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Origin of the Residual NMR Linewidth of a Peptide Bound to a Resin under Magic Angle Spinning

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The tetrapeptide Ala–lle–Gly–Met bound to a Wang resin via the methionine residue was studied by NMR under MAS conditions and compared to the same peptide in solution. The bound peptide exhibits average linewidths superior to those observed for the peptide in solution. The origin of the residual NMR linewidth observed for the bound form was investigated. The dynamics of the peptide is shown to be only marginally responsible for the increased linewidth; the major cause of the line broadening appears to be nonaveraged magnetic susceptibility differences.

Key Words: **high-resolution MAS; resin; line broadening; dynamics; magnetic susceptibility.**

The emergence of new technology in the design of high-resolution magic angle spinning (MAS) (*1, 2*) probes has opened new avenues for the study of molecules that belong neither to the liquid nor to the solid state but which lie somewhere between the two. The domain of application of this new technique ranges from the study of organic molecules and peptides bound to a solid resin support (*3–10*) to polymers, lipids (*11*), and human and animal tissues (*12, 13*).

The technique requires the sample under study to have some degree of mobility. In the case of a pure solid like a resin or a polymer, a solvent has to be added in order to swell up the sample. This condition is already satisfied for human and animal tissues. The sample is then spun at the magic angle (54.7°) in order to average out the remaining interactions.

In this Communication, we report the study of the tetrapeptide Ala–lle–Gly–Met bound to a Wang resin via the methionine residue in DMF. The 1D 400-MHz proton spectra of the tetrapeptide bound to the resin and in solution recorded at the magic angle are shown in Fig. 1. Although the average linewidth of the bound peptide is remarkably small compared to the dry resin under static conditions (data not shown), it is clear that the peptide in solution has sharper lines. On average, at 400 MHz the proton linewidths observed for the bound peptide are about three times larger than those observed for the peptide in solution. The purpose of this work is to investigate the possible causes of that residual line broadening.

In order to differentiate between a homogeneous and a heterogeneous line broadening, the dynamic behavior of the bound and of the free peptide was first analyzed using relaxation time measurements. The T_1 values obtained for ^{13}C at 300 and 500 MHz are presented in Table 1 along with the uncertainty of the measurements. Except for the alanine α-carbon, the $T_1(^{13}C)$ values of the bound peptide are in the same range of values as those of the free peptide. This indicates that the dynamic behavior of the peptide is not very different in both cases. The weak field dependence of $T_1(^{13}C)$ implies that the extreme narrowing condition can be assumed and that consequently $T_2 = T_1$ for carbon. Under these conditions, the contribution of transverse relaxation to the line broadening $(1/\pi T_2)$ is weak and cannot explain the linewidths of the ^{13}C spectra of the bound peptide. The interpretation of proton T_1 measurements is complicated by the presence of NOE interactions and spectral overlap, therefore making the comparison of the $T_1(^1H)$ values of the bound and free peptide less straightforward. The values obtained for the bound and free peptide are nevertheless in the same range. Measurement of $T_2(^1H)$ values is again complicated by spectral overlap and scalar coupling between the proton nuclei. However, for some protons the measurement is still possible. A value of 360 ms was measured for the $T_2(^1H)$ value of the CH_3 group of the Met residue of the bound peptide. This value corresponds to a theoretical $(\Delta\nu_{1/2})_{th}$ of 0.9 Hz whereas the $(\Delta\nu_{1/2})_{mes}$ measured at 500 MHz is 12.3 Hz. All these results support the fact that most of the residual linewidth is not due to relaxation processes.

Another possible cause of line broadening could be an incomplete averaging of chemical shift and homonuclear dipolar interactions. However, experiments carried out at higher spin rates do not show any differences in the linewidth of the sample. The proton linewidth decreases up to a speed of 2 kHz and then remains constant (spectra were recorded up to 6 kHz). The solvent that is added to the sample and that swells up the resin is apparently reintroducing enough mobility to considerably reduce the chemical shift anisotropy (CSA) and the homonuclear proton dipolar interaction.

