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Enantiomeric analysis using natural abundance deuterium 3D NMR spectroscopy in polypeptide chiral oriented media

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ABSTRACT

The use of natural abundance deuterium (NAD) three-dimensional (3D) NMR experiment in chiral liquid crystals (CLC) is reported and discussed. This homonuclear autocorrelation 3D experiment allows assigning deuterium signals of weakly aligned molecules at natural isotopic abundance. It provides an efficient strategy for identifying the spectral enantio-discriminations on NAD spectra. We demonstrate that NAD 3D NMR is feasible within reasonable experimental times (14 h) using the 3D Quadrupole Double-Quantum NMR sequence [O. Lafon, P. Lesot, Chem. Phys. Lett. 404 (2005) 90] and a 14.1 T NMR spectrometer equipped with a selective 5 mm deuterium NMR cryoprobe. The analytical potentialities of this technique are illustrated in the case of (±)-but-2-yn-1-ol dissolved in poly-γ-benzyl-L-glutamate/$CHCl_3$ mesophase.

1. Introduction

Despite the low natural isotopic abundance of deuterium (0.015%) and its low magnetogyric ratio ($\gamma(^1H)/\gamma(^2H) = 6.515$), proton-decoupled natural abundance deuterium (NAD) NMR spectroscopy in polypeptide chiral liquid crystals (CLCs) provides an efficient tool for enantiomeric and enantiotopic analysis [1–4]. The main advantage of NAD NMR as compared to the NMR of labelled compounds, is that all possible deuterium sites in a molecule can be simultaneously probed, consequently increasing the possibility of observing more enantiomeric discriminations.

However, too much information of enantiomeric discrimination can become a disadvantage. NAD NMR 1D spectra of chiral solutes weakly aligned in CLCs are difficult to analyse for two reasons: (i) in such phases the enantiomers have different orientational ordering, and hence each of them has, in principle, its own NAD spectrum; (ii) in contrast to thermotropic liquid crystals [5,6], ^2H quadrupolar splittings, $\Delta\nu_Q$'s, in these weakly aligning media have approximately the same magnitude as the ^2H chemical shift dispersions. Consequently the components of quadrupolar doublets that are centred at different chemical shifts may overlap which hampers their assignment.

In other words, the analysis of congested NAD spectra definitely necessitates the use of multidimensional NMR spectroscopy [7,8]. In NAD-NMR spectroscopy, the probabilities in finding a deuteron coupled to a ^{13}C or another deuterium nucleus are extremely low and therefore ^2H–^{13}C and ^2H–^2H correlation experiments are precluded nowadays [9,10]. The assignment of NAD spectra relies exclusively on multidimensional autocorrelation ^2H experiments, which allow the correlation between the two components of the quadrupolar doublets, and hence facilitate their identification on the basis of the ^2H chemical shifts.

In order to extend the analytical potentialities of NAD-NMR spectroscopy, we explore here a new approach: the use of NAD 3D experiments. Recently, proton-decoupled deuterium 3D NMR pulse sequences (denoted as *Q*-DQ, *δ*, *Q*-resolved and *δ*-resolved/*Q*-COSY) were developed in order to investigate perdeuterated solutes dissolved in the poly-γ-benzyl-L-glutamate (PBLG) mesophase [11]. These NMR pulse sequences offer the advantage over autocorrelation 2D techniques that ^2H signals are distributed over three different spectral axes, thereby improving the readability of NAD signals. NAD NMR 1D and 2D experiments were previously performed on a routine NMR spectrometer operating at 61.4 MHz (9.4 T) and equipped with a 5 mm selective ^2H probe [3,4,12]. Therefore, applying 3D experiments to analyse chiral solutes at natural isotopic abundance was precluded by the low sensitivity of deuterium.

Recently, Lesot et al. have demonstrated the analytical potential of NAD NMR in CLCs using a modern high-field NMR spectrometer (14.1 T) equipped with a cryogenically cooled selective 5 mm ^2H NMR probe [13–16]. With this new technological device, the gain in sensitivity becomes so important (a factor 4–5 compared to classical probes) [17] that NAD spectra of very high molecular weight solutes can be recorded within reasonable experimental time (around 14 h of acquisition) [15] while for small chiral molecules or for relatively large amount of solute (50–100 mg), the experimental time of NAD 1D experiments with satisfying S/N ratio can

