from Figure 5) and its occurrence would presumably involve cooperative motions of many chains.

It is to be emphasized, however, that while the structure we suggest seems the most likely in terms of presently available evidence, there is at least one other possibility. Alignment of chains along, or close to, one diagonal of the c-axis projection seems certain, but a different c-axis stagger along that diagonal is conceivable that would result in a different obliquity of b and c axes. This structure would not allow optimal interdigitation of methylene hydrogens but departure from this condition would be modest but, since the packing of methylene sequences is loose to begin with, this is not a telling argument. In neither case is it clear why the d_{001} spacing is essentially the same in the two crystal forms; this spacing is determined both by chemical repeat distance and by stagger between molecular sheets depending largely upon interactions that do not involve ester linkages in a crucial way and are, therefore, difficult to assess ab initio. When the symmetry of the new polymorph is better characterized, it may prove necessary to revise some of the tentative conclusions in part 1 regarding its optical properties. It will be recalled that the only disagreement of substance in our quantitative analysis of optical behavior arose in connection with this polymorph, though a credible explanation was proposed that did not require a drastic change in viewpoint.

It is suggested that the stable monoclinic form of the polymer should hereafter be designated α-poly(ethylene sebacate) and the metastable polymorph be called β-poly(ethylene sebacate).

Conclusions

Principal conclusions are (a) that the new polymorph of poly(ethylene sebacate) is metastable, (b) that it transforms to the stable monoclinic form at temperatures above 70 °C, apparently within the solid state, (c) that nucleation of this form is suppressed by traces of moisture or sebacic acid (probably other polar contaminants as well), and (d) that its crystal structure differs from that of the stable form principally in that the plane of the central chain in the unit cell lies parallel to those of the corner chains and not at a large angle to them as in the stable monoclinic form. This difference in structure accounts qualitatively for all the known properties of the polymorph, including the sensitivity of nucleation to polar contaminants. A full determination of structure and symmetry, however, requires an X-ray diffraction study of an oriented fiber free of the stable form. Production of such a fiber has not yet been achieved but work is continuing.

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Two-Dimensional J Spectroscopy: ^{1}H NMR of Polysaccharides. Application to Capsular Heteroglycans and Labeled Cellulose Triacetate

Didier Y. Gagnaire, François R. Taravel,* and Michel R. Vignon*

Centre de Recherches sur les Macromolécules Végétales (CNRS),† 53X, 38041 Grenoble Cédex, France. *Received March 23, 1981*

ABSTRACT: ^{1}H two-dimensional J spectroscopy has been investigated in the case of polysaccharides: capsular heteroglycans and cellulose triacetate. The results show the usefulness of this technique regarding identification of overlapping protons and coupling constant measurements, leading to an easier analysis of ^{1}H spectra. In particular, anomeric configurations of rhamnose-containing polysaccharides were confirmed accurately. Another application of this technique concerned deuterated cellulose triacetate, for which an improved resolution allowed a differentiation of the possible isotopomers on the basis of their isotopic effect.

Although many important studies of proton two-dimensional spectroscopy have already been published,[1-4] none of them has been devoted to polysaccharides or other stereoregular polymers. As a matter of fact, it is well-known that for polysaccharides and polymers, magnetic field inhomogeneity effects, high-viscosity solutions, and short spin–spin relaxation times (T_2) contribute to broaden the lines in normal one-dimensional ^{1}H NMR spectra. With the substantial resolution enhancement obtained by two-dimensional NMR experiments and with the possibility of measuring chemical shifts and coupling constants in a region of the spectrum where many signals overlap (as shown by Hall et al.[3]), problems relevant to polysaccharide NMR should be overcome, particularly in the case of complex polysaccharides such as capsular polysaccharides of *Klebsiella* (containing more than one residue in the repeating unit) and labeled polysaccharides containing several isotopomers, i.e., a mixture of multiply labeled compounds.

