SOME OPTICAL PROPERTIES OF DIADENOSINE-5′-PHOSPHATES

By J. F. Scott and P. C. Zamecnik

JOHN COLLINS WARREN LABORATORIES, MASSACHUSETTS GENERAL HOSPITAL, BOSTON

Communicated October 3, 1969

Abstract.—The absorption spectra, optical rotatory dispersion, and circular dichroism of a series of diadenosine-5′-phosphates, differing in the length of the phosphate bridge, indicate that in this set of compounds the adenine residues form an intramolecular stacked conformation. The differences in the optical properties suggest that this arrangement is different from that deduced by others for diadenosine-3′,5′-monophosphate (ApA(3′,5′)) but is of comparable stability.

The optical properties of oligomers and polymers of adenylic acid have been carefully studied by a number of investigators[1–5] and the geometry of these 3′, 5′-linked chains has been determined for oriented fibers by X-ray diffraction[6] and deduced from the circular dichroism of solutions of these compounds[2] using the theoretical treatment used by Tinoco and his co-workers.[7] In the course of an investigation of the association of the compounds involved in the reaction leading to the aminoacylation of tRNA, it was observed that a dialyzable product was formed which was subsequently identified as P^1,P^4-di(adenosine-5′)tetraphosphate[8, 9] (Ap_4A). At that time it was noted that the optical rotatory dispersion of this compound was qualitatively similar to ApA(3′,5′) but of opposite sign.[10] We have since studied a series of di(adenosine-5′)phosphates which differ in the length of the phosphate linkage, and we wish to report here the results of this investigation which suggest that the adenine residues may "stack" in more than one geometrical relation which is different from that of ApA(3′,5′) or of single-stranded poly A at neutral pH.

Materials and Methods.—The following compounds were the generous gift of Dr. John W. Moffatt: P^1,P^2-di(adenosine-5′)diphosphate (Ap_2A), P^1,P^3-di(adenosine-5′)-triphosphate (Ap_3A), and Ap_4A. ApA(3′,5′) was obtained from Miles Laboratory.

The optical rotatory dispersion curves were obtained with a Cary model 60 spectropolarimeter fitted with thermostated cuvettes. The circular dichroism curves were prepared with the same instrument using a Cary model 6001 circular dichroism attachment. Absorption spectra were done with a Cary 11 spectrophotometer with jacketed cuvettes. The path length of the cuvettes was 0.1, 1.0, 5.0, or 10 mm, as required.

Solvent base line curves were recorded before and after each sample spectrum in the case of optical rotatory dispersion and circular dichroism curves, and the data were reduced by point-by-point subtraction. These data are expressed as mean residue rotation (m) and $\Delta\epsilon([\epsilon_L - \epsilon_R])$, respectively. The concentration of adenosine residues was calculated from the $A_{260\ m\mu}$ in 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0 as a standard solvent, coupled with determinations of phosphate.[11] The circular dichroism unit was calibrated with d-10-camphorsulfonic acid. The absorbance of the sample solutions did not exceed 1.6, and the residue ellipticity was not a function of absorbance in the range used.

The choice of solvents and temperatures were experimental variables and are specified in the captions and text.

Results.—The absorption, circular dichroism, and optical rotatory dispersion curves of ApA(3′,5′), Ap_2A, Ap_3A, and Ap_4A are presented in Figure 1. The data

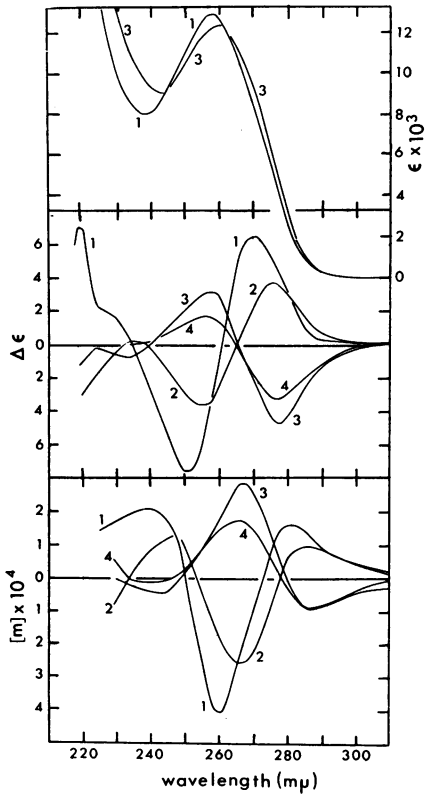

FIG. 1.—From top panel to bottom: absorption, circular dichroism, and optical rotatory dispersion spectra. In all panels, the numbering is as follows: Curve 1, ApA(3′,5′); Curve 2, Ap_2A; Curve 3, Ap_3A; Curve 4, Ap_4A. The absorption spectra for the 5′,5′ set of compounds are very similar and only one such is presented. Solvent: 2×10^{-3} *M* EDTA, pH 8.5.

