4. L. A. McFadden, thesis, University of Hawaii, Honolulu (1983).
5. G. J. Veeder, D. L. Matson, E. F. Tedesco, *Icarus* **55**, 177 (1983); E. F. Tedesco *et al.*, *Am. Astron. Soc. Bull.* **15**, 825 (1983).
6. G. J. Veeder and H. P. Larson, in *Asteroids*, T. Gehrels, Ed. (Univ. of Arizona Press, Tucson, 1979), p. 724.
7. Asporina is 2.70 astronomical units (AU) from the sun, and recent observations give its diameter as 60 km and its geometric albedo as 0.14 (R. H. Brown and D. Morrison, in preparation). Nenetta is located at 2.87 AU; E. F. Tedesco, J. C. Gradie, and D. J. Tholen (unpublished observations) give its diameter as 29 km and its albedo as 0.29. Neither object is listed as a member of a family, as defined by orbital similarities [J. G. Williams, in *Asteroids*, T. Gehrels, Ed. (Univ. of Arizona Press, Tucson, 1979), p. 1040]. Asteroid 246 Asporina was observed on 15.6 April 1983 at an average air mass of 1.08 and was ratioed to the star 70 Vir (G5 V) observed at a similar air mass on the same night. Asteroid 289 Nenetta was observed on 16.2 April 1983 at an average air mass of 1.09 and was ratioed to the star HR 3881 (G1 V) observed at a similar air mass on the same night. We have measured the small differences between the near infrared colors of these stars and the sun (G2 V) and find that they are negligible in this study.
8. C. R. Chapman and M. J. Gaffey, in *Asteroids*, T. Gehrels, Ed. (Univ. of Arizona Press, Tucson, 1979), p. 655.
9. ———, in *ibid.*, p. 1064.
10. M. Feierberg, H. P. Larson, C. R. Chapman, *Astrophys. J.* **257**, 361 (1982).
11. M. J. Gaffey, *J. Geophys. Res.* **81**, 905 (1976); R. B. Singer, *ibid.* **86**, 7967 (1981).
12. G. W. Wetherill *et al.*, in *Basaltic Volcanism on the Terrestrial Planets* (Pergamon, Elmsford, N.Y., 1981), p. 944, table 7.2.9; C. E. Nehru, M. Prinz, J. Delaney, *Lunar Planet. Sci. Abstr.* **14**, 552 (1983).
13. R. G. Burns, *Am. Mineral.* **55**, 1608 (1970).
14. G. Hunt and J. Salisbury, *Mod. Geol.* **1**, 285 (1970).
15. M. Miyamoto, A. Mito, Y. Takano, N. Fujii, *Mem. Natl. Inst. Polar Res. Spec. Issue No. 20* (1981), p. 346.
16. R. Dymek, A. Albee, A. Chados, *Proc. Lunar Sci. Conf.* **6**, 301 (1975).
17. R. T. Dodd, *Meteorites* (Cambridge Univ. Press, Cambridge, England, 1981).
18. R. Greenberg and C. R. Chapman, in preparation.
19. L. D. Ashwal, J. Warner, C. A. Wood. *J. Geophys. Res.* **87** (Suppl.), A393 (1982); G. Ryder, *ibid.*, p. A401; C. A. Wood and L. D. Ashwal, *Proc. Lunar Planet. Sci. Conf.* **7B**, 1359 (1981).
20. D. Bogard, C. A. Wood, J. Melosh, private communication.
21. We thank C. Chapman, D. Tholen, M. J. Gaffey, L. A. McFadden, E. F. Tedesco, and M. Prinz for helpful comments. D.P.C. and W.K.H. are visiting astronomers at the NASA Infrared Telescope Facility, which is operated by the University of Hawaii under contract with NASA. Supported by NASA grant NGL-12-001-057 (D.P.C.) and by NASA contract NASW 3134 (W.K.H.). This is Planetary Science Institute contribution No. 191. The Planetary Science Institute is a division of Science Applications, Inc.

