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New insights into the bonding arrangements of L- and D-glutamates from solid state ^{17}O NMR

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Abstract

Magic angle spinning (MAS) from L- and D-glutamic acid–HCl at 14.1 T produces highly structured and very similar NMR spectra. Lines from all 4 oxygen sites are readily distinguished and assigned. These ^{17}O NMR spectra are very different from the previously reported ^{17}O spectrum of the D,L-form presumably because that was a racemic crystal. ^{17}O NMR from L-monosodium glutamate–HCl is very different again requiring the application of double angle rotation and 3 quantum MAS NMR to provide resolution of 5 different sites. Hence high resolution ^{17}O solid state NMR techniques offer possible new insight into biochemical bonding processes.

1. Introduction

The importance of oxygen, as the most abundant element in the Earth's crust, and its ubiquity throughout living systems, might imply that it could play a central role in biological NMR studies. However, ^{17}O, the only NMR-active oxygen isotope, has a spin $I = 5/2$ so that it possesses a quadrupole moment (which often leads to significantly broadened lines from solids) which along with a low natural abundance (0.037%), makes its study by NMR still relatively uncommon. In recent years, despite these difficulties, with the advent of higher magnetic fields, faster magic angle spinning (MAS) and techniques for improving resolution there has been a significant increase in ^{17}O NMR reports from inorganic materials such as gels, glasses, zeolites and mineral analogues [1–8]. However, there have been many fewer reports of ^{17}O NMR from organic materials as they present even more of a challenge due to the larger quadrupole interaction. Recent reports of ^{17}O NMR from organic materials have included hemeproteins [9], polypeptides [10], strongly hydrogen-

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bonded carboxylic acids [11], amino acids [12] and nucleic acid bases [13].

L-glutamate plays a significant role in many biochemical processes acting as one of the most important neurotransmitters and by activating several families of brain-receptors [14]. Glutamate is very widely used within the food industry with ~1.5 million tons of monosodium glutamate (MSG) used each year [15]. It is established that L-glutamate (but not D) imparts a unique taste, termed 'umami' [16], and is added to a wide variety of foodstuffs to improve their flavour [17]. The obvious interest in this has led to several proposed molecular mechanisms to explain taste transduction but as yet none is fully accepted. A key experimental challenge is to provide high quality, detailed and unambiguous atomic scale information about the molecular bonding arrangement and changes that occur upon ligand–receptor interaction. This demands the development of experimental probe techniques to deliver this information. Solid state NMR is one non-perturbing approach which can be used for the study of ligand–receptor interactions where molecular size is not limiting and crystallinity not a requirement [18,19]. Here it is shown that the ^{17}O NMR spectra from D and L-forms of glutamic acid are very similar but very different from the D,L-form, and that 5 sites in MSG can be distinguished, opening up new possibilities for insight into molecular mechanisms responsible for the biological activity of glutamate.

2. Experimental details

L-glutamic acid–HCl ($C_5H_9NO_4 \cdot HCl$) was prepared following a literature procedure [20] using 20 at% ^{17}O H_2O. The D-form was purchased from Sigma-Aldrich, Gillingham (UK). and ^{17}O-enriched by exchange 20 at% ^{17}O H_2O. L-MSG monohydrate ($NaC_5H_8NO_4 \cdot H_2O$) was prepared by dissolving L-glutamic acid in water and adding NaOH until neutral. The sample was recrystallised from water and the excess water was evaporated under vacuum. All samples were single phase to better than 99%.

Most of the NMR was carried out on a Chemagnetics Infinity 600 spectrometer at a frequency of 81.345 MHz. MAS and 3Q MAS experiments used a 4 mm probe spinning at ~16 kHz. For the MSG, where the direct MAS spectrum provided no distinctive spectral features from which to obtain the interaction parameters, variable field double angle rotation (DOR) was carried out. These experiments were undertaken using odd-order sideband suppression by triggering the acquisition between consecutive scans at two positions of the outer rotor differing by 180° [21]. The outer rotor speed was varied between 1300 and 1700 Hz to determine the centrebands. ^{17}O DOR spectra were acquired at magnetic fields of 8.45 and 14.1 T at respective frequencies of 48.8 and 81.43 MHz. Spectra were referenced to water at 0 ppm. Spectral simulations were carried out using dmfit software [22].

