

Figure 2. Infrared spectra in the carbonyl region in D_2O solution of poly(I) Li salt as a function of temperature in the presence of different alkali metal salts. In 0.17 M LiCl (top), an ordered structure is formed to a small extent at 0 °C, but without added salt (inset: lowest absorbance measured at 5 °C, highest at 61 °C) no structure is formed. In 0.1 M KCl the carbonyl band has ν_{\max} 1684 cm^{-1} , $\Delta\nu_{1/2}$ 16 cm^{-1} , and ϵ_{\max} 1190 at 0 °C. The helix is very stable (T_m , 58 °C in 0.1 M KCl) and even in the absence of added salt (left inset) it is the predominant species at ambient temperature. In 0.1 M CsCl (bottom) rapid cooling leads to the spectrum observed at 4 °C with ν_{\max} 1691 cm^{-1} and ϵ_{\max} 1050. Heating the metastable complex causes a progressive shift to the spectrum seen at 34 °C (ν_{\max} 1683.5 cm^{-1} (ϵ_{\max} 1070)). On recooling from 34 °C the spectrum remains unchanged with ν_{\max} 1683.5 cm^{-1} . Heating from either 4 or 34 °C then leads to a single transition (T_m , 53 °C) to the random coil (ν_{\max} 1675.5 cm^{-1} (ϵ_{\max} 1035)).

by ~ 3.4 Å.¹⁴ In 0.0026 M K^+ , the site occupancy is ≤ 0.5 and the transition breadth is ~ 19 °C.

In 0.1 M solutions of RbCl or CsCl, poly(I) forms a helix only slightly less stable than that formed with KCl (Figure 1). Poly(I) with each of these salts has both stable and metastable ordered forms (cf. ref 15) and exhibits large kinetic effects.

The carbonyl oxygen atoms lining the central cavity of the poly(I) helix (Figure 1) provide two distinct binding sites which differ in metal-ligand distance and coordination number. The first site is the center of the planar array which is ~ 2.2 - 2.3 Å from the centers of the four carbonyl oxygens. The second site is also on the helix axis but located midway between the planes of the tetrameric array of bases. This point is ~ 2.8 Å from each of eight carbonyl oxygens. Observed metal-oxygen ligand distances indicate that the first of these sites may be considered for Na^+ and Li^+ and the second for larger cations: Li-O, 2.0-2.2 Å;¹⁶ Na-O, 2.25-2.36 Å;¹⁷ K-O, 2.7-3.1 Å;^{18,19} Rb-O, 2.7-3.2 Å;¹⁸ Cs-O, 3.0-3.6 Å.^{18,20} Li^+ is presumably too small to bridge the first binding site effectively. The usual Cs-O distances¹⁸ are probably too large for the second site, but uncommonly short distances have been reported²⁰ and may occur in poly(I).

The present finding of large specific alkali metal ion effects on stability of a polynucleotide helix may have implications for biological functions of the nucleic acids, such as translation, transcription, or intramolecular interactions of ribosomal RNA. The requirement for specific alkali metal complexing in polynucleotides would be simply that the organic ligand

provide enough electronegative atoms (usually oxygen) with suitable geometry to form a cavity of appropriate size. It appears that the usual double helices do not meet this requirement, but biologically important looped structures may more readily do so. The oxygen ligands could be supplied by carbonyl groups, phosphate oxygens, and sugar hydroxyl or ether groups in different combinations. Single-strand polymer conformation could change, as does that of many ionophores, to facilitate specific site binding of metals. One possible effect of such metal complexing, for example, may be to shift the equilibrium between looped and paired structures in favor of the loop in a manner dependent on the nature or concentration of the alkali metal ion. If such a shift occurred, clearly the relative stabilities of different helical regions and of protein-nucleic acid interactions could also be affected.