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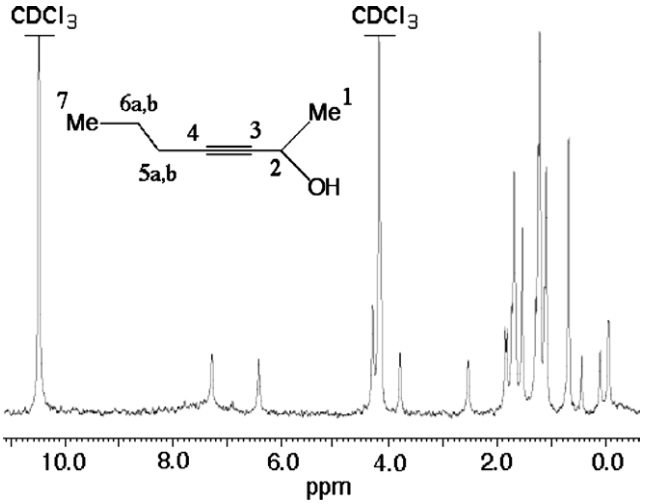

Fig. 1. 92.1 MHz NAD 1D spectrum of (±)-but-2-yn-1-ol (**1**) in PBLG/$CHCl_3$ recorded at 301 K in 20 min with 4k data points and accumulated 1400 scans. No filtering is applied.

be reduced to less than 1–2 hours [14]. Under these conditions, 3D NMR techniques can be successfully applied in order to analyse isotopically unmodified molecules.

In this communication we report the NAD 3D NMR experiments performed in weakly aligning chiral liquid crystals. The 3D experiment chosen uses the 3D *Q*-Double Quantum (*Q*-DQ) sequence because it has the highest sensitivity among the ^{2}H 3D experiments reported in Ref. [11]. As an illustrative example, we investigated a chiral, isotopically unmodified molecule, (±)-but-2-yn-1-ol (**1**), which is a potential building block in the synthesis of various bioactive natural macrolactins (family of Dolastatine) [4]. Although this chiral molecule possesses just seven non-equivalent ^{2}H sites, its NAD NMR 1D spectrum in the PBLG/$CHCl_3$mesophase (Fig. 1), is very congested and hence requires multidimensional experiments to determine the doublets assignments.

2. A brief description of the *Q*-DQ 3D NMR sequence

The *Q*-DQ 3D NMR sequence excites double-quantum coherences (DQC's) of any isolated deuteron (spin $I = 1$) in order to correlate the two components of a quadrupolar doublet. As seen in Fig. 2, this 3D sequence derives from the *Q*-DQ 2D strategy. The fixed defocusing delay τ is converted into a variable time period, t_1, in order to optimize the build-up of DQC's during a time, t_2 [11]. Thus, in contrast with the *Q*-DQ 2D map, the peak intensities of *Q*-DQ 3D spectrum do not depend on the magnitude of quadrupolar splittings, ω_Q. Consequently *Q*-DQ 3D experiments can be used, in principle, for quantitative measurement of the enantiomeric excess. The main characteristics of the *Q*-DQ 3D experiment can be found in Ref. [11]. After a four-step phase cycle and disregarding all phase terms, the expression for the positions of the signals for a single deuteron, $S(t_1,t_2,t_3)$, in the three time domain is

$$S(t_1,t_2,t_3) \propto \sin[\omega_Q t_1]\exp\left[-\frac{t_1}{T_2}\right] \times \sin[2\omega_D t_2]\exp\left[-\frac{t_2}{T_{2D}}\right] \times \left(\sin[\omega_Q t_3]e^{i\omega_D t_3}\right)\exp\left[-\frac{t_3}{T_2}\right] \quad (1)$$

where $\omega_D = 2\pi\nu_D$ and $\omega_Q = \pi\Delta\nu_Q$ denote the deuterium offset frequency and quadrupolar splittings. T_2 and T_{2D} are the relaxation times of respectively single- and double-quantum coherences. This expression shows that, after triple Fourier transform (FT), chemical shift and quadrupolar splittings appear together in the F_3 dimension, while they are separated in F_1 and F_2 dimensions ($\Delta\nu_Q$ in F_1 and $2\nu_D$ in F_2). As described in Ref. [11], the *Q*-DQ 3D sequence allows obtaining peaks phased in pure absorption in three dimensions, ensuring the best achievable resolution for a 3D experiment. Finally, as single-quantum coherences (SQC's) are defocused with respect to quadrupolar splitting during t_1 delay and then refocused during t_3 period, the signal is proportional to $\sin(\omega_Q t_1)$ and $\sin(\omega_Q t_3)$. These sine-modulations with respect to the $\Delta\nu_Q$'s lead to an antiphase character of the quadrupolar doublets in the F_1 and F_3 dimensions. Consequently when the peaks are phased in pure absorption, half of them are positive while the rest are negative.