Calculations of the NMR parameters were performed using GAUSSIAN 98 on different size clusters. Whilst calculations on a large enough cluster agree quite well (<1 ppm for the shift) with experiment for e.g., ^{29}Si in inorganic systems, for ^{17}O agreement is less good [6]. Nevertheless with the relatively large differences observed in the experimental determined shifts it was felt that calculations would be a useful aid in site assignment. Calculations were performed using the GIAO method with different basis sets (up to 6.311G**) on different size clusters up to three molecules in extent. In the three molecule calculations the two neighbouring molecules of the specific oxygen site being considered were used.

3. Results and discussion

The ^{17}O MAS NMR spectrum from L-glutamic acid shows a highly featured spectrum with two main resonances centred at ~260 and 125 ppm (Fig. 1a). Each of these resonances shows a number of singularities, and is composed of two strongly overlapping lines that are nevertheless readily separated in the spectral simulation. It should be noted that the features in the lower shift resonance only became clear when high (100 kHz) ^{1}H-decoupling powers were used. The simulation parameters for these 4 sites are summarised in Table 1. For comparison DOR NMR data was obtained at 14.1 T (Fig. 1b) and 8.45 T. There is a

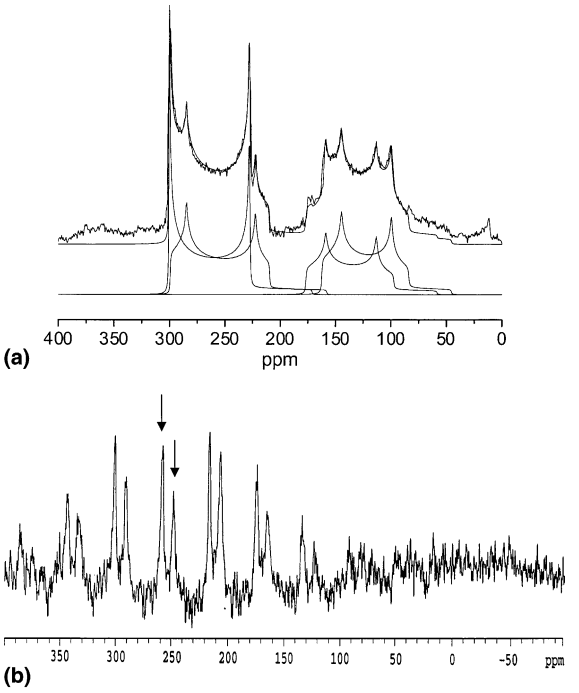

Fig. 1. (a) 14.1 T ^{17}O MAS NMR spectrum of L-glutamic acid together with a simulation for the centreband only and (b) 14.1 T DOR data collected with the outer rotor spinning at 1700 Hz (↓ indicate the centrebands).

large increase in the resolution of the spectra by employing DOR which shows a series of narrow lines and associated spinning sidebands. By varying the spinning speed from 1300 to 1700 Hz the centrebands can be identified, with only 2 centrebands observed at both fields. The peak position (δ_{peak}) in DOR spectra is given by

$$\delta_{\text{peak}} = \delta_{\text{cs,iso}} - \frac{3}{40} F(I) \frac{C_{\text{Q}}^2}{\nu_0^2} \left(1 + \frac{\eta^2}{3}\right) \qquad (1)$$

where $\delta_{\text{cs,iso}}$ is the isotropic chemical shift, C_{Q} is the quadrupole interaction parameter, ν_0 is the Larmor frequency, η is the quadrupole asymmetry parameter and $F(I)$ is a spin-dependent factor which for $I = 5/2$ is 2/25. Analysis at multiple applied magnetic fields [1,6,23] allows the NMR interaction parameters to be deduced as a product termed the quadrupole product $P_Q = C_Q\sqrt{(1+\frac{\eta^2}{3})}$ which is dominated by C_{Q}. For the two observed peaks from L-glutamic acid–HCl P_{Q} and $\delta_{\text{cs,iso}}$ are 8.22 ± 0.10 MHz and 320.7 ± 0.5 ppm, and 8.41 ± 0.10 MHz and 314.6 ± 0.5 ppm. There is very good agreement between these parameters and the sites labelled 1 and 2 observed in MAS (Table 1).

