,[5] it has hitherto remained under utilized. In connection with our interest[6] in molecular assembly involving cyanuric acid and its analogues as DNA nucleobase mimics, we were in search of a technique to study self-assembles that defy crystallization. In this communication, it is demonstrated through the well-known example of cyanuric acid (**CA**)**:**melamine (**MA**) assembly,[4] that solid state magic angle spinning (MAS) NMR involving naturally abundant (^{13}C, ^{15}N) and easily deuterated (^{2}H) nuclei gives structural information in reliable agreement with that from X-ray technique and hence may offer newer opportunities to study noncrystalline molecular assemblies.


CA


MA

Cyanuric acid (**CA**), a six-membered cyclic imide with alternate arrangement of hydrogen bond donors and accep-

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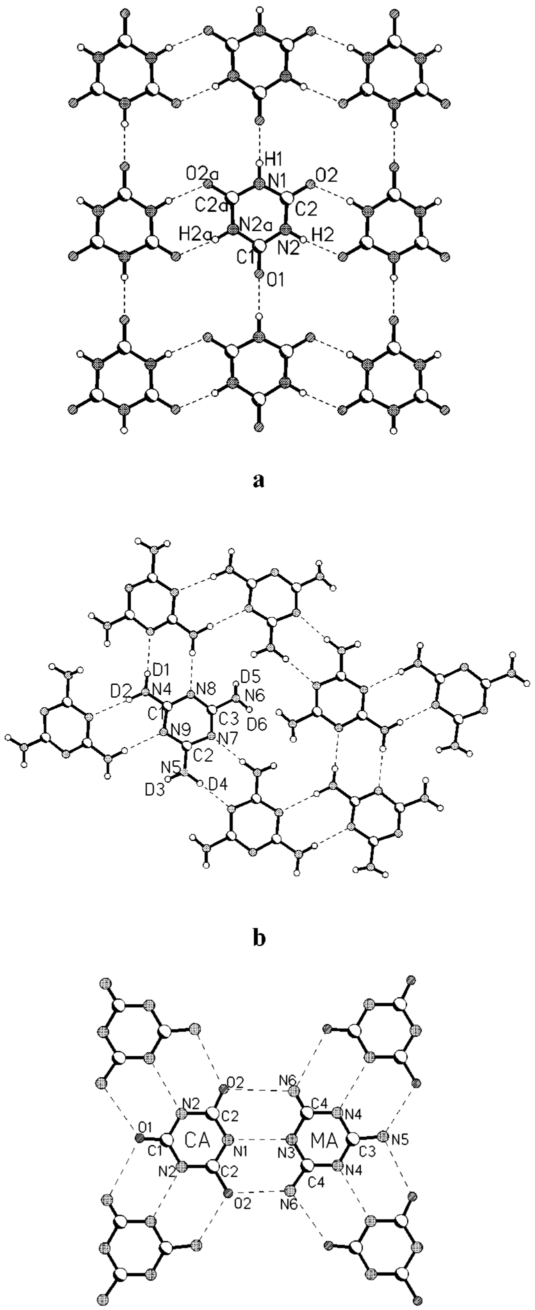
tors, forms a network of well-defined robust hydrogen-bonded systems arranged on a molecular tape[7] (Figure 1a).



a

b

Figure 1. Hydrogen bonding patterns in (a) cyanuric acid (**CA**), (b) melamine (**MA**), and (c) **CA:MA** 1:1 complex derived from their crystal structures.[7,8] In (c), D1 is linked to N1, D2 is linked to N2, D3 linked to N5, D4, D5 linked to N6.

The successive tapes are held together by single hydrogen bonds from C(1)O of one chain with N(1)H of the other chain. **CA** is symmetric in monomeric form, but in the hydrogen-bonded supramolecular form, the C(1) and N(1) atoms located on the 2-fold symmetric axis have a hydrogen-bonded environment that is different from other C(2) and N(2). Melamine (**MA**) the supramolecular hydrogen-bonded complement of **CA** is likewise symmetrical in monomeric form. In the supramolecular crystal[8] structure, the three exocyclic amino groups are hydrogen-bonded differently (Figure 1b), leading to a nonequivalence in the system. In the 1:1 **CA:MA** complex, the full complementation of hydrogen bond donor and acceptor sites leads to a hexagonally symmetrical rosette structure[4] (Figure 1c), and the asymmetry imposed by the supramolecular environment in the monomers disappears. The main endeavor of this paper is the application of ^{2}H, ^{13}C and ^{15}N solid state MAS NMR techniques to lyophilized, noncrystalline samples of **CA**, **MA**, and 1:1 **CA:MA** complex, to examine whether the NMR data reliably reflect the structural features seen in crystal structures. The results have implications for extending the solid state NMR as a potential technique to study molecular self-assembly in noncrystalline or poorly crystalline complexes.