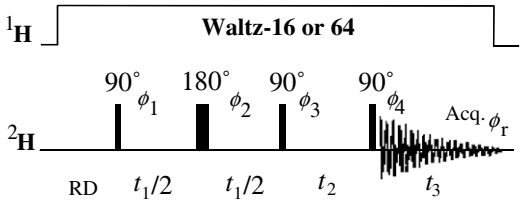

Fig. 2. Pulse scheme of basic *Q*-DQ 3D pulse sequence with $\phi_1 = \phi_2 = 16(x)$; $\phi_3 = 4(x), 4(-x), 4(y), 4(-y)$; $\phi_4 = 4(x,y,-x,-y)$; $\phi_r = -x, y, x, -y, y, x, -y, -x, x, -y, -x, y, -y, -x, y, x$. Composite pulses can be applied to reduce offset effects and non-ideal flip angles.

3. Experimental

The sample of (±)-but-2-yn-1-ol ((±)-**1**) in PBLG was prepared using 100 mg of PBLG (DP = 512), 100 mg of solute, and 350 mg of chloroform. Other details concerned with the sample preparation can be found in references [2,3]. The experiments were carried out at 301 K using a Bruker Avance II 600 MHz spectrometer equipped with a selective 5 mm cryoprobe (92.1 MHz for ^{2}H). Proton broadband decoupling was achieved using the WALTZ-16 composite pulse decoupling. The 3D spectrum was recorded with 8 scans *per* FID and $90 \times 110 \times 832$ data points in t_1, t_2, t_3 dimensions, respectively. Given the short relaxation time, T_1 of deuterons (0.1–0.3 s), the recycling time was set to 0.5 s to reduce the total experimental time to 14 h. The 3D matrix was zero-filled to a resolution of $128(F_1) \times 512(F_2) \times 1024(F_3)$ data points prior to the triple FT. A Lorentzian filtering was applied in all three dimensions (LB = 3 Hz). Neither linear prediction nor maximum entropy process were performed to reconstruct the NMR signals [18].

4. Results and discussion

The narrow frequency distribution of resonances in the 1D NAD spectrum of **1** (see Fig. 1) complicates the task of pairing of the two components for each doublet, and thereby their assignment. Overcrowding in the 1D spectrum is augmented by the presence of pairs of diastereotopic deuterons in each methylene group that are non-equivalent (even in an isotropic solvent). Indeed, if all sites are spectrally diastereo- and enantio-discriminated and disregarding the hydroxyl group, there are seven non-equivalent sites in each enantiomer and we should observe a maximum of 14 doublets centred at seven different chemical shifts in a CLC.

In practice only 15 peaks of the solute are resolved on the 1D spectrum (see Fig. 1). Such a loss of information can arise from unresolved chiral discriminations and from fortuitous overlapping of quadrupolar doublets centred at different chemical shifts. Undoubtedly, the undecipherable spectral region between 0 and 2 ppm in Fig. 1, shows numerous overlapping of peaks. These can be resolved by the multi-dimensional NMR strategy. Fig. 3 presents the NAD *Q*-DQ 3D spectrum of (±)-**1**. Here the positive (blue)[1] and

[1] For interpretation to color in Fig. 3, the reader is referred to the web version of this article.

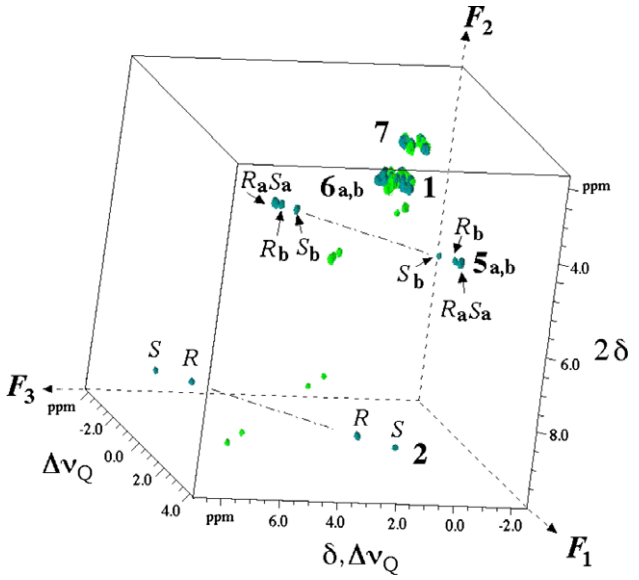

Fig. 3. NAD *Q*-DQ 3D spectrum of **1**. The spectral information in 2D planes F_1/F_3, F_1/F_2 and F_2/F_3 are $(\Delta\nu_Q/\delta,\Delta\nu_Q)$, $(\Delta\nu_Q/2\delta)$ and $(2\delta/\delta,\Delta\nu_Q)$, respectively. The NAD doublet of chloroform is not displayed. The assignment of doublets is given. For deuterons 5a,b, the assignment shown corresponds to assignment 1 (see Table 1).

the negative (green)[1] peaks are shown together, but half of peaks can be removed from the display in order to facilitate the legibility of the NMR spectral cube. All spectral data extracted from the analysis of the 3D spectrum and 2D planes are presented in Table 1.