8 August 1983; accepted 12 September 1983

Native Cellulose: A Composite of Two Distinct Crystalline Forms

Abstract. *Multiplicities in the resonances of chemically equivalent carbons, which appear in the solid-state carbon-13 nuclear magnetic resonance spectra of native celluloses, have been examined at high resolution. The patterns of variation are consistent with the existence of two distinct crystalline forms. One form is dominant in bacterial and algal celluloses, whereas the other is dominant in celluloses from higher plants.*

The resonance multiplicities reported in the solid-state ^{13}C nuclear magnetic resonance (NMR) spectra of native celluloses (*1, 2*) have been examined at higher resolution for a variety of native forms and for a sample of regenerated cellulose I. The pattern of variation among spectra of the native forms suggests that they are all composites of two distinct crystalline forms of cellulose. This observation provides a basis for reassessing some of the conflicting interpretations of data concerning the structures of native celluloses.

Significant questions remain with respect to the structure of native celluloses. These include the symmetry of the unit cell and its application in the analysis of diffraction data (*3*) as well as the conformational differences, or lack thereof, between native cellulose and its most common alternate polymorph (*4*). The solid-state ^{13}C NMR spectra represent an important source of new information that can help to resolve these questions.

A number of groups have reported spectra of different celluloses (*1, 2, 5*). Although spectra of pure samples of cellulose II could be rationalized in terms of nonequivalent sites within a unique unit cell, the spectra of native celluloses reveal multiplicities that cannot be so explained. The observations summarized here result from our effort to interpret these multiplicities in spectra of higher resolution.

We recorded the spectra by applying the cross polarization–magic angle spinning (CP-MAS) technique in a high-field instrument (*6*). This method involves cross polarization to enhance the ^{13}C signal, high-power proton dipolar decoupling to eliminate dipolar line-broadening due to protons, and spinning of the sample about an axis at a particular angle to the static field to eliminate chemical shift anisotropy. The methods and considerations involved in the acquisition and interpretation of the spectra have been discussed (*2, 7*). Those central to the point of this report will be reviewed briefly.

The samples included a bacterial cellulose from *Acetobacter xylinum*, an algal cellulose from *Valonia ventricosa*, and two fibrous celluloses, cotton linters and ramie. Finally, celluloses of the pure polymorphic forms I and II were regenerated by procedures developed in one of our laboratories (*8*).

The spectra of the native celluloses, many of which have been reported (*2*), are shown together with that of the regenerated cellulose I in Fig. 1. The accepted assignments to the different carbons in the anhydroglucose units are as indicated at the top of Fig. 1. The features between 70 and 81 parts per million are assigned to C-2, C-3, and C-5 collectively, because at present there is no basis for individual assignment. The assignments of the resonances due to C-1, C-4, and C-6, however, are firmly established on the basis of comparison with oligosaccharides and model compounds. The structural implications of the resonance profiles of each of these carbons are our central concern.

The regions of the spectrum corresponding to C-4 and C-6 are similar in that in most of the spectra both include a cluster of sharper resonances and a broader upfield wing. Each of the broader wings is associated both with the surfaces of crystalline domains, on the basis of morphological considerations, and with regions of three-dimensional disorder, on the basis of comparisons of the cotton linters spectrum with spectra of a hydrocellulose and an amorphous cellulose. In the case of C-1, it appears that the sharper components overlap the resonance associated with the less ordered domains.

The sharper components of the C-4, C-6, and C-1 resonances possess multiplicities that suggest that they arise from magnetically nonequivalent sites within crystalline domains. The most important feature in Fig. 1 is the pattern of variation of the multiplets, primarily at C-4 but also at C-1 and C-6. These lines differ among the samples; the relative intensities are not constant, and they are not in the ratios of small whole numbers as would be expected if they arose from different sites within a single unit cell. The native celluloses are, therefore, composites of two or more crystalline forms. But the spectral intensities are not consistent with the possibility of three independent crystal forms, each giving rise to a single resonance in the C-1, C-4, and C-6 regions. A model based on two independent crystalline forms remains the simplest plausible proposal. And indeed a decomposition of the spectra on the basis of such a model has been accomplished.

The approximate component spectra of the two forms are illustrated in Fig. 2

together with a spectrum of a high-crystallinity cellulose II. The latter spectrum is included to distinguish the heterogeneity of crystalline forms proposed here for native celluloses from the well-recognized polymorphy of cellulose.