Experimental Methods

Samples. The undeuterated or deuterated cellulose triacetate was obtained by acetylation of biosynthesized cellulose by *Acetobacter xylinum* from glycerol or *sn*-[1,1,2,3,3-$^2H_5$]-glycerol as a carbon source.

sn-[1,1,2,3,3-$^2H_5$]-Glycerol was prepared according to Koch and Stuart's method[5] in the presence of Raney nickel catalyst. Deuteration was effected to the extent of 80%. Bacterial cellulose was grown in water according to Hestrin's experimental conditions.[6] The deuterated cellulose was labeled at approximately 46% on each of the six carbon atoms, as determined by one-dimensional proton analysis.[7]

† Laboratoire propre du CNRS, associé à l'Université Scientifique et Médicale de Grenoble.

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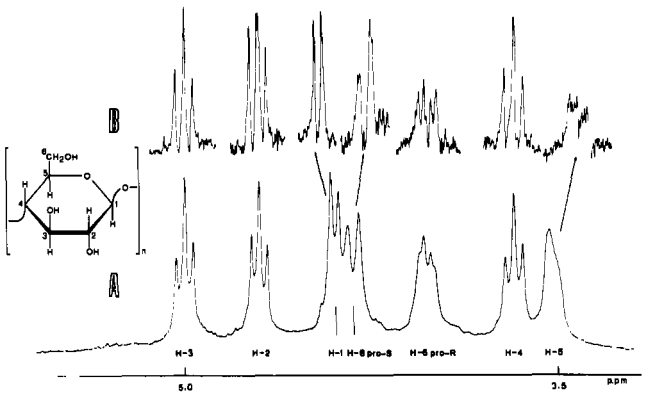

Figure 1. (A) Normal ^1H FT spectrum (250 MHz) of cellulose triacetate (1% solution) in $CDCl_3$ at 323 K. (B) Corresponding two-dimensional J spectra. The data were obtained with the following spectrometer settings: F_2 frequency range, 1800 Hz over 8K data size (0.439 Hz per point); F_1 frequency range, ±28.1 Hz with 256 data size (0.220 Hz per point). Sixteen pulse sequences and a delay time of 3 s were used.

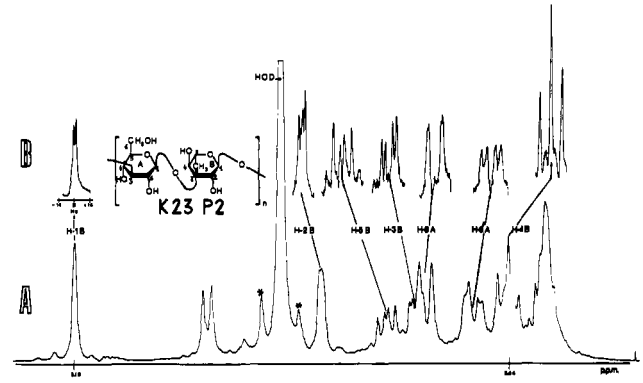

Figure 2. (A) Normal ^1H FT spectrum (250 MHz) of K23P2 in D_2O at 360 K. (B) Partial two-dimensional J spectra of protons H-1B, H-2B, H-3B, H-4B, and H-5B of the rhamnose residue and H-6A, and H-6'A of the glucopyranose unit. See Figure 1 for spectrometer settings.

Klebsiella K23 capsular polysaccharide has been investigated by Dutton et al.[8] The structure was found to consist of a tetrasaccharide repeating unit with two residues (α-L-rhamnopyranose and β-D-glucopyranose) in the main chain and two residues (β-D-glucuronic acid and α-D-glucopyranose) in the side chain. The carboxyl-reduced polysaccharide was subjected to a Smith degradation,[9] and a polymeric product was obtained following dialysis and lyophilization. Methylation analysis of this material, denoted K23P2, showed it to consist of a disaccharide repeating unit composed of one glucosyl and one rhamnosyl residue, i.e., (-3-α-L-Rhap-(1→3)-β-D-Glcp-(1→)$_n$. This compound was freeze-dried from D_2O and then dissolved in 99.95% D_2O (1% solution).