^{13}C, ^{15}N and ^{2}H NMR of CA and MA. Figure 2 shows the cross polarization-magic angle spinning (CP-MAS)^{1}H−^{13}C (panel A), ^{1}H−^{15}N NMR spectra[9] (panel B), and MAS ^{2}H NMR[10] (panel C) of (i) **CA**, (ii) **MA**, and (iii) 1:1 **CA:MA** complex, lyophilized from methanol.[11] The ^{13}C, ^{15}N, and ^{2}H resonance assignments and chemical shifts derived for the donor and acceptor groups in **CA**, **MA**, and **CA:MA** are given in Table 1. Despite the chemical equivalence of C atoms in the monomer, the ^{13}C NMR spectra [Figure 2A (i) and (ii)] reveal two signals in a 2:1 intensity ratio.[12] This is in complete agreement with their crystal structures,[7,8] which show nonequivalence arising from a differently hydrogen-bonded environment in the supramolecular assembly. The 2-fold axis through the central C, N atoms of **CA** and **MA** rings facilitates the assignments to unique (C1, N1, D1) and symmetry-related (C2, N2, D2) atoms.[7,8]

In ^{15}N NMR, two signals in a 2:1 ratio are seen for the ring nitrogens of **CA** [Figure 2B (i)] and **MA** [Figure 2B (ii), δ 170−175], while the three exocyclic NH_2 groups of **MA** result in separate signals in the region δ 80−95. The observed 3-fold multiplicity for the $^{15}NH_2$ resonances in **MA**

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(11) (a) The high-resolution solid-state MAS experiments were performed on a Bruker DRX-500 FT-NMR spectrometer at ambient probe temperature (295 K). To reduce the prohibitively long proton T_1 (> 500 s) for an efficient data collection in the ^{1}H−^{13}C and ^{1}H−^{15}N CP-MAS experiments, paramagnetic Cu^{2+} doping was done by lyophilizing the nondeuterated samples with 0.01% $CuCl_2$. Spinning speed was maintained at 8 kHz. Typically 400, 4000, and 600 transients were accumulated for ^{13}C, ^{15}N, and ^{2}H experiments. Resolution enhancement with a sine-squared window function was used prior to Fourier transformation. (b) The samples of **CA**, **MA**, and **CA:MA** (1:1) were lyophilized twice from D_2O followed by methanol for recording the ^{2}H NMR spectra.

(12) The relative intensities in ^{13}C and ^{2}H MAS spectra were estimated by considering the center and spinning sideband intensities. The sideband intensities at 8 kHz spinning were found to be negligible in ^{15}N spectra. CP dynamics experiments in the 1:1 **CA:MA** complex show similar ^{13}C CP behavior for the ring carbons, irrespective of Cu^{2+} doping (T_{IS} = 5.1, 5.8 ms, $T_{1\rho}{}^{H}$ = 16.4, 16.7 ms), at 50 kHz rf field, thus validating ^{13}C relative intensity measurements at a single mixing time (1 ms).