We obtained the spectra designated I_α and I_β by taking appropriate linear combinations of the spectra of the regenerated cellulose I and of the cellulose from *Acetobacter xylinum*. These two celluloses were judged to be closest to the two extremes on the basis of a two-component model. The spectra in Fig. 1 indicate the following ranking with respect to the relative amounts of I_α as a fraction of the total: *Acetobacter xylinum* ≅ *Valonia ventricosa* > cotton ≅ ramie > regenerated cellulose I.

The spectra of Fig. 2 must be regarded as approximations at this point, because correction for noncrystalline components remains incomplete. Nevertheless, the narrow component features in the C-1, C-4, and C-6 regions are correct indicators of unit cell magnetic nonequivalences. The I_α form has singlets at C-1 and C-6 and a closely spaced doublet at C-4. The I_β form, on the other hand, has doublets at C-1, C-4, and C-6. Although the C-4 doublets in both spectra are somewhat asymmetric, there are indications that this may be a consequence of differences in line width rather than differences in intensity. Thus, the criterion that component intensities occur as ratios of small whole numbers is met, within the experimental uncertainty, for the narrow portions of the resonances of C-1, C-4, and C-6.

The proposal of multiple crystal forms in native celluloses is novel in that it implies that all native celluloses are composites of two distinct forms. At the same time it confirms earlier reports (*9*) that *Acetobacter* and *Valonia* celluloses are structurally different from other celluloses such as cotton and ramie. A very crude estimate based on our model suggests that *Acetobacter* cellulose is 60 to 70 percent I_α, whereas cotton is approximately 60 to 70 percent I_β (*10*).

Our observations carry implications for a number of areas of basic inquiry concerning cellulose. Questions concerning structure are important both inherently and because structural models have been the basis for the identification of cellulose and for speculation concerning mechanisms of biosynthesis.

Models based on diffractometry must be reassessed because, in most instances, the diffraction patterns of systems of mixed structure are not simple superpositions of the patterns of individual components (*11*). Interpretations in terms of a unique unit cell have a serious constraint built into them, the implications of which require reexamination.

A reassessment is also necessary for the use of diffraction patterns to complement chemical analysis in the identification of cellulose. Some departures from the more common patterns may no longer be taken as negative evidence.

The two celluloses in which I_α is dominant correspond to lower forms in which the biosynthesis is simultaneous with extension of the microfibril network and the architecture of the aggregates is rather less complex. On the other hand, I_β is dominant in the celluloses from higher plants where the major component is the secondary wall, usually formed after the geometry of the cell has been established and frequently possessing a complex architecture.

Our results represent yet another instance of the importance of spectroscopic observations in studies of macromolecular structure (*12*).

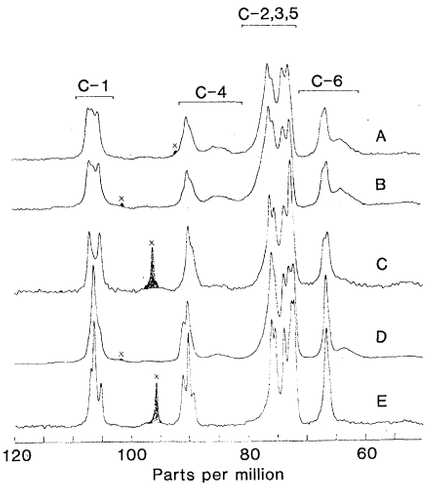

Fig. 1. The ^{13}C CP-MAS spectra of various celluloses: (A) ramie; (B) cotton linters; (C) regenerated cellulose I; (D) *Acetobacter xylinum* cellulose; (E) *Valonia ventricosa* cellulose. The x marks the small first spinning side band of linear polyethylene added as an internal chemical shift standard; its center band at 33.6 parts per million is not included in this display.