The synthesis of ^{13}C-labeled nigeran has already been described by one of us.[10]

NMR. NMR spectra were recorded at 250 MHz on a Bruker XM 250 spectrometer equipped with an Aspect 2000 computer. Two-dimensional J-resolved spectroscopy was performed with the Bruker FT NMR two-dimensional program (version 801010). The data were obtained with the following spectrometer settings: F_2 frequency range, 1800 Hz over 8K data size (0.439 Hz per point); F_1 frequency range, ±28.1 Hz with 256 data size (0.220 Hz per point). Sixteen pulse sequences and a delay time of 3 s were used.

Before Fourier transformation, the data were multiplied in both directions by window functions in a cosine form (cos (πt[2·(AQT)]$^{-1}$), with AQT, the acquisition time, =2.27 s). The pulse sequence used was 90°-$t_{1/2}$-180°-$t_{1/2}$-fid (t_2). By arranging for different experimental conditions to prevail during t_1 and t_2, we were able to separate the effects of chemical shifts and scalar couplings into two frequency dimensions. Selected sections projected onto the F_1 axis have been used to represent the two-dimensional J spectra shown in this paper.

Results and Discussion

Resolution enhancement is illustrated in Figure 1 for the case of cellulose triacetate. While the normal proton one-dimensional spectrum shows only a triplet for H-2, the corresponding two-dimensional J spectrum appears as a well-resolved quadruplet with ^3J(H-1, H-2) = 7.6 Hz and ^3J(H-2, H-3) = 9.7 Hz. Similarly, a ^3J(H-6 *pro-S*, H-5) of 2.0 Hz can be accurately measured in the two-dimensional J signal of H-6 *pro-S*, which, by this technique, presents no overlapping with H-1. In addition, ^3J(H-6 *pro-R*, H-5) = 5.5 Hz and ^3J(H-4, H-5) = 9.5 Hz have been determined, analyzing the two-dimensional J signals of H-6 *pro-R*, H-5, and H-4. Furthermore, a long-range coupling constant (^4J) with H-1 or H-3 can be detected for H-5, in agreement with what we can observe for monosaccharide models.[3,11] Such a small coupling constant has never been observed in normal one-dimensional spectra of polysaccharides. We mention here that well-resolved cross-sectional spectra could be very useful for assigning resonance lines in complex molecules.

Now if we compare by simulation the shape of the lines for both spectra shown in Figure 1, we find that the best fit is obtained with a line width of 4 Hz for the normal spectrum and 2 Hz only for the two-dimensional spectrum. As shown in the case of H-2 the two-dimensional line resolution reached is at least 2 Hz; in the meantime peaks appear sharper. These remarks suggest that cancellation of field inhomogeneity effects by the spin-echo technique involved during two-dimensional NMR experiments should provide a good estimation of spin–spin relaxation times (T_2) using half-line-width measurements, particularly when no other possibility is available.

In the case of capsular polysaccharides of *Klebsiella*, normal proton one-dimensional spectroscopy has usually been used to assign anomeric configurations (by means of measured coupling constants). However, when the repeating unit contains mannose or rhamnose residues, the only available way of specifying the anomeric configuration has been based on chemical shift considerations (α form, $\delta > 4.95$; β form, $\delta < 4.95$).[12,13] Until now, the poor resolution of one-dimensional polymer proton spectra did not allow differentiation of the configurations on the basis of coupling constants obtained for monomeric models (α form, ^3J(H-1, H-2) = 1.8 Hz; β form, ^3J(H-1, H-2) = 1.1 Hz[14]). Figure 2 reproduces the proton one-dimensional spectrum and the corresponding two-dimensional spectrum of *Klebsiella* polysaccharide serotype K23P2 (on which the side chain of the repeating unit has been removed). Thus H-1 of the rhamnose residue appears as a well-resolved doublet with ^3J(H-1B, H-2B) = 1.9 Hz, confirming that it is α linked to the glucose residue. Also, by this technique the same coupling constant can be deduced from the quadruplet signal of H-2B as well as ^3J(H-2B, H-3B) = 3.5 Hz, leading to unambiguous assignment of H-3B, interfering with one of the H-6's from the glucose residue in the one-dimensional proton spectrum but well resolved and separated in the two-dimensional J spectrum. A further analysis, supported by the spreading of peaks according to their J splittings, is thus easier and very helpful for step-by-step unambiguous assignment, provided that we are dealing with weakly coupled spin systems.