The likely assignment of this spectrum is that lines 1 and 2 can be identified with the two carbonyl oxygens (O2 and O3). This assignment agrees with the shifts observed for carbonyls in other amino acids as well as from carboxylic acids. The line with the largest shift has an asymmetry parameter of near zero indicating axial local symmetry which means it most probably comes from the O3 site where the local geometry is more closely planar (Fig. 2) [24]. This view is reinforced from the calculations which always produce a slightly larger chemical shift for O3. The other two lines then come from OH oxygens (O1 and O4), as indicated by the distinctive shifts and the sensitivity of their spectrum to proton decoupling. This

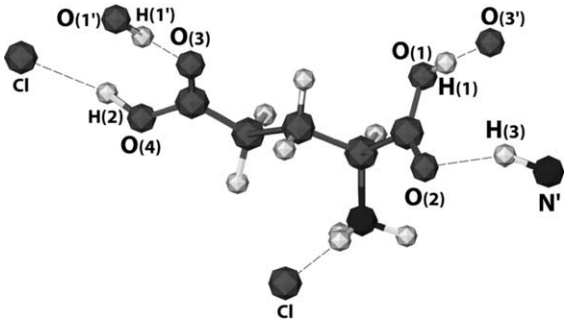

Fig. 2. Structure of L-glutamic acid.

Table 1
^{17}O NMR interaction parameters for L-glutamic acid–HCl

Site	$C_{\text{Q}}/(\text{MHz}) \pm 0.03$	$\eta \pm 0.02$	$\delta_{\text{iso}}/(\text{ppm}) \pm 0.5$	Assignment
L-form 1	8.16	0.0	322.0	O3
2	8.31	0.17	315.0	O2
3	7.49	0.25	187.0	O1
4	7.45	0.25	172.5	O4

is also the reason why these two signals are lost from the DOR spectrum as this was acquired on a single channel probe with no decoupling. Relatively strong dipolar coupling will not be averaged by spinning, as the outer rotor frequency is only 1.7 kHz, thus the magnetisation from these peaks will dephase comparatively rapidly and be lost from the spectrum. The electric field gradient for lines 3 and 4 is very similar with a ~15 ppm shift difference. Calculations over a range of cluster sizes consistently show that O1 has a larger isotropic chemical shift by ~10–15 ppm than O4, thus line 3 is assigned to O1 and line 4 to O4. The richness of the NMR spectrum from the solid is lost in solution with only two Lorentzian-like lines are observed with shifts of 263 and 251 ppm. The details of the quadrupole interaction are lost because of the motional averaging so that the two carbonyls become indistinguishable as do the two hydroxyls. The very different shifts in solution indicate that the carbonyls and hydroxyls are much more similar here due to the different hydrogen bonding in the solid.

^{17}O MAS NMR from D-glutamic acid was also collected and, to within experimental error, the NMR parameters obtained from simulation were the same as those given in Table 1 for the L-form. This data from the L- and D-glutamic acids individually makes interesting comparison with that reported recently for D,L-glutamic acid [12] where the spectrum showed two resonances in approximately the same position as each of the pairs of lines that are observed here for both the L- and D-glutamic acids. Even under 3Q conditions there was no resolution into separate lines [12]. There was, in addition, an extra peak of twice the intensity centred around ~200 ppm intermediate to the two peaks observed here in L- and D-glutamic acids. The assignment of the resonances in that report [12], where hydroxyls are assigned to the outer two peaks and carbonyls to the inner resonance, is at variance to that suggested here. The very unusual shift for the hydroxyl was ascribed to the $O-H\cdots Cl$ bond. However, the equivalent HCl compounds are used here so that a possible reinterpretation of the spectrum from the D,L-form can be made. Thus the outer two resonances are from carbonyls and hydroxyls that are closely similar to those present in the L and D structures since their parameters are in close agreement with the mean parameters observed for the two pairs of sites observed here. Then the intermediate peak comes from oxygens (appearing to have similar NMR parameters) that are very different to those in the chiral forms and result from the differing bonding in the D,L form. The D,L form of the glutamate could be either be a racemic conglomerate of chiral crystals or a racemic crystal. However, the presence of the additional resonance in the spectrum with ~50% of the total intensity strongly implies the latter. The D,L-glutamic acid–HCl investigated by Wu et al. [12] was enriched from the D,L monohydrate. This same material was used by Dunitz and Schweizer [25] to produce racemate anhydrous D,L-glutamic acid whose conformation was found to differ from the two chiral modifications of the monohydrate [25]. No detailed structure for racemic D,L-glutamic acid–HCl could be located in the literature. However, inspection of the structure of the racemic monohydrate [25] shows that whilst two oxygen sites have similar C–O bond lengths to the L-glutamate–HCl form the other two oxygens have bond lengths intermediate in length consistent with the intermediate shift observed. It is clear that, even from the limited numbers of oxygen observations that have already been made in the solid state, oxygen parameters can vary strongly with the details of the bonding arrangement. Hence differences in the packing conformation between the chiral forms and the D,L racemate leads to changes in the oxygen bonding of the network so that in the racemate half the hydroxyls and carbonyls must be in very different (hydrogen) bonding environments. The ability to detect small non-covalent interactions is indicative of the sensitivity of this approach in opening up a great wealth of new detailed information.

