Oligonucleotide Studies. Part V. A New Nearest Neighbor Interaction Parameter Obtained from Optical Rotatory Dispersion Data for the Ribonucleotide Sequence $\widehat{\text{ApAp}}$ in Ap$\widehat{\text{ApAp}}$Cp*

Yasuo Inoue, Masahiro Masuda and (in part) Shohei Aoyagi

Department of Chemistry, Tohoku University, Sendai, Japan

Received April 15, 1968

In previous studies of optical rotatory dispersion (ORD) spectra of oligoadenylates and polyadenylic acid (poly A), it was noticed that the shape of the ORD curve of poly A can not be calculated exactly from the ORD data for dinucleoside phosphate ApA and mononucleotide pA (Cantor and Tinoco, Jr., 1965; Holcomb and Tinoco, Jr., 1965; Cantor et al., 1966). Poly A exhibits its long wavelength trough in the ORD curve at 256 mμ at pH 7 whereas the corresponding trough of ApA appears at 260 mμ at the same pH value (Cantor and Tinoco, Jr., 1965). This indicates that the fractional degree of interactions between nearest neighbor bases are not the same in the dinucleoside phosphate ApA and in longer oligonucleotides, e. g., ApApXp, XpApApYp, etc., and polynucleotides in which the two bases ApA are nearest neighbors, although the optical properties can be assumed to be accounted for by nearest neighbor interactions between the bases. Furthermore, the conformation of ApA is not completely the same as that of the ApA sequence in longer oligomers and polymers.

In this communication we have studied optical rotatory dispersion of ApApCp and Ap$\widehat{\text{ApAp}}$Cp to isolate the optical contribution from the sequence $\widehat{\text{ApAp}}$ in Ap$\widehat{\text{ApAp}}$Cp with a view to account for the ORD shape of poly A in terms of nearest neighbor calculations using the ORD data for $\widehat{\text{ApAp}}$.

Materials used in this study were obtained from digestion of high molecular weight RNA with ribonuclease T_1 followed by pancreatic RNase digestion. Since

*Part IV in this series is by Yasuo Inoue and Shohei Aoyagi, Biochem. Biophys. Res. Comm., 28, 973 (1967).

details of the preparation of the tetranucleotides (ApApApCp, ApApApGp, and ApApApUp) and their spectrophotometric constants are not given in this paper, we report analytical data for the base composition as a purity criterion and molar extinction coefficient at 260 mμ at pH 7 for ApApApCp are shown below:

Base composition ratio A/3 : C		ϵ_{260} at pH 7
1.00	1.06	3.99×10^4

The ORD spectra were measured on a Jasco recording spectropolarimeter model ORD/UV-5 with sample solutions of which absorbancies at 260 mμ are appoximately 1.0 to 1.1 and the ionic strength 0.1. Although the shapes of the ORD curves of ApCp, ApApCp, and ApApApCp are quite similar, a large change was observed in the amplitude of the first Cotton effect in going from ApCp to ApApCp and ApApApCp, and also a significant drift of the long wavelength trough toward shorter wavelength was noticed (A detailed ORD study has been reported for ApApCp by Vournakis et al., 1966). Similar effects are also observed in as yet unpublished results for two sets of oligonucleotides, $(Ap)_iXp$ where $i = 1$, 2, and 3, and X = G and U.

Tinoco and his co-workers have predicted the ORD curve of poly A at pH 7 by nearest neighbor calculations using the following simple equation (Cantor et al., 1966)

$$[\phi]_{\text{poly A}} = 2[\phi]_{\text{ApA}} - [\phi]_{\text{pA}} \qquad \text{Eq. 1}$$