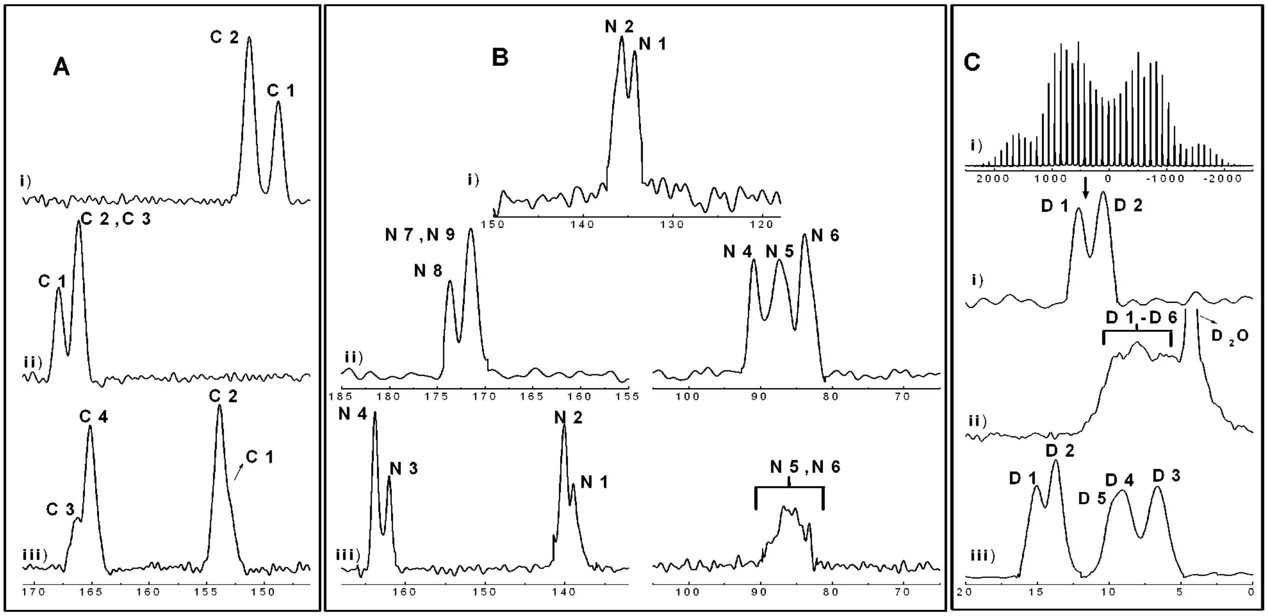

Figure 2. Trinuclear (11.7 T) ^{13}C (125.758 MHz) (A), ^{15}N (50.678 MHz) (B), and ^{2}H (76.773 MHz) (C) MAS NMR spectra of cyanuric acid (**CA**) (i), melamine (**MA**) (ii), and 1:1 cyanuric acid:melamine (**CA:MA**) complex (iii). The labeling of atoms for i, ii, and iii follow that shown in Figure 1a−c, respectively.

(N4−N6, Figure 2B) arises from a magnetic nonequivalence imposed on them by the supramolecular environment in which the three chemically identical amino groups individually form 2, 1, and no hydrogen bonds in the supramolecular network. This inequivalence is not reflected in the ^{15}N or ^{13}C resonances of ring atoms of **MA**, perhaps because of smaller chemical shift dispersion. Despite the complex arrangement of **MA** in the crystal, the ^{13}C and ^{15}N signals can be tentatively assigned on the basis of the hydrogen bond environments as well as strengths.[8] Intermolecular hydrogen bonding involving deuterons is evident from the correlation of observed ^{2}H chemical shifts with the hydrogen bonding distances (r_{D1-O1} = 1.758, r_{D2-O2} = 1.774 Å).[8]

^{13}C, ^{15}N, and ^{2}H NMR of CA:MA (1:1) Complex. ^{15}N chemical shielding effects have long been used as an effective tool for investigating H-bonding interactions.[13] In nucleosides, the significant shielding effects (lower frequency shift) observed for H-bonded pyridine-type N atoms and decreased shielding (high-frequency shift) of N-H moieties are considered to be characteristic of proton acceptor and donor sites, respectively.[14] In comparison with the asymmetric spectra of individual components [(Figure 2 (i) and (ii)], the solid state NMR of the 1:1 complex [(Figure 2 (iii)] exhibited a more symmetric pattern, expected of a molecular self-assembly (Figure 1c). In the 1:1 **CA:MA** complex, the ring nitrogens of **MA** (N3, N4) experience a large low-frequency shift ($\Delta\delta \approx -10$ ppm) compared to that in neat **MA** (N7−N9), whereas that of **CA** (N1 and N2) showed a reverse trend ($\Delta\delta \approx +5$ ppm). This is in agreement with their respective role as stronger H-bonding acceptor and donor. The ^{15}N chemical shift dispersion for amino nitrogen of **MA** in the complex is not large enough to resolve all the expected resonances, and hence a direct comparison of chemical shift differences upon complexation was rendered difficult.