A much more likely mechanism for line broadening could arise

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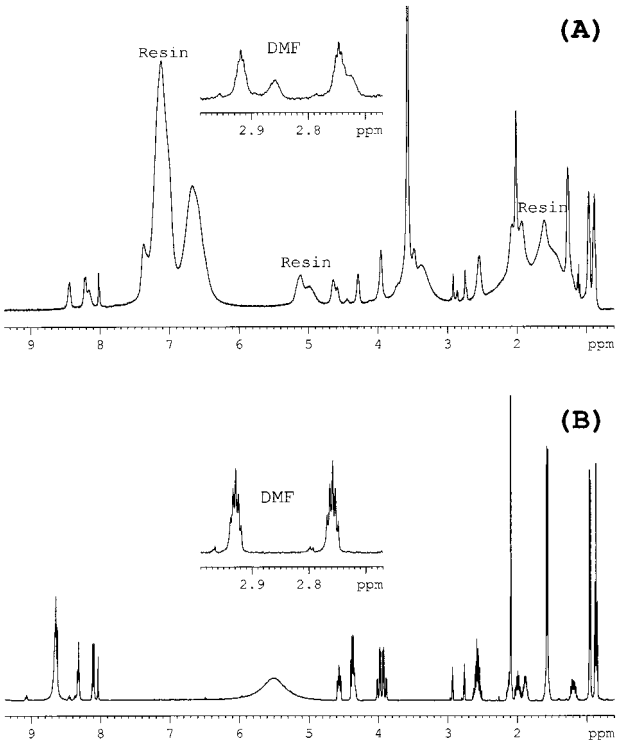

FIG. 1. 1D proton spectra of the tetrapeptide Ala–lle–Gly–Met bound to a Wang resin swollen in DMF (A) and of the peptide in solution in DMF (B). Both spectra were recorded on a Bruker Avance 400-MHz spectrometer equipped with a $^{1}H/^{13}C/^{2}D$ HRMAS gradient probe. A 4-mm rotor and a speed of 3 kHz were used for both spectra.

from the important differences of magnetic susceptibilities present in the sample at the interface between the resin beads and the peptide. The resin bead can be viewed as a macroscopic dipole that will create a dipolar field in its environment with a $(3\cos^2\theta - 1)$ spatial dependence (*14–16*). The spins that belong to the peptide are therefore involved in a dipolar interaction with the resin beads. This effect is similar to a homonuclear proton–proton dipolar interaction and should therefore be removed by MAS. However, the key point here is that MAS will only average out the isotropic part of the bulk magnetic susceptibility tensor κ (IBMS) and will leave the anisotropic part (ABMS) unaffected (*14, 15, 17*). In the case of a complex sample like a resin, the magnetic susceptibility tensor is very likely to be anisotropic. This anisotropy is all the more probable because the Wang resin to which the peptide is attached is made of polystyrene with numerous aromatic groups. The molecular magnetic susceptibility of the aromatic groups is highly anisotropic and the ABMS that results will strongly contribute to the line broadening of the peptide resonances.

Spectra recorded at 300 and 500 MHz (Fig. 2) exhibit a line broadening that is proportional to the H_0 field, which is in agreement with the fact that differences in magnetic susceptibilities are the major cause of the residual line broadening. Further proof that differences in bulk magnetic susceptibility are not completely averaged out by MAS is the observation of four distinct resonance lines for the methyl groups of the DMF that is used to swell the resin in a 4:3 ratio (Fig. 1A). Two of these resonances correspond to the usual lines of the DMF spectrum at 2.75 and 2.92 ppm, whereas the two remaining ones, at 2.73 and 2.86 ppm, originate from the DMF that is trapped inside the resin. Moreover, the resonances that belong to the DMF in solution are better resolved than those that belong to the DMF trapped inside the resin.