Preliminary analysis of the cube indicates that the deshielded NAD signals correspond to the methyne deuteron (two doublets centred at 4.38 ppm) and exhibits large spectral enantiodiscrimination (160 Hz). Since the enantiomeric mixture is racemic, a direct assignment of stereodescriptors, *R*/*S*, for each of doublet, observed in the 3D spectrum, is *a priori* not possible [16]. However a previous NAD NMR analysis of enantiopure **1**, (*R*)-isomer, dissolved in the same PBLG mesophase (in almost the same temperature) has allowed specifying *R*/*S* descriptors corresponding to each quadrupolar doublets [4].

The diastereotopic deuterons belonging to methylene group 5 can be also assigned easily from their chemical shift values (2.02 ppm). Indeed, these methylene deuterons are deshielded by the carbon–carbon triple bond. Note that similar to the deuterons at position 2, deuterons at position 5 exhibit large quadrupolar splittings (see Table 1). Besides excluding alignment of these C–D bonds along the magic angle directions [2,3], the higher orientational ordering may be related to the fact that carbons 2 and 5 are adjacent to the acetylenic rigid part of the molecule. Indeed in this particular location, time-averaging of C–D bonds order parameter, due to conformational dynamics, is diminished. For site 5, we expect to detect two pairs of quadrupolar doublets (centred on two slightly different chemical shifts) if both deuterons are spectrally enantiodiscriminated [8]. Experimentally, only three doublets are observed centred exactly on the same chemical shift. Two of the doublets have equal intensities whereas the third one is twice intense. As no difference in chemical shift can be measured between diastereotopic deuterons, it is not possible to separate their signals. Nevertheless, the previous analysis of (*R*)-**1** in PBLG permits the identification of the doublets related to each enantiomer.

Finally, NAD spectrum of deuterons at site 5 may arise from two situations: (i) chiral discrimination (64 Hz) is only observed for one of diastereotopic deuterons while for the second no spectral separation occurs (assignment 1), (ii) both diastereotopic deuterons show chiral discriminations (95 and 31 Hz), leading to a fortuitous overlap between doublets of deuterons 5a and 5b belonging to enantiomers *R* and *S*, respectively (assignment 2). Given the difficulty in predicting experimental chiral discriminations, it is not possible to decide in favour of either spectral situations. Recording the NAD 3D spectrum of (±)-**1** in an achiral mesophase might help to solve this problem [19].

The most shielded signal corresponds to the methyl group 7. The small magnitude of quadrupolar splittings at this site partly results from fast spinning of the methyl group about C6–C7 bond, thus decreasing the orientational ordering of C–D bonds. No chiral discrimination is detected because of the large distance of the methyl C–D bonds from the stereogenic centre and the conformational dynamics of flexible alkyl chain.

Using the above described assignments, the rest of the NAD signals, located between 1.0 and 1.5 ppm, can only correspond to deuterons at positions 1 and 6. These signals, however, cannot be directly assigned from the 3D cube given the small dispersion of resonances. In this case the analysis of 2D slices is required. Here the best view is obtained when considering the F_1/F_3 plane displayed in Fig. 4. This 2D zoom of the 3D spectrum formally corresponds to an untilted *Q*-resolved 2D experiment. Indeed the deuterium chemical shifts are refocused in the F_1 dimension.

As the *Q*-DQ 3D experiment is quantitative, the most intense signals in Fig. 4 correspond to the three magnetically equivalent

Table 1
Deuterium data extracted from the NAD 3D spectrum

δ/ppm[a]	$\lvert\Delta\nu_Q^R\rvert/\lvert\Delta\nu_Q^S\rvert$/Hz[b]	$\lvert\Delta\Delta\nu_Q\rvert$/Hz	DOE (^{2}H)[c]	^{2}H Site
0.86	48/48	0	0	7
1.37	55/25	30	0.75	1
1.40	36/68	32	0.61	6a
1.39	50/68	18	0.31	6b
2.02[d]	402/402[e]	0	0	5a
	371/307[e]	64	0.19	5b
	402/307[f]	95	0.27	5a
	371/402[f]	31	0.08	5b
4.38	358/518	160	0.36	2

