treated with deoxyribonuclease, or treated with alkali, its 1680 cm^{-1} band is much weakened. They also found that the low-polymer sodium deoxyribonucleate does not show the strong 1680 cm^{-1} band.

All these facts indicate, according to the interpretation based on the criterion suggested above, that the base paring takes place only in the high-polymer ribonucleic and deoxyribonucleic acids but not in the low-polymer nor in the denatured forms.

We have also observed 6-μ-region spectra of various nucleic acids and nucleoproteins in D_2O solution. The results of the observations and the interpretations on the basis of the criterion suggested here will be detailed elsewhere.

This work was supported by a grant from the Scientific Research Fund of Ministry of Education of Japan.

T. SHIMANOUCHI
M. TSUBOI
Y. KYOGOKU

Department of Chemistry, Faculty of Science, Tokyo University, Hongo, Tokyo (Japan)

I. WATANABE

Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto (Japan)

[1] T. SHIMANOUCHI, M. TSUBOI, Y. KYOGOKU AND I. WATANABE, *Nature*, submitted.
[2] Y. KYOGOKU, M. TSUBOI AND T. SHIMANOUCHI, *Abstr. 13th Annual Meeting Chem. Soc. of Japan, Tokyo, April, 1960*, p. 239.
[3] E. R. BLOUT AND H. LENORMANT, *Biochim. Biophys. Acta*, 17 (1955) 325.

Received October 13th, 1960.

Biochim. Biophys. Acta, 45 (1960) 195–196

Infrared spectra of the three-stranded helices formed by polyuridylic acid with polyadenylic acid and with tetraadenylic acid

Infrared spectra in D_2O solutions have been used to determine the tautomeric forms of nucleotides and polynucleotides and to study the interactions of polynucleotides in aqueous solution[1]. Poly A and poly U* can interact to form either a two-stranded or a three-stranded helix depending upon conditions[2]. The infrared spectra of the two-stranded helix[1] showed a decrease in intensity of the 1632 cm^{-1} band and frequency increase of this band and of the 1660 cm^{-1} band. This note reports the spectra of the three-stranded helices formed between poly A and poly U and between tetra A and poly U. For the necessary related spectra, the assignment of bands, and the experimental methods used, previous papers[1] should be consulted.

The most striking result with the three-stranded helix concerns the 1630 cm^{-1} band. As stated above, this band is decreased in intensity in the two-stranded helix[1]. It has disappeared as a separate peak in the case of poly (A + 2 U) and of tetra A + 2 poly U (Fig. 1).

* The polyadenylic acid (poly A) and polyuridylic acid (poly U) were prepared with polynucleotide phosphorylase (M. GRUNBERG-MANAGO AND S. OCHOA, *J. Am. Chem. Soc.*, 77 (1955), 3165). The tetraadenylic acid (tetra A = pApApApA)[3] + 2 poly U solution was the generous gift of Dr. MARIE LIPSETT AND Dr. LEON HEPPEL[3].

It has been shown that polynucleotide helices[3,4] dissociate reversibly upon heating, the melting temperature depending upon the polymers used and upon the kind and concentration of salt. By using heated solutions it has been possible to follow the thermal dissociation of these three-stranded helices (Fig. 1; Table I) by their infrared spectra. The hot solution of tetra A + 2 poly U shows a fully developed