The sensitivity of oxygen to changes in bonding is emphasised by comparison of the acid with the sodium salt where there are two distinct but very similar glutamate anions [26] doubling, in principle, the number of oxygen sites. The well separated pairs of resonances with highly structured quadrupolar lineshapes for the acid are replaced by a single, almost featureless line with some minor

structure in the MAS spectrum (Fig. 3a). This peak is at an intermediate shift to the signals from L-glutamic acid–HCl. To help resolve the different sites DOR and 3Q MAS were applied producing a large increase in the resolution of the spectra. DOR (Fig. 3b) shows a series of narrow lines and associated spinning sidebands. By varying the spinning speed from 1300 to 1700 Hz the centrebands can be identified. It appears that at least 5 isotropic lines can be resolved which is confirmed by the 3Q MAS NMR which also shows 5 lines in the centreband in Fig. 3c. The DOR and isotropic MQ spectra are differently affected by the quadrupole interaction [23] introducing an additional multiplicative factor of $-19/12$ in Eq. (1) for 3Q experiments for $I = 5/2$. Plotting the DOR and 3Q data allows an estimate of the combined quadrupole parameter, P_Q, and shift for each line (Fig. 3d) [23]. These NMR parameters were consistent with simulations of the anisotropic slices of the 3Q data set where the asymmetry parameter, η, was found to be typically $\sim$0.4–0.5 (Table 2) and a composite of the 5 lines produced a good simulation of the almost featureless MAS lineshape which could even reproduce some of the fine structure on the main peak (Fig. 3a). The shifts for all these lines cover a 43 ppm range centred on 274 ppm very different from L-glutamic acid. The electric field gradients are also very different, C_Q is within $\sim$5% of 7.4 MHz, much less than O2 and O3 in L-glutamic acid and the asymmetry parameters of all sites are much larger. These changes must be due to the changed bonding in MSG. Although all 8 sites cannot be resolved, even with a resolution of better than 1 ppm, inspection of the structure [26] shows that the oxygens occupy 4 pairs of very similar sites. One of