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Low-Temperature Natural-Abundance ^{13}C NMR Spectroscopy of Matrix-Isolated Species. The Anisotropy of the Shielding Tensor in Ethylene

Sir:

Low-temperature matrix isolation of reactive species has become an important method for their preparation and study.¹ With the vigorous current expansion of this technique to relatively complex organic molecules, it is clear that the two most powerful organic structural identification methods of mass spectroscopy and NMR must be added to those already available for matrix isolation work. Recently, secondary ion mass spectrometry has been successful in doped argon matrices,² and for the first time we now report the ^{13}C NMR spectrum of molecules trapped in an argon matrix.

The ^{13}C spectra were obtained on natural abundance sam-

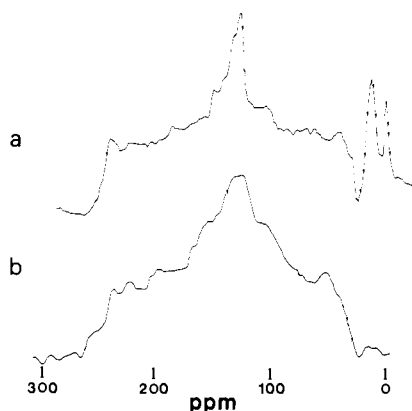


Figure 1. Low-temperature ^{13}C NMR spectra of solids: (a) ethylene-ethane-methane (70:20:10), 100 scans; (b) ethylene-argon (1:100), ~ 0.4 mmol of ethylene, 35 000 scans.

ples by the cross-polarization (CP) technique of Pines, Gibby, and Waugh³ with a 2-ms mixing time and 3-s repetition rate. A Varian XL-100 instrument equipped with a home-built single-coil double-resonance probe,⁴ decoupler channel, and pulse programmer was used in acquiring the data. With this probe, a rotating field of 12 G for ^1H spin locking and decoupling can be produced with 90 W of power. The Hartmann-Hahn⁵ condition can be matched by adjusting the output power of the ^{13}C transmitter (1 kW maximum) in steps of 0.1 dB. The matrices were deposited inside a 12-mm sample chamber on a sapphire finger attached to the end of a copper rod extending from the cold (10 K) end of an Air Products Displex 202B closed-cycle helium refrigerator.

The spectra, shown in Figure 1, exhibit a favorable signal-to-noise ratio, particularly considering the extreme anisotropy of the ethylene shielding tensor. These results demonstrate the viability of natural-abundance ^{13}C NMR spectroscopy on low-temperature matrix-isolated samples where the Boltzmann factor greatly enhances sensitivity. The diagonal elements of the shielding tensor are $\sigma_{11} 238 \pm 2$, $\sigma_{22} 126 \pm 2$, $\sigma_{33} 29 \pm 2$ ppm downfield from methane. The same values were obtained on less dilute samples in argon and on solid ethylene-ethane-methane mixtures at temperatures up to 40 K. The separations between tensorial shielding components do not compare very favorably with previous semiempirical calculations,^{6,7} but agree fairly well with ab initio results using a large basis set ($\sigma_{11} 270.8$, $\sigma_{22} 108.5$, $\sigma_{33} 13.1$ ppm).⁸ The huge shielding anisotropy is in line with published results on the substituted ethylenes, dihydromuconic acid,⁹ and hexamethyl(Dewar benzene).¹⁰ The isotropic average 131 ppm of the measured

shielding components relative to methane compares well with the difference of the room temperature isotropic shifts¹¹ of ethylene and methane.

We conclude that natural-abundance ^{13}C NMR measurements on argon-matrix-isolated species are feasible with today's technology and will provide an excellent method for characterization of extremely reactive molecules. It is encouraging that the average of the three shielding tensor components in solid ethylene relative to methane is comparable with the isotropic liquid chemical shift. Furthermore, the argon matrix did not alter the magnitude of the anisotropy. It should be noted that solid NMR work at cryogenic temperatures, whether in matrix isolation¹² or in neat solids, offers an excellent means of acquiring chemical-shift anisotropies in small molecules, for which the most accurate wave functions can be calculated. Low temperatures are required to solidify many low-molecular-weight organic materials and to optimize sensitivity.

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