The results are reproduced in Fig. 1. Qualitatively the agreement between the experimental and calculated ORD curves of poly A is quite good. But the trough of the first Cotton effect is shifted to short wavelength from the value predicted from Eq. 1 with ApA. At the same time, the amplitude of poly A is larger than that predicted by nearest neighbor calculations with the dinucleoside phosphate. To minimize these discrepancies, the rotation of the sequence $\widehat{\text{ApAp}}$ in $\underline{\text{ApAp}}\widehat{\text{ApCp}}$ was calculated from those of $\underline{\text{ApAp}}$Cp, $\underline{\text{ApAp}}\widehat{\text{ApCp}}$ and pA using Eq. 2.*

* Similarly, the rotational contribution of the sequence $\underline{\text{ApAp}}$ can be estimated from an equation based on data obtained from ApApAp and pA:

$$[\phi]_{\underline{\text{ApAp}}} = \frac{2[\phi]_{\text{ApApAp}} + [\phi]_{\text{pA}}}{4}$$

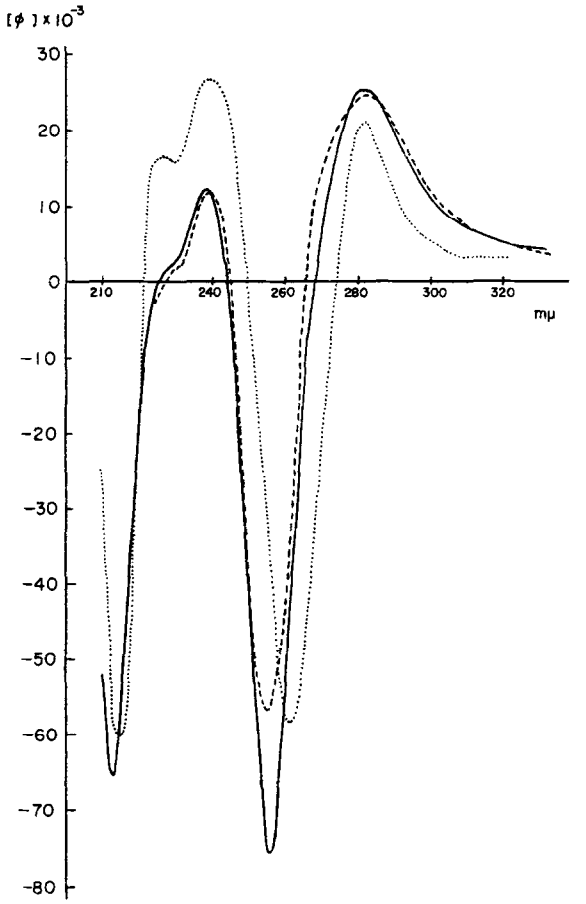

Fig. 1. The optical rotatory dispersion of poly A at pH 7.
——— experimental (Holcomb and Tinoco, Jr., 1965)
..... calculated from Eq. 1 using ApA (Cantor et al., 1966)
------- calculated from Eq. 1 using $\widehat{\text{ApAp}}$ in $\underline{\text{Ap}\widehat{\text{ApA}}}$pCp

$$[\phi]_{\widehat{\text{ApAp}}} = \frac{4[\phi]_{\underline{\text{Ap}\widehat{\text{ApA}}}\text{pCp}} - 3[\phi]_{\underline{\text{ApA}}\text{pCp}} + [\phi]_{\text{pA}}}{2} \qquad \text{Eq. 2}$$

The results are shown in Fig. 2 together with the rotation of the dinucleoside phosphate ApA for comparison. Although the amplitude of the first Cotton effect is not so significantly different, the trough of the first Cotton effect of $\widehat{\text{ApAp}}$ appears at shorter wavelengths than that of the dimer, indicating that, of the above two discrepancies (i. e., in position of the first trough and amplitude of the first Cotton effect), the former one, at least, should be removed when the rotation of poly A

is calculated from $\widehat{ApAp}$ using the equation 1. In fact the agreement between the experimental and calculated ORD of poly A is excellent as can be seen from Fig. 1. The calculated ORD curves using Eq. 1 with $\widehat{ApAp}$ from $\underline{ApAp\widehat{ApCp}}$, $\underline{ApAp\widehat{ApGp}}$, and $\underline{ApAp\widehat{ApUp}}$ are almost identical, indicating that the rotational contribution of the sequence $\widehat{ApAp}$ in $\underline{ApAp\widehat{ApXp}}$ is greatly influenced by the presence of X but not so much by the nature of X.