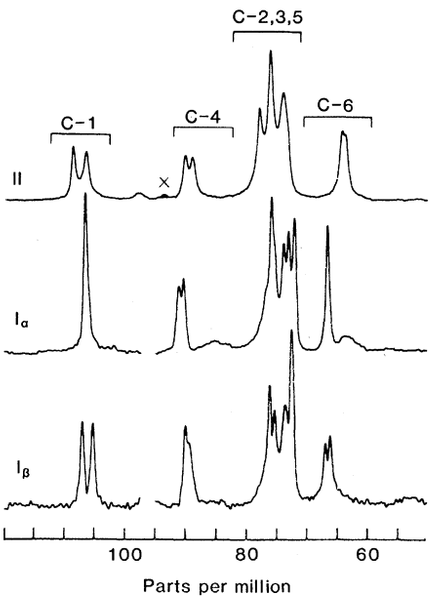

Fig. 2. Comparison of the ^{13}C CP-MAS spectrum of cellulose II and the derived spectra of the two proposed crystalline forms of cellulose I, namely, I_α and I_β. An x or a gap marks the location of the first spinning side band of the linear polyethylene chemical shift standard.

RAJAI H. ATALLA
Chemical Sciences Division, Institute of Paper Chemistry, Appleton, Wisconsin 54912
DAVID L. VANDERHART
Polymers Division, National Bureau of Standards, Washington, D.C. 20234

References and Notes

1. R. H. Atalla, J. C. Gast, D. W. Sindorf, V. J. Bartuska, G. E. Maciel, *J. Am. Chem. Soc.* **102**, 3249 (1980); W. L. Earl and D. L. VanderHart, *ibid.*, p. 3251.
2. W. L. Earl and D. L. VanderHart, *Macromolecules* **14**, 570 (1981).
3. D. W. Jones, in *Cellulose and Cellulose Derivatives*, vol. 5, part 4, *High Polymers*, N. M. Bikales and L. Segal, Eds. (Interscience, New York, 1971), p. 117; O. Ellefsen and B. A. Tonnesen, in *ibid.*, p. 151; B. A. Tonnesen and O. Ellefsen, in *ibid.*, p. 265; R. H. Atalla, *Adv. Chem. Ser., No. 181* (1979), p. 55.
4. M. Norman, *Text. Res. J.* **33**, 711 (1963); T. Petitpas, M. Oberlin, J. Mering, *J. Polym. Sci. Part C* **2**, 423 (1963); R. H. Atalla, *Appl. Polym. Symp.* **28**, 659 (1976); *Proceedings of the International Symposium on Wood and Pulping Chemistry*, Stockholm, June 1981, SPCI Report No. 38 (1981), vol. 1, p. 57.
5. G. E. Maciel, W. L. Kolodziejski, M. S. Bertran, B. E. Dale, *Macromolecules* **15**, 686 (1982); R. L. Dudley *et al.*, *J. Am. Chem. Soc.* **105**, 2469 (1983).
6. The NMR spectrometer used to record the spectra is a Bruker CXP200, operating at frequencies of 50.3 MHz for ^{13}C and 200 MHz for protons. Linear polyethylene was usually added to the samples to provide a chemical shift reference; it also permits monitoring of the precision of the magic angle setting, which is known to be critical for good resolution.
7. D. L. VanderHart and R. H. Atalla, in *Symposium on Microstructure of Cellulose, Proceedings of the International Dissolving and Specialty Pulps Conference, 1983* (TAPPI Press, Atlanta, in press); D. L. VanderHart and R. H. Atalla, *Macromolecules*, in press.
8. The procedure was as follows: 85 g of microcrystalline cellulose, wet with 62 ml of distilled water, was dissolved in 1590 ml of 85 percent phosphoric acid. After it was allowed to stand for 6 weeks, the mixture was filtered through fine glass (pore size, 4 to 5.5 μm) and then it was added to three times its volume of distilled water. The precipitate was separated by centrifugation, and the clear liquid saved. A portion of the precipitate, which was cellulose II of relatively high crystallinity, was then boiled in 4*N* HCl for 48 hours. The residue, in 3.3 percent yield, was cellulose I. A similar sample had been prepared by hydrolysis of another portion of the precipitate in water at 210°C, in a pressure vessel, for 2 hours. The x-ray diffractograms were similar to one reported earlier [R. H. Atalla and S. C. Nagel, *Science* **185**, 522 (1974)] and leave no doubt that the samples are the pure cellulose I polymorph. The clear liquid from the centrifugation was added to an equal volume of methanol. A small amount of additional precipitation occurred; this was the cellulose II of the highest crystallinity used in recording the signature spectrum. Both regenerated celluloses I and II were of a degree of polymerization below 20.