This magnetic susceptibility-induced broadening effect is very localized and affects only the proton resonances close to the resin (*16*). By mixing a swollen Wang resin and the peptide in solution in DMF in the same rotor, a solution-like spectrum

TABLE 1
Proton Assignment and Comparison of the ^{13}C T_1 Values Obtained for the Peptide Bound to a Resin and for the Peptide Free in Solution at 300 and 500 MHz

	$\delta(^1H)$ ppm		$T_1(^{13}C)$ (ms) 300 MHz		$T_1(^{13}C)$ (ms) 500 MHz	
	Liquid	HRMAS	Liquid	HRMAS	Liquid	HRMAS
CH_3M	2.13	2.06	1900 ± 240	1650 ± 200	2750 ± 350	2450 ± 300
$CH_{3\gamma}I$	0.99	1.01	640 ± 100	540 ± 70	780 ± 100	670 ± 80
$CH_{3\delta}I$	0.90	0.93	1000 ± 150	1000 ± 140	1200 ± 160	960 ± 120
CH_3A	1.60	1.31	550 ± 90	610 ± 120	600 ± 70	660 ± 80
$CH_{2\gamma}M$	2.60	2.59	—	—	—	—
$CH_{2\gamma}I$	1.23–1.60	1.25–1.65	500 ± 70	240 ± 50	280 ± 40	380 ± 70
$CH_{2\beta}M$	2.13	2.12	300 ± 40	310 ± 60	380 ± 60	340 ± 60
$CH_\beta I$	1.92	1.97	500 ± 80	410 ± 70	430 ± 50	460 ± 80
$CH_\alpha M$	4.60	4.67	410 ± 70	310 ± 100	400 ± 60	430 ± 100
$CH_\alpha I$	4.40	4.33	410 ± 100	380 ± 100	430 ± 70	390 ± 80
$CH_\alpha A$	4.38	3.66	480 ± 70	690 ± 90	470 ± 50	780 ± 130
$CH_\alpha G$	3.98	4.00	260 ± 60	210 ± 60	230 ± 40	260 ± 60

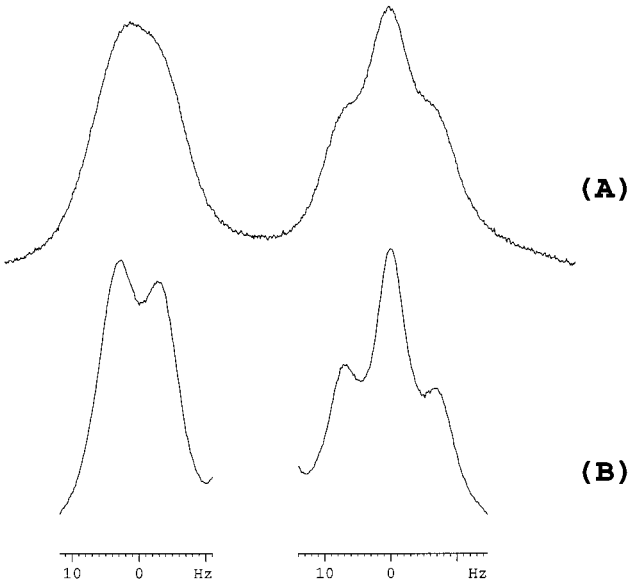

FIG. 2. Expansion of the 1D proton spectrum of the tetrapeptide Ala–lle–Gly–Met bound to a Wang resin swollen in DMF showing the γ (left) and δ (right) methyl groups of the isoleucine residue. Spectrum (A) was recorded at 500 MHz and spectrum (B) at 300 MHz.

was observed for the peptide, indicating that the peptide was not affected by ABMS effects.

In conclusion, we have shown that the residual NMR linewidth observed in the spectra of a peptide bound to a resin originates mainly from anisotropic bulk magnetic susceptibility differences that are not averaged out by MAS. It is most likely that the presence of strongly anisotropic phenyl groups contributes significantly to this phenomenon, and the use of resins that do not contain any aromatic groups is therefore strongly advocated.

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