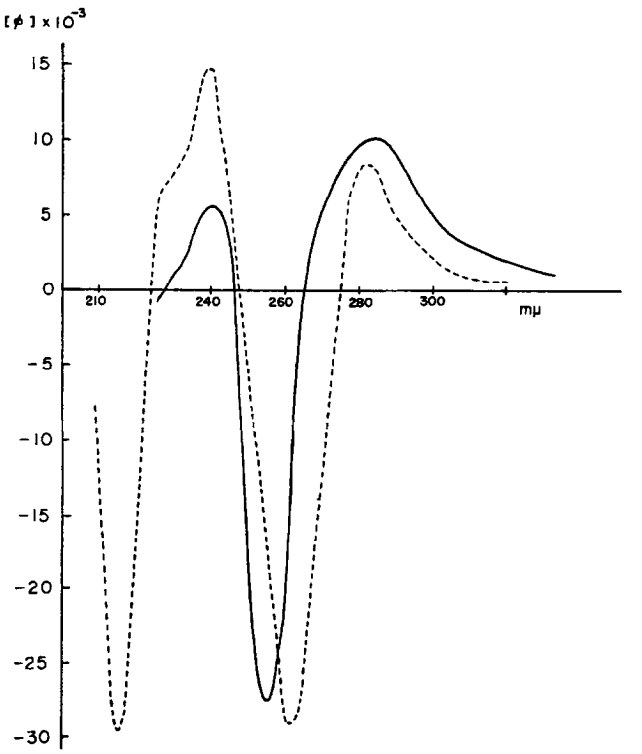

Fig. 2. The optical rotatory dispersion of ApA and $\widehat{ApAp}$ at neutral pH.
--------- ApA (Warshaw et al., 1965)
———— $\widehat{ApAp}$ (calculated from Eq. 2 based on data obtained from ApApApCp, ApApCp, and pA.

According to Tinoco and his co-workers the disagreement between experimental and calculated ORD curves for homopolynucleotides arises from the presence of electrostatic and geometric constraints in the polymers which are not present in the dinucleoside phosphates. The present finding indicates that these constraints begin to show their presence in tetramers by forcing these molecules to adopt a conformation with a single helical sense. Thus, the discrepancy in the position of

the first positive Cotton effect completely disappears when nearest neighbor calculations are made, based on $\overset{\frown}{\text{ApAp}}$; the position of the trough is governed by the short range conformation. However, errors in calculating the magnitude of the high wavelength Cotton effect in the ORD of the polymer, though reduced to some extent, are still present when the calculations are based on $\overset{\frown}{\text{ApAp}}$, showing that such homopolymers have long range conformations for which allowance is not being made in these calculations of a simple additive form based on data obtained from oligonucleotides. Therefore, the prediction of the ORD of higher oligo- or polynucleotide having very regular structure (e. g., poly A, poly C, etc.) from the data for dinucleoside phosphates should be regarded as being approximate.

References

Cantor, C. R., Jaskunas, S. R., and Tinoco, I., Jr., J. Mol. Biol., 20, 39 (1966).
Cantor, C. R., and Tinoco, I., Jr., J. Mol. Biol., 13, 65 (1965).
Holcomb, D. N., and Tinoco, I., Jr., Biopolymers, 3, 121 (1965).
Vournakis, J. N., Scheraga, H. A., Rushizky, G. W., and Sober, H. A., Biopolymers, 4, 33 (1966).
Warshaw, M. M., Bush, C. A., and Tinoco, I., Jr., Biochem. Biophys. Res. Comm., 18, 633 (1965).