9. H. J. Marrinan and J. Mann, *J. Polym. Sci.* **21**, 301 (1956).
10. The proposal that native celluloses are composites of I_α and I_β is distinct from, although complementary to, the structural model proposed by R. H. Atalla [*Appl. Polym. Symp., No. 37* (1983), p. 295] on the basis of spectroscopic studies. The distinct conformations identified as k_I and k_{II} in that model correspond to the forms of cellulose here represented as I_β and II. The spectroscopic studies that formed the basis of the conformational model involved native celluloses in which I_β is dominant.
11. M. Kakudo and N. Nobutami, *X-ray Diffraction by Polymers* (Elsevier, New York, 1972), p. 224.
12. ———, *ibid.*, p. 285; H. Tadokoro, *Structure of Crystalline Polymers* (Wiley, New York, 1979).
13. The Bureau of Engraving and Printing provided partial support for the work of D.L.V. The work of R.H.A. was supported through institutional and academic research programs of the Institute of Paper Chemistry.

28 February 1983; accepted 31 October 1983

Catalytic Activity of an RNA Molecule Prepared by Transcription in vitro

Abstract. *Ribonuclease P is a ribonucleoprotein that cleaves precursors to transfer RNA (tRNA) molecules to yield the correct 5′ terminal sequences of the mature tRNA's. The RNA moiety M1 RNA of ribonuclease P from* Escherichia coli *and the unprocessed transcript prepared in vitro of the gene for M1 RNA can both perform the cleavage reactions of the canonical enzyme in the absence of the protein moiety. When the transcript of the M1 RNA gene is combined with the protein moiety not only is a tRNA precursor cleaved but also the precursor to 4.5S RNA from* Escherichia coli.

The transcripts of all transfer RNA (tRNA) genes in *Escherichia coli* are processed by ribonuclease P to yield the correct 5′ terminal sequences of the tRNA's (*1*). Ribonuclease P consists of a protein moiety, C5, and an RNA moiety, M1 (*2*), but it has been shown that M1 RNA alone can cleave precursors of tRNA molecules with the correct site specificity (*3*). In vivo M1 RNA is generated from a longer gene transcript (abbreviated pM1 RNA) by the removal of 36 or 37 nucleotides from the 3′ terminus (*4, 5*). We now report that pM1 RNA, prepared by transcription in vitro of the gene for M1 RNA, can perform the same cleavage of the precursor to tyrosine tRNA ($tRNA^{Tyr}$) from *E. coli* as can M1 RNA purified from *E. coli*. These experiments were undertaken to eliminate the possibility that a protein contaminant in the M1 RNA preparations could account for the observed catalytic activity. Our results show that an unprocessed gene transcript, prepared in vitro, has the properties of a biochemical catalyst and extend our previous observation that an RNA molecule can execute enzymatic functions previously thought to be reserved for proteins.

pM1 RNA made in vitro by transcription of the gene for M1 RNA (*4*) can cleave the precursor to *E. coli* $tRNA^{Tyr}$ (Fig. 1, lane 2). The products of this reaction (which is carried out in buffer containing 60 m*M* Mg^{2+}) have the same mobility as the products generated by cleavage of the same substrate by M1 RNA or ribonuclease P (Fig. 1, lanes 4, 5, and 9). In the experiments shown in Fig. 1, lanes 2 and 9, pM1 RNA and M1 RNA were present in the respective reaction mixtures in equal amounts. Portions of these mixtures were assayed during the period of linear kinetics of product accumulation. It is apparent that the initial rates of both reactions are similar (Fig. 1, lanes 2 and 9). However, when reconstituted ribonuclease P complexes (*3, 6*) consisting of pM1 RNA and the protein subunit, C5, of *E. coli* ribonuclease P were assayed for activity, the rate of reaction was about seven times faster than with pM1 RNA alone (compare Fig. 1, lane 2 to lane 4 and lane 5 to lane 7) (*7*). When C5 protein was mixed with mature M1 RNA in equimolar amounts to reconstitute ribonuclease P, there is only a twofold stimulation in the rate of reaction as compared to the rate when M1 RNA is used alone (*3*). In the experiments we describe here, C5 protein is added in hundredfold molar excess. Under these conditions the rates of the reactions carried out by complexes made with pM1 RNA or with M1 RNA are stimulated to an equal extent (data not shown). This stimulatory effect, which we are studying further, may be due to the alteration by C5 protein of the effective concentration of the RNA's (by disaggregation of RNA complexes) or it may be an indication that C5 protein is needed in more than equimolar amounts for optimum activity of the protein-RNA complexes.