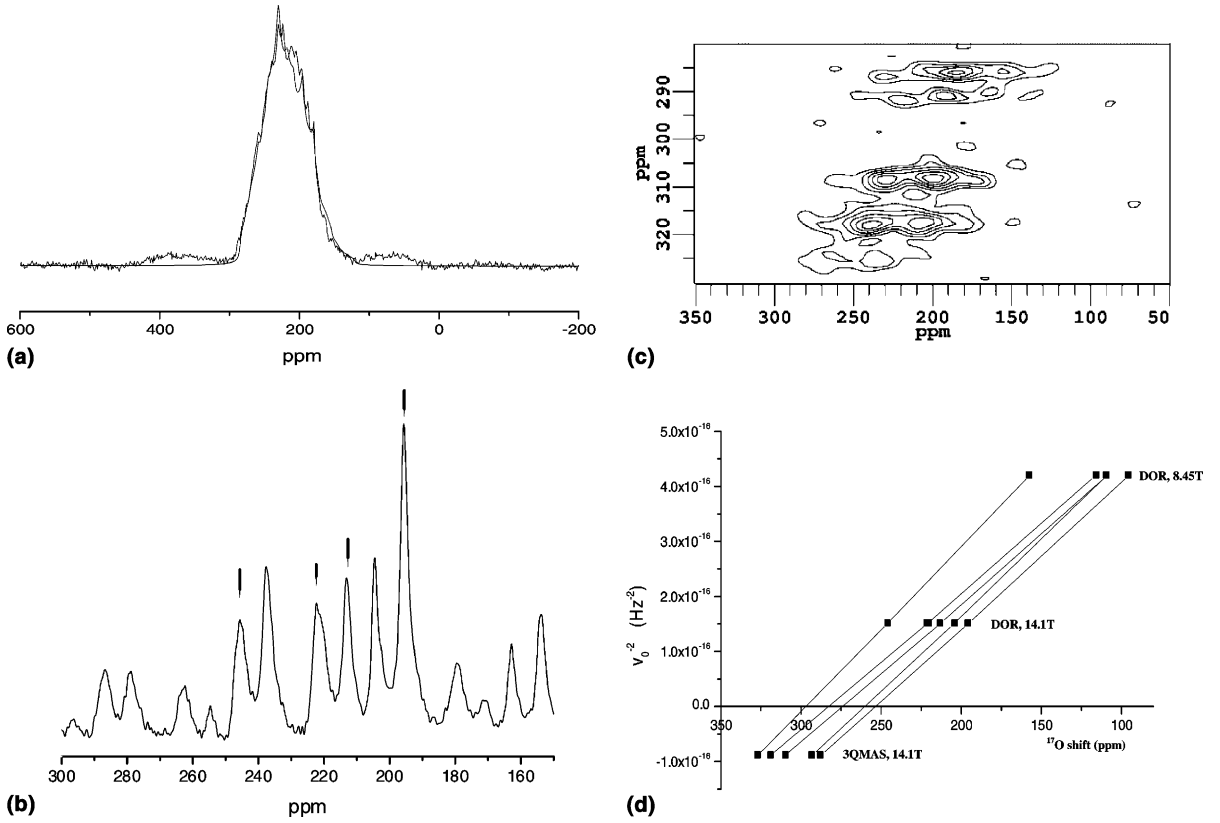

Fig. 3. (a) 14.1 T ^{17}O MAS NMR spectrum of L-MSG together with a simulation based on the 5 components deduced from the higher resolution data. (b) 14.1 T DOR (↓ indicate the centrebands), (c) the centreband of the 14.1 T 3Q MQMAS NMR data and (d) the field dependence of the isotropic position from the DOR and 3Q data.

Table 2
^{17}O NMR interaction parameters for L-MSG P_Q and δ_{iso} are taken from the DOR results, η and C_Q from the MQ slice simulations

Site	$P_Q/(\text{MHz}) \pm 0.05$	$\delta_{iso}/(\text{ppm}) \pm 1.5$	$C_Q/(\text{MHz}) \pm 0.2$	$\eta \pm 0.05$
1	7.93	254	7.4	0.47
2	7.76	260	7.2	0.50
3	8.11	274	7.6	0.45
4	8.15	283	7.7	0.40
5	7.45	297	7.0	0.45

these pairs, possibly O5/O7 which show the greatest difference in the bond distances (0.10 Å), splits to give the 5 lines observed.

4. Conclusion

The data presented here has further demonstrated the exquisite sensitivity of solid state ^{17}O NMR to changes in the bonding environment with specific application to biochemically significant molecules. The differences between the D,L- and L- and D-forms of glutamic acid–HCl must reflect changes in the (hydrogen) bonding within the compounds which determines the molecular packing. The chemical shift and electric field gradient of L-MSG formed from the acid indicate significant changes in the bonding at the oxygen sites occur. Higher resolution techniques than MAS are necessary to reveal these differences. 3QMAS at a single field would be sufficient but the quality and accuracy of the data is greatly improved by the use of DOR. Neither ^{1}H, where the spectra begin to show signs of resolution for the hydroxyl protons only in glutamic acid at ultra high spinning speeds (>40 kHz), nor ^{13}C offers anywhere near the degree of structural discrimination of ^{17}O. The ability to resolve the different sites and to detect changes from ^{17}O NMR spectra suggest that this will be a fruitful experimental approach to elucidate molecular pathways of biochemical recognition and could find widespread application.

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