Similarly, the signal of H-5B can be easily identified by means of its ^3J(H-5, H-6) ≃ 6.0 Hz, as this coupling constant can also be determined from the characteristic upfield doublet of the methyl group (H-6).

Figure 3 shows another application of two-dimensional J spectroscopy in the case of deuterated cellulose triacetate (obtained by *Acetobacter xylinum* biosynthesis from

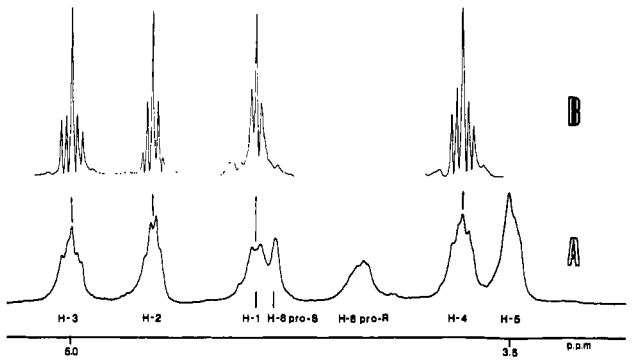

Figure 3. (A) Normal ^1H FT spectrum (250 MHz) of deuterated cellulose triacetate (biosynthesized from *sn*-[1,1,2,3,3-$^2H_5$]-glycerol by *Acetobacter xylinum*) in $CDCl_3$ at 323 K. (B) Corresponding two-dimensional *J* spectra (protons H-1, H-2, H-3, and H-4). Spectrometer settings as in Figure 1. In the case of H-1, note the near-equality for the occurrence of the isopotomers in which H-1 is coupled to H-2 and in which H-1 is coupled with a deuterium atom at C-2.

sn-[1,1,2,3,3-$^2H_5$]-glycerol[7]). Due in particular to the existence of several isotopomers, the proton one-dimensional spectrum is very complex, and only relative labeling ratios for the different sites can be deduced by quantitative analysis. Information concerning the probabilities for the occurrence of the possible isotopomers, which would be very useful for elucidating the biosynthetic mechanism, is almost impossible to extract accurately.

In contrast, the two-dimensional *J* spectra (shown in Figure 3 for H-1, H-2, H-3, and H-4) lead to an easy determination of the combinations of different ^2H or ^1H sites. Thus H-1 appears either as a doublet corresponding to all the isotopomers with a hydrogen atom at the C-1 and C-2 positions (and a hydrogen atom or a deuterium atom at the other positions) or as a singlet corresponding to isotopomers with a hydrogen atom at C-1 and a deuterium atom at C-2. The singlet must be shifted upfield by 0.25 Hz relative to the chemical shift of the doublet, allowing differentiation of the species, as they should be represented by different cross sections, provided that a sufficient resolution in the F_2 direction has been reached. In our experiment the F_2 digital resolution was not sufficient to make this phenomenon clear and the resonances of the observed signals appear only centered about the same frequency zero. However, a quantitative estimation of the different species is still possible if the two-dimensional spectra are recorded with an appropriate relaxation delay.

In the same way H-2, H-3, and H-4 can be distinguished and appear as follows: (a) a well-resolved triplet (a quadruplet for H-2) corresponding to isotopomers with hydrogen atoms; H-$(i-1)$/H-(i)/H-$(i+1)$ with $2 \leq i \leq 4$; (b) well-resolved doublets (two possibilities) corresponding to isotopomers monodeuterated on one neighboring site or the other: ^{2}H-$(i-1)$/H-(i)/H-$(i+1)$ or H-$(i-1)$/H-(i)/^{2}H-$(i+1)$; (c) a singlet corresponding to isotopomers with two deuterated neighbors: ^{2}H-$(i-1)$/H-(i)/^{2}H-$(i+1)$. As for H-1, an improved resolution in the F_2 direction will differentiate each species on the basis of its isotopic effect (the mono- and dideuterated isotopomers showing a resonance at a higher field than the unlabeled one).