[a] The centre of the $CHCl_3$ doublet is calibrated at 7.23 ppm. The splitting is equal to 581 Hz.
[b] The *R*/*S* assignment is derived from a previous NAD 2D NMR analysis of (*R*)-**1**.
[c] DOE: differential ordering effect is defined as: [3] $\mathrm{DOE}(^2\mathrm{H}) = 2\lvert\Delta\Delta\nu_Q\rvert/[\lvert\Delta\nu_Q^R\rvert + \lvert\Delta\nu_Q^S\rvert]$.
[d] Depending on the assignment made on the diastereotopic deuterons 5a and 5b.
[e] Assignment 1.
[f] Assignment 2.

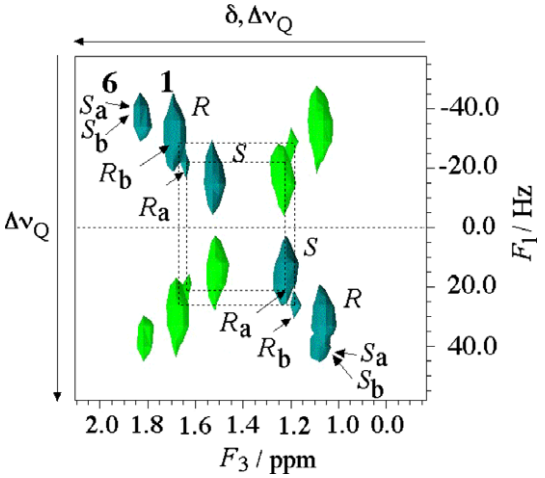

Fig. 4. Partial 2D map centred on the methylene group 6 and the methyl group 1. It corresponds to F_1/F_3 projection of 2D slices, located between 1.30 and 1.50 ppm in F_2 dimension. The corners of the dashed rectangles indicates the position of deuteron 6 signals, partly overlapping with those of deuterons 1.

deuterons of the methyl group at position 1. Similarly to the methyl group 7, the magnitude of quadrupolar splittings for deuterons in methyl group 1 is rather small due to its fast spinning. Still, their NAD signals consist of two quadrupolar doublets centred on the same chemical shift, indicating that spectral discrimination occurs. The methyl group at position 1 is directly bonded to the asymmetric carbon, and thus experiences stronger enantio-discriminating interactions.

The assignment of the doublets for the methylene group, 6, is less trivial because these weak signals overlap with those of the methyl group 1. Due to the double rotation of the C–D direction in this methylene group around the C6–C5 and C5–C4 internuclear axes, the magnitude of quadrupolar splittings for the deuterons at site 6 is comparable with the one obtained for the methyle group 1. However, a careful analysis of quadrupolar doublet distribution in Fig. 4 shows that the methylene group 6 exhibits three distinct quadrupolar splittings (see Table 1). Fortuitously for the *S*-enantiomer, the signals of diastereotopic deuterons 6a and 6b overlap. Here again this conclusion was confirmed with the data derived from the previous analysis of (*R*)-**1**. Thus, both diastereotopic sites of methylene group 6 exhibit chiral discriminations.

Finally the perusal of Table 1 indicates that the magnitude of spectral enantio-discrimination is not simply related to orientational ordering. For example, the DOE of methyl group 1 is maximal whereas its average quadrupolar splitting is minimal. Furthermore, whatever the assignment chosen for deuterons at position 5, they present smaller DOE's than those of methylene group 6. This observation is counter-intuitive. Indeed, since the deuterons 5 are closer to the stereogenic centre, we could expect higher DOE's. This result emphasizes the difficulty to predict and assign chiral discriminations experimentally observed, in particular for flexible enantiomers.

5. Conclusion

In the present work, we demonstrate that NAD 3D NMR spectroscopy is possible and permits the assignment of quadrupolar doublets belonging to chiral compounds oriented in a polypeptide chiral liquid crystal. The feasibility of the method is shown for the case of isotopically unenriched enantiomers, that are involved in the synthesis of natural products as chiral building blocks. The large spatial distribution of resonances in the 3D spectrum facilitates the identification of chiral discriminations in NAD NMR spectra. NAD 3D experiments especially suit the study of weakly aligned solute because the small magnitude of ^{2}H quadrupolar splittings (generally <1000 Hz) requires a reduced number of point data in all dimensions, thus limiting the total experimental time. Finally, we demonstrate the considerable advantage of using cryogenically cooled NMR probes for analysing weakly naturally abundant nuclei with low sensitivity such as deuterons.

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