Further evidence for hydrogen bonding in the **CA:MA** assembly is provided by the ^{13}C data, which displayed a lower frequency shift ($\Delta\delta = -2.5$ ppm) for the aromatic carbons of **MA** and a higher frequency shift ($\Delta\delta = +2.5$ ppm) for the ring carbons of **CA**. The small magnitude of $\Delta\delta$ in ^{13}C is attributable to the disposition of carbons that

Table 1. ^{13}C, ^{15}N, and ^{2}H Chemical Shifts in **CA**, **MA**, and **CA:MA** Complex[a]

	chemical shifts (ppm)		
	^{13}C	^{15}N	^{2}H
CA	151.3 (C2), 149.0 (C1)	135.7 (N2), 134.3 (N1)	12.0 (H1), 10.5 (H2)
MA	167.8 (C1), 166.2 (C2,C3)	90.9 (N4), 87.4 (N5), 83.9 (N6), 173.7 (N8), 171.6 (N7,N9)	9.7−5.6 (H1 − H6)
CA:MA	165.2 (**MA**), 153.6 (**CA**)	163.8, 162.0, 85.1 (**MA**), 140.1, 139.1 (**CA**)	8.5, 6.5 (**MA**), 14.9, 13.8 (**CA**)

[a] Chemical shifts are externally referenced with respect to TMS for ^{13}C and ^{2}H and liquid ammonia for ^{15}N. For assignments and labeling of carbons, nitrogens and deuteriums see Figure 1.

are two bonds away from the hydrogen-bonding site. The hydrogen-bonding effects are also seen from the anisotropic ^{13}C chemical shielding parameters that were obtained from experimental simulation.[15]

All the deuterium in the donor and acceptor groups of the **CA:MA** complex are detected with a 1:2 multiplicity for ^{2}H in chemically similar environments (Figure 2C), affirming the 2-fold symmetry in the **CA:MA** complex. A significant high-frequency shift ($\Delta\delta = 3$ ppm), decrease in quadrupole coupling constant (e^2Qq/h),[15] and increase in asymmetry parameter (η)[14] for the electric field gradient tensor observed for the N−D of **CA** unit is in accord with strong hydrogen bonds[16] in the supramolecular structure. The ^{2}H chemical shift dispersion for the amino deutrons are not large enough to resolve all the expected six resonances, whereas a good dispersion is seen for them in the **CA:MA** complex, with the observance of expected three resonances. The ^{2}H quadrupole coupling constant and asymmetry parameter for ^{2}H of ND_2 showed very little change[15] after complexation with **CA**, suggesting that the strength of hydrogen bonding for the amide group in the neat as well as the complexed structure is similar.

Significantly, the trinuclear NMR approach presented here discerns the number of crystallographically inequivalent C and ring N sites to be two each for **CA** and **MA** and the total number of nonequivalent deuterons (protons) to be 2 for **CA**. For the molecular self-assembly of **CA** and **MA**, four carbons, six nitrogens, and five protons are the minimum number of atoms required to represent a **CA:MA** repeat unit. Thus the three-dimensional NMR view of the **CA**, **MA**, and **CA:MA** molecular assembly is consistent with the inequivalence of **CA** and **MA** units seen in the crystal structure. Most importantly, in the X-ray study, ring carbons and nitrogens of **CA** and **MA** could not be distinguished from one another because of a perfect ring stacking in a three-dimensional connectivity of **CA** and **MA** in the rosette structure.[4] However using solid state NMR, a full identity of the constituent atoms in the **CA:MA** molecular assembly is rendered possible as a result of variations in their chemical shifts.[17]

In conclusion, this report demonstrates that trinuclear (^{13}C, ^{15}N, and ^{2}H) MAS NMR is a complementary approach to study hydrogen-bond-mediated assembly in supramolecular systems that are not easily crystallizable. The NMR approach establishes asymmetry imposed by the self-association in the monomers **CA** and **MA**, and the hydrogen bonding in the 1:1 **CA:MA** complex conforms to the supramolecular architecture[4] held by complementary hydrogen bonding. In the complex, the X-ray data does not unequivocally distinguish the two stacked rings because of their same size and shape, and this is alleviated by the solid-state NMR approach. The **CA:MA** example chosen here is for demonstration of the concept and check for the agreement of solid-state NMR results with that of crystallography. It provides opportunities to study assemblies generated by lyophilizing samples at different pH and solvent conditions where crystallization may not always be successful. Further, the precise estimates of hydrogen bonding distances[18] in principle can be derived from ^{13}C−^{2}H and ^{15}N−^{2}H REDOR experiments. Such studies are in progress.

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Supporting Information Available: $\Delta\delta$ and η parameters derived for ^{13}C and ^{2}H; comparative crystal structure data for **CA**, **MA** and **CA:MA** complex; experimental/theoretical ^{2}H (3) and ^{13}C (4) slow MAS NMR for **CA**, **MA** and **CA:MA** complex at different rotor speeds; and ^{1}H−^{13}C polarization dynamics for different ring carbons of **CA**, **MA** and **CA:MA**. This material is available free of charge via the Internet at http://pubs.acs.org.

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