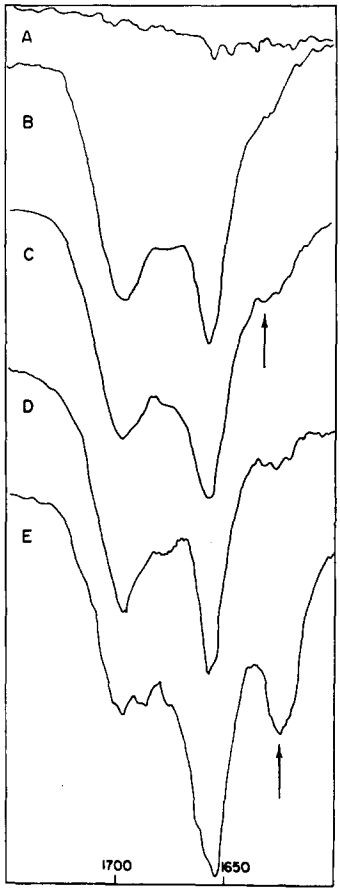

Fig. 1. Infrared absorption spectra in D_2O of polynucleotide solutions. Abscissa, frequency of absorption in cm^{-1}. Ordinate, % transmission on arbitrary scale. Conditions indicated in footnote to Table I. A, D_2O *vs.* D_2O to show compensation obtained. B, Poly A + 2 poly U at 30°. C, Poly A + 2 poly U at about 60°; the arrow at 1632 cm^{-1} indicates the slight increase in absorption. D, Tetra A + 2 poly U at 30°. E, Tetra A + 2 poly U at about 60°; the arrow indicates the large increase in absorption at 1625 cm^{-1}.

peak at 1625 cm^{-1} (Fig. 1E), indicating complete dissociation of the helix. As the solution slowly cools there is a gradual decrease in intensity of this peak until the original spectrum (Fig. 1D) is again observed at room temperature. In the case of poly (A + 2U) this spectral effect was much less pronounced (Fig. 1C) since the attainable temperature was not high enough to cause extensive dissociation. It has been proposed that a major factor contributing to the changes in the infrared spectra of polynucleotides upon helix formation is the decrease in dielectric constant as the water is forced away from the planar aromatic rings by the formation of the very tightly packed helix[5]. When a second strand of poly U is added, the solvent would be further excluded from the vicinity of the adenine rings. It is possible that this further decrease in dielectric constant around the adenine rings may contribute to the further decrease in intensity of the adenine absorption, though the effect of induced

dipoles or other more specific interactions may also be important. For important recent work on the effect of solvent on the frequency and intensity of carbonyl absorption, the reader is referred to refs. 6–8 and the refs. cited in these articles.

TABLE I

INFRARED SPECTRA IN D_2O SOLUTION

Material	*Temp.*	*vmax (cm^{-1})*
Poly (A + 2U)	30°	1659; 1699
Poly (A + 2U)	~ 60°	1632; 1657; 1698
Tetra A + 2 poly U	30°	1654; 1697
Tetra A + 2 poly U	~ 60°	1625; 1659; 1697

The spectra were measured with a Beckman IR-7 spectrophotometer at high scale expansion, using matched CaF_2 cells of 0.025-mm path length. The instrument was purged with dry air. The high-polymer solutions were approx. 0.01 *M* in repeating units of AU_2 and 0.1 *M* in NaCl, 0.01 *M* in sodium cacodylate, 0.01 *M* in $MgSO_4$, pH 7.0. The tetra A + poly U solution was approx. 0.005 *M* in repeating units of AU_2, 0.004 *M* in $MgCl_2$, 0.01 *M* in sodium cacodylate, pH 7.2. No NaCl was added.

The high-temperature measurements were made by warming the filled cells to about 60°, measuring the spectra immediately, and then scanning repeatedly as the cells cooled to room temperature.

The above result with the oligonucleotide constitutes additional evidence in support of the three-stranded structure for tetra A + 2 poly U[3].

National Institutes of Health, Bethesda, Md. (U.S.A.) H. TODD MILES

[1] H. TODD MILES, *Biochim. Biophys. Acta*, 22 (1956) 247; 27 (1958) 46; 30 (1958) 324; 35 (1959) 274, and references there cited.
[2] G. FELSENFELD, D. R. DAVIES AND A. RICH, *J. Am. Chem. Soc.*, 79 (1957) 2023.
[3] M. N. LIPSETT, L. A. HEPPEL AND D. F. BRADLEY, *Biochim. Biophys. Acta*, 41 (1960) 175.
[4] P. DOTY, H. BOEDTKER, J. R. FRESCO, R. HASELKORN AND M. LITT, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 482.
[5] H. TODD MILES, *Nature*, 181 (1959) 1814.
[6] L. J. BELLAMY AND R. L. WILLIAMS, *Trans. Faraday Soc.*, 55 (1959) 14.
[7] G. L. CALDOW AND H. W. THOMPSON, *Proc. Royal Soc., A*, 254 (1960) 1.
[8] G. L. CALDOW, D. CUNLIFFE-JONES AND H. W. THOMPSON, *Proc. Royal Soc., A*, 254 (1960) 17.

Received September 26th, 1960

Biochim. Biophys. Acta, 45 (1960) 196–198