Additional information can be obtained locally when different coupling constants are involved. For example, in the case of H-2 the two doublets corresponding to the two isotopomers ^{2}H-1/H-2/H-3 and H-1/H-2/^{2}H-3 should be detectable, due to the large difference between ^3J(H-1,H-2) = 7.6 Hz and ^3J(H-2,H-3) = 9.7 Hz. However, only one doublet was observed with a coupling constant of 9.7 Hz. This observation agrees well with a predominant probability for the occurrence of one of the two possible isotopomers, i.e., ^{2}H-1/H-2/H-3. The other monodeuterated species, H-1/H-2/^{2}H-3, is not detectable. The particular action of two enzymes, triosephosphate isomerase and aldolase, involved in the head-to-head condensation of triose fragments[15] (glycerol) during the biosynthesis of cellulose, leading to the formation of a molecule with six carbon atoms, can explain this discrepancy. Both enzymes act in such a way that exhanges occur preferentially at positions 3 and 4 of the new molecule formed. Quantitative analysis confirms the corresponding deuteration at these positions.

The knowledge of the contribution of the different isotopomers seen for each site (as obtained by two-dimensional spectroscopy) and the determination of the labeling percentage on each specific position (as obtained by one-dimensional analysis) give the possibility of correlating the various data in order to estimate the occurrence of all the possible species.

This kind of analysis constitutes a good approach in the complete elucidation of reaction mechanisms in synthetic and biosynthetic studies.

In order to investigate line widths observed by two-dimensional spectroscopy (in connection with the relaxation phenomena, in particular, spin–spin relaxation time T_2), a ^{13}C-enriched polysaccharide was also studied. This polysaccharide, [1-^{13}C,6-^{13}C]-nigeran, which is a polyglucan with α-D-glucopyranosyl residues alternately linked 1→3 and 1→4, was obtained by biosynthesis with *Aspergillus aculeatus* from D-[1-^{13}C]-glucose.[10] The normal proton spectrum analysis has shown that the ^{13}C nuclei offer an additional relaxation mechanism for the anomeric protons. Thus the ^{13}C satellites of the two anomeric protons (55% enrichment) have a relaxation time (T_1) 2 times smaller than the T_1 of the corresponding unlabeled protons.[16] Furthermore, the satellite lines appear as wide, unresolved peaks, while the corresponding ^{12}C protons are well-resolved doublets. The simplest explanation of this phenomenon seems to be directly related to the observed difference in the relaxation rates. In order to elucidate this problem, more studies are needed, especially two-dimensional *J* experiments, and these are now in progress. However, even though by this technique it has been possible to measure a line width of 2 Hz for the ^{12}C anomeric protons, only an approximate value (3 Hz) of the line width for the ^{13}C satellites was determined due to a poor signal-to-noise ratio. From these estimations, T_2 values of 0.1 and 0.16 s can be deduced for the ^{13}C and ^{12}C anomeric protons, respectively. They are different from the T_1 values already measured (0.26 and 0.68 s, respectively)[16] and could lead, assuming that the equations defined by Doddrell et al.[17] apply to polysaccharides, to a rotational correlation time (τ_R) near 10^{-9} s, in agreement with the value found from NOE experiments.[16]

Conclusion

Even though two-dimensional *J* experiments are time-consuming, the above results clearly show the usefulness of this technique when applied to polysaccharides or, more generally, to polymers, in addition to all NMR techniques available to the practicing spectroscopist. Resolution enhancement, identification of overlapping protons, and accurate determinations of coupling constants lead to an easier interpretation of proton spectra and to information capable of providing insight into structural, sterochemical, and conformational details (particularly in the case of mannose- or rhamnose-containing polysaccharides). As shown in the case of deuterated cellulose from *Acetobacter xylinum*, two-dimensional experiments open a new route

to obtain a better understanding of synthetic reactions or biosynthetic pathways.

