Infrared spectra and tautomeric structure of polyinosinic and polycytidylic acids in D_2O solution

It has been shown that poly A and poly U interact[1-4] with formation of a two-stranded helix[2,3] of structure similar to DNA and that poly I and poly C form a helix[5] of which the detailed structure is unknown. Earlier infrared studies[6,7] have demonstrated the tautomeric forms existing in poly A + poly U; this note presents evidence that poly I definitely exists in the keto form and poly C probably in the amino form both before and after interaction. The observation[5] that the poly I + poly C helix has an X-ray diffraction pattern resembling that of RNA suggests that a study of this system may provide information which is pertinent to the structure of RNA.

Since inosine, IMP, and poly I all have their bands at the same frequency as the N-alkylinosine model and have no absorption near the frequency of the O-alkylinosine model compound (Table I), it follows that the tautomeric form is keto rather than enol. (For a discussion of this method of determining tautomeric form see

TABLE I

INFRARED SPECTRA* IN D_2O SOLUTION

Material	*Frequency (cm^{-1})*		
	Neutral solution	*Acid solution*	*Basic solution*
Inosine	1675 (S)		1597 (S); 1563 (M)
IMP	1674 (S)		1597 (S); 1563 (M)
1-Benzylinosine**, ***	1675 (S)		
6-Methoxy-9-β-D-ribofuranosylpurine**	1610 (S); 1590 (M)		
Cytidine	1650 (S); 1618 (M)	1711 (S); 1657 (S)	
1-β-D-Glucopyranosyl-4-dimethylamino-2-pyrimidone[9]	1650 (S); 1625 (M)	1712 (S); 1656 (S)	
Poly I§	1678 (S)		
Poly C§	1655 (S); 1640 (W; shoulder) 1620 (M)		
Poly I + Poly C§	1697 (S); 1651 (S); 1640 (W; shoulder)	1625 (VW)	

* The spectra were measured on a Beckman IR-7 spectrophotometer in 25 μ matched CaF_2 cells, solvent compensated. A scale expander on the % T scale was used in these experiments.

** The author is very much indebted to Dr. E. SHAW for a sample of 1-benzylinosine (*J. Am. Chem. Soc.*, 80 (1958) 3899), and to Dr. H. SCHAEFFER for the gift of some 6-methoxy-9-β-D-ribofuranosylpurine (*J. Am. Chem. Soc.*, 80 (1958) 4896).

*** This compound was measured as a mull because of its insolubility. The difference that this might make in the frequency (*e.g.*, $\Delta\nu = 5$ to 10 cm^{-1}) is small compared to the frequency difference between the keto and the enol models ($\Delta\nu = 70$ cm^{-1} when both are measured as mulls).

§ The poly C was prepared with polynucleotide phosphorylase from *E. coli* generously supplied by Dr. M. SINGER and Dr. L. HEPPEL. The poly I was the generous gift of Dr. D. DAVIES and was prepared with the *Azotobacter vinelandii* enzyme. The solutions were 0.05 *M* in NaCl and 0.01 *M* in sodium cacodylate, the pH (microelectrode) 7.3 for poly I, 7.1 for poly C and 7.4 for poly I + poly C, concentration approximately 0.01 *M* in repeating units for each polynucleotide. The same result was obtained for the mixture in 0.1 *M* NaCl and 0.05 *M* buffer, pH 7.0, concentration of each polynucleotide about 0.006 *M* in repeating units. The ultraviolet spectrum found for the mixture of poly I and poly C was the same as that reported by DAVIES AND RICH[5].

Abbreviations: Poly A, polyadenylic acid sodium salt; poly U, polyuridylic acid sodium salt; DNA, deoxyribonucleic acid; poly I, polyinosinic acid sodium salt; poly C, polycytidylic acid sodium salt; RNA, ribonucleic acid; IMP, inosinic acid sodium salt.

ref. [8] and [9]). It has been stated previously[8,9] that cytidine probably has the amino form, and the close similarity of the poly C bands indicates that it also is probably in the amino form. The same data show that the interaction of the polymers causes no change of tautomeric form in the inosine units and probably none in the cytidine units*.

It has been proposed that the observed increase in frequency of the poly I peak (a large decrease would be required if there had been a change to the enol form) and decrease in intensity of the poly C peak upon interaction of the polymers may be due primarily to a decrease in the dielectric constant of the immediate environment of the vibrating groups[10]. If this proposal is correct, then it would follow that all or most of the hypoxanthine rings are in approximately the same dielectric environment.

It is also apparent that no hydrogen-bonding scheme which involves placing a charge on the hypoxanthine or cytosine rings is possible, in view of the absence of any strong bands near 1600 cm^{-1} or 1711 cm^{-1} (Table I; it is possible, though unlikely, that the 1711 cm^{-1} band might be shifted to lower frequency and obscured by the inosine band).

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Received June 5th, 1959

* In the case of poly A + poly U, the facts that the structure of the helix is known[2] and that the keto form of uridine is definitely established[6,8,9] makes it possible to say that the adenine units are definitely in the amino form (even in the absence of the desirable imino model compound) since only this tautomer could form a hydrogen-bonding scheme in a helix of this structure. In the case of poly I + poly C, however, such a definite statement cannot be made about poly C since the detailed structure of the helical interaction product is not known.

Transfer of RNA-bound amino acids to microsomal proteins

Evidence for incorporation of amino acids into soluble RNA has been reported[1-6]. Recently, HOAGLAND *et al.*[6] demonstrated that isolated RNA-bound amino acids are transferred to peptide-bound material in microsomes. The present communication describes some studies on the rat-liver soluble fraction which catalyzes incorporation

Abbreviations: RNA, ribonucleic acid; ATP, adenosine 5'-triphosphate; GTP, guanosine 5'-triphosphate.