analytically pure (±)-1 from 4.5 g of cyanoenone 3. Furthermore, the approach is readily amenable to modification for production of other *Lycopodium* alkaloids. For example, by using another cuprate in addition to enone 3, we have prepared cyanodione 14, which is an attractive intermediate for conversion into lycodine.[18] Further applications of the route in *Lycopodium* alkaloid synthesis are under investigation.

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Poly(I) Helix Formation. Dependence on Size-Specific Complexing to Alkali Metal Ions

Sir:

Poly(I) forms an ordered helical structure, originally thought to be three stranded[1] but more recently accepted as four stranded.[2,3] The hydrogen-bonding scheme (Figure 1) has a fourfold rotation axis in the center of the planar tetrameric array of inosines, and the polarity of the four strands is parallel. The observed stability of the helix (e.g., $T_m \simeq 25\ °C$ in 0.2 M Na^+)[4] has been surprising since only a single hydrogen bond joins each base to its neighbor, and a presumably destabilizing hole or cavity exists in the center of the tetrameric arrangement of bases (Figure 1). The results presented below suggest that an unoccupied central hole would indeed be destabilizing, but that the dimensions and chemical composition of the cavity permit stabilizing interactions which are essential for helix formation. The recent discovery[5] of specific alkali metal ion effects on formation of ordered complexes by a nucleotide, 5'-GMP, led us to examine the effect of these metals on helix formation by poly(I). In general, alkali metal ions show very little difference in their affinities for nuclei acid helices[6-9] or polyphosphates.[10] In contrast, we show here a striking de-
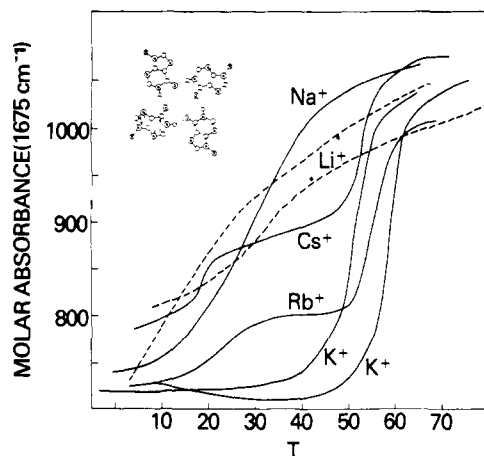

Figure 1. Inset: hydrogen bonding scheme of poly(1) helix. Infrared melting curves of helical poly(1) were measured at or slightly below the frequency maximum of the random coil polymer. Poly(1): 0.045 M Li^+ salt, unless otherwise indicated. Added salts: Li^+, upper curve, none; Li^+, lower curve 0.17 M LiCl; Na^+, 0.10 M NaCl; Cs^+, 0.10 M CsCl (measured at 1670 cm^{-1}); K^+ (left), 0.021 M KCl, 0.17 M LiCl, 0.042 M poly(1) Li salt; K^+ (right), 0.10 M KCl. The ordinate scale applies to the 0.1 M KCl curve, but numerical values are somewhat different for the others, plotted at lower frequencies. The first steps of the Rb and Cs curves are not equilibrium melting curves, but represent the conversion of a metastable to a stable helix (see text).

pendence of poly(I) helix formation on the nature of the alkali metal cation. We believe this to be the first demonstration of control of ordered polynucleotide structures by size-selective complexation with alkali metal ions.

Helix formation of poly(I) was monitored by infrared spectroscopic observation of the inosine carbonyl stretching vibration[11] at 1676 cm^{-1}, using methods described and discussed previously.[4,12,13]

Poly(I) (P-L Biochemicals, lot no. 200-15, K^+ salt) was converted to the Li salt by extensive dialysis against LiCl. Alkali metal chlorides were the ultrapure grade of Alfa Inorganics.

The infrared spectrum of poly(I) Li^+ salt without added counterion shows no helix formation, and in 0.17 M LiCl, only ~50% helix at 0 °C (Figure 2).

In 0.1 M NaCl, the carbonyl band shifts to 1684.5 cm^{-1} at 4 °C (ϵ_{max} 1045, $\Delta\nu_{1/2}$ 26 cm^{-1}). Helix formation is slow, but is largely complete after 6 h at 4 °C. The melting curve (Figure 1) is cooperative though broad ($\sigma \simeq 28\ °C$) with $T_m = 28$ °C.

The increase in helix stability on going to the next alkali metal, K^+, is dramatic. The K^+ salt of the polymer in the absence of added counterion is largely helical at 4 °C and still extensively structured at 40 °C (Figure 2). In 0.1 M KCl the thermal transition is highly cooperative ($\sigma = 8\ °C$) with $T_m = 58$ °C (Figure 1). This T_m is 30 °C above that in 0.1 M NaCl, and at least 50 °C above that in 0.1 M LiCl. These results may be compared with differences of less than ~5 °C in T_m of double helical polynucleotides in 0.1 M solutions of the different alkali metal ions.[7,9]

Experiments with low concentrations of K^+ (0.02–0.0026 M) and a higher constant concentration of Li^+ (0.21 M) showed that K^+ controlled the formation of a stable helix with a cooperative transition (Figure 1). For 0.01, 0.005, and 0.0026 M KCl, $T_m = 46$, 43, and 36 °C, respectively, in 0.2 M Li^+. From these results we conclude that Li^+ and K^+ (at high ratios of Li^+ to K^+) have their effects at different binding sites, the former satisfying the electrostatic screening requirements of helix formation and the latter complexing at specific, size-selective binding sites (see below). With all sites occupied, the helix would have an axial channel filled with K^+ ions separated
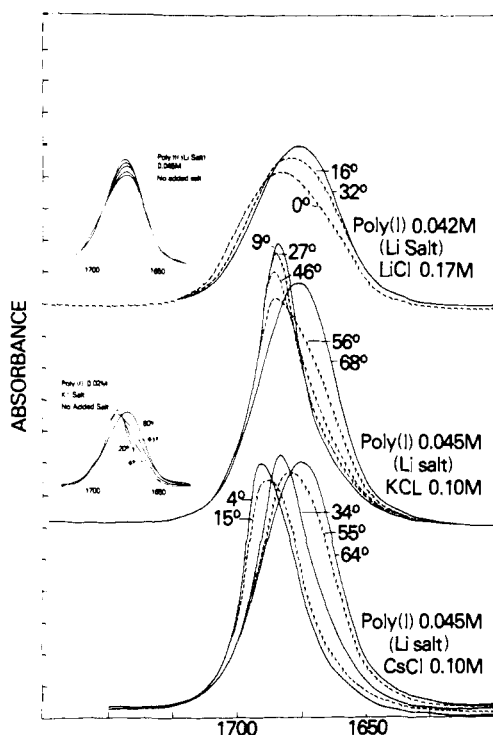
Figure 2. Infrared spectra in the carbonyl region in D_2O solution of poly(I) Li salt as a function of temperature in the presence of different alkali metal salts. In 0.17 M LiCl (top), an ordered structure is formed to a small extent at 0 °C, but without added salt (inset: lowest absorbance measured 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 $\sim$3.4 Å.[14] In 0.0026 M K$^+$, the site occupancy is $\leq$0.5 and the transition breadth is $\sim$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 $\sim$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 $\sim$2.8 Å from each of eight carbonyl oxygens. Observed metal–oxygen ligand distances indicate that the first of these sites may be considered for Na$^+$ 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 distances[18] are probably too large for the second site, but uncommonly short distances have been reported[20] 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 13C 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 13C NMR spectrum of molecules trapped in an argon matrix.

The 13C spectra were obtained on natural abundance sam-