More generally, when in a spectrum two overlapping signals exist, as they correspond to two different forms of the same site (by isotopic exchange or other phenomena), two-dimensional J spectroscopy will be able to differentiate between them, as far as their induced chemical shift variation is in agreement with the experimental digital resolution. Furthermore, quantitative analysis will be made easier and will lead to a better conception of the phenomena involved. Of course, it will be advantageous to use the two-dimensional technique with higher field spectroscopy in order to overcome most of the problems related to strongly coupled spin systems.

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NMR Study of Nylon 66 in Solution (^{1}H, ^{13}C, and ^{15}N NMR Using Adiabatic J Cross Polarization)

Brenda S. Holmes* and William B. Moniz

Chemistry Division, Naval Research Laboratory, Washington, D.C. 20375

Raymond C. Ferguson

Central Research and Development Department,† E. I. du Pont de Nemours, Wilmington, Delaware 19898. *Received March 9, 1981*

ABSTRACT: We have utilized ^{15}N NMR in conjunction with ^{1}H and ^{13}C NMR chemical shifts to study nylon 66 in acid solutions. Using the adiabatic J cross-polarization (AJCP) technique, we obtained signal enhancements which resulted in ~100-fold time savings and eliminated the need to match exactly the radio-frequency amplitudes of the ^{1}H and ^{15}N during polarization transfer. The effects of solvent acidity, variation of polymer concentration, and variation of degree of polymerization (DP) on nylon 66 in solution have been studied. ^{15}N chemical shifts were more responsive to variation of the experimental parameters than the corresponding proton and ^{13}C data. ^{15}N data exhibited deshielding with increasing solvent acidity, deshielding with decreasing polymer concentration, but little variation with respect to degree of polymerization below 25% (w/v). At higher nylon concentrations, however, the DP of the polymer apparently affects the extent of protonation and/or hydrogen bonding, which causes an increase in the shielding at the ^{15}N nucleus of the amide group. Using the ^{15}N NMR data in conjunction with the ^{1}H and ^{13}C data of the nylon 66, we have confirmed the site of protonation/hydrogen bonding as the oxygen of the amide carbonyl in the presence of excess acid.

Introduction

In our previous paper we studied the ^{15}N nuclear magnetic resonance (NMR) chemical shifts of polyamides in solution with respect to solvent acidity, concentration, and temperature.[1] We found that the ^{15}N chemical shifts of nylon 66 in trifluoroacetic acid (CF_3COOH, TFA) were most sensitive to changes in concentration. Nylon 66 in 1,1,1,3,3,3-hexafluoro-2-propanol ($(CF_3)_2CHOH$, HFIP) solutions had ^{15}N chemical shifts least sensitive to concentration changes. These two systems were chosen for this study using ^{1}H, ^{13}C, and ^{15}N NMR techniques in order to determine if protonation and/or hydrogen bonding varies with changes in concentration. We also studied two different molecular weights of nylon 66 to determine if the changes were dependent on degree of polymerization.

Schilling and Kricheldorf[2] have shown that ^{13}C NMR chemical shifts of carbonyl carbons of polyamides and peptides vary with solvent acidity, as do ^{15}N chemical shifts of polyamides;[1,3] the ^{15}N shifts, however, are more sensitive to sequence variation than ^{13}C chemical shifts in polyamides and peptides.[3] The small magnetogyric ratio (γ) and low natural abundance, in addition to long T_1 values of ^{15}N nuclei, have made natural-abundance ^{15}N NMR more tedious than ^{13}C NMR.

The J cross-polarization (JCP) technique,[5] however, was used to increase the sensitivity of ^{15}N NMR in the previous study of polyamides in solution. In the present study, we used the adiabatic JCP (AJCP) technique[6] to examine the ^{15}N NMR chemical shifts of the nylon 66/TFA and nylon 66/HFIP solutions.

Experimental Section

Samples. Two different molecular weights of nylon 66 were provided by du Pont. The number-average molecular weights,

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