The products generated by cleavage of the precursor to $tRNA^{Tyr}$ by pM1 RNA, by the pM1 RNA–C5 protein complex, or by M1 RNA alone have the same electrophoretic mobilities. Analyses of two dimensional oligonucleotide separation patterns (fingerprints) of the reaction products generated by either pM1 RNA alone or by the pM1 RNA–C5 protein complex show that the products are the same as those produced by M1 RNA or by cleavage by ribonuclease P of the precursor to $tRNA^{Tyr}$. In particular, the 5′ terminus of the large fragment containing the tRNA sequence is pGGU as expected (G, guanine; U, uracil) (*8*).

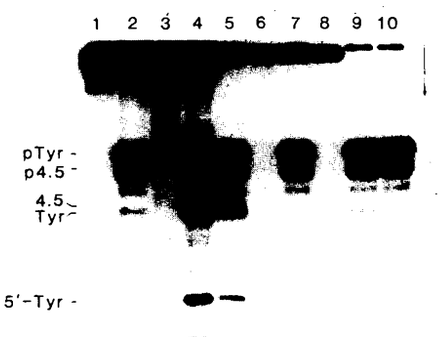

Fig. 1. Cleavage of precursor to $tRNA^{Tyr}$ by pM1 RNA. pM1 RNA was made in vitro generally as described (*4*) with sufficient unlabeled ribonucleoside triphosphate added so that the product had a specific radioactivity of 7×10^6 to 8×10^6 count/min per microgram of RNA (*12*). Assays for ribonuclease P activity were carried out and analyzed in a 10 percent polyacrylamide gel (*3*); the buffer compositions are given in (*13*). The substrate was a mixture of the precursor to $tRNA^{Tyr}$ (abbreviated pTyr; 560 fmole was added where indicated below) and the precursor to 4.5*S* RNA (abbreviated p4.5). The substrate preparations and procedures for the reconstitution of ribonuclease P have been reported in some detail (*3*). The cleavage products generated in the reactions shown in the figure are abbreviated as Tyr and 4.5 for the segments containing the mature RNA sequences and 5′ Tyr for the fragment of the precursor to $tRNA^{Tyr}$ that contains precursor-specific nucleotides. The 5′-terminal fragment of the 4.5*S* precursor molecule is not distinguishable from fragments of pM1 RNA generated by radioautolysis in lane 3 or from background in lane 4. (Lane 1) pM1 RNA (7.5 fmole) assayed in buffer C, no pTyr was added; (lane 2) as lane 1 except that pTyr was added; (lane 3) pM1 RNA (7.5 fmole) reconstituted with C5 protein (750 fmole) by direct mixing (*3*) in buffer B and no pTyr was added; (lane 4) as lane 3, but pTyr was added; (lane 5) pM1 RNA (15 fmole) was reconstituted with C5 protein (1500 fmole) by dialysis (*3*) in 20 μl and 2 μl was assayed in buffer A, and pTyr was added; (lane 6) as lane 5 except that pTyr was not added; (lane 7) pM1 RNA (15 fmole) was treated as in the reconstitution experiments for lanes 5 and 6 and assayed in buffer C, with pTyr added; (lane 8) as in lane 7 except that pTyr was not added; (lane 9) nonradioactive M1 RNA (7.5 fmole) was assayed in buffer C, with pTyr added; (lane 10) pTyr only.