for ApA(3′,5′) are included as a reference of convenience, and they are in agreement with the results of others[2, 5] for this compound. The circular dichroism bands of the 5′,5′ compounds exhibit the splitting into oppositely signed components, suggesting that in all cases the chromophores are coupled as described by Tinoco *et al.*[12] The optical rotatory dispersion data are consistent with this interpretation. It is clear from both sets of data, however, that there are qualitative differences between the 5′,5′- and 3′,5′-linked dinucleosides. The set of 5′,5′-linked compounds shows approximately the same crossover point in the circular dichroism spectra (as well as wavelength of maximum rotation), which is located at a longer wavelength (see Table 1) than that for ApA(3′,5′). Among the 5′,5′ compounds, the circular dichroism of Ap_2A has the same sign as ApA(3′,5′) while Ap_3A and Ap_4A show bands of opposite sign. The wave number differences between the positive and negative maxima are similar in all cases (Table 1).[12] Differences in the absorption spectra are less striking and are described below.

As expected from the evidence for interaction between the chromophores, it was found that the optical rotatory power was sensitive to temperature and to ionic environment (Table 1). Somewhat surprisingly, however, while the addition of first Na^+ and then Mg^{++} increases the amplitude of the Cotton effect in ApA(3′,5′), the opposite effect is seen in Ap_3A and Ap_4A, while little effect is

TABLE 1. *Amplitude of residue rotation.**

T solvent†	ApA(3′,5′) 1	2	3	Ap₂A 1	2	3
5°	40.3	42.0	49.7	21.3	21.5	21.2
20°	28.6	31.8	35.6	17.9	17.8	17.7
40°	19.4	20.0	20.0	12.5	13.3	13.8
60°	11.8	12.7	12.4	9.1	9.0	9.4
85°	6.8	...	...	5.5	...	...
λ_{max}‡	260.0	260.0	260.0	267.0	267.0	267.0
$\Delta\nu$§	2790	2800	2920	2910	2970	2900
ϵ(P)¶		12.7 ± 0.07 × 10^3			11.8 ± 0.10 × 10^3	

T solvent†	Ap₃A 1	2	3	Ap₄A 1	2	3
5°	22.4	19.1	14.3	16.6	13.8	12.1
20°	19.8	15.7	11.7	13.0	11.8	8.8
40°	14.8	11.2	7.1	9.1	8.2	7.4
60°	10.3	8.0	4.4	7.5	6.2	7.7
85°	6.8	...	...	5.7	...	...
λ_{max}‡	267.5	267.0	266.0	267.0	267.0	270.0
$\Delta\nu$§	3160	3070	3070	2960	2950	2380
ϵ(P)¶		12.0 ± 0.07 × 10^3			12.5 ± 0.07 × 10^3	

* The amplitude measured on the long wavelength limb of the Cotton effect and expressed in degrees × 10^{-3}.

† The compounds were dissolved in Solvent 1 (0.002 *M* EDTA, pH 8.5). After recording the optical rotatory dispersion curves enough solid NaCl was added to make the solution 0.15 *M* in NaCl. Next, 1 *M* Tris:HCl, pH 7.6, was added to a final molarity of 0.05 *M* and the curves repeated (Solvent 2). Then 1 *M* $MgCl_2$ was added to a final concentration of 0.005 *M* (Solvent 3) and the curves again repeated.

‡ λ_{max} is the wavelength (mμ) of maximum rotatory power (regardless of sign) at 20° in each of the solvents.

§ The difference in the frequencies (wavenumber) of the + and − extrema of the circular dichroism curves at room temperature.

¶ The residue absorptivity ± standard error of the mean of four measurements determined as described in the text. By this method the value for AMP is 14.6 ± 0.07 × 10^3.

noted in the case of Ap_2A. Elevation of the temperature in all cases leads to a noncooperative type of decrease in amplitude.

The absorption data reveal small but significant changes in the spectra of the 5′,5′ compounds considered as a group when compared with ApA(3′,5′). The absorption spectra for ApA(3′,5′) and, as a representative of the 5′,5′ group of compounds, for Ap_3A are plotted in Figure 1. The principal feature of these spectra is the shift of the absorption maxima of the 5′,5′ group of compounds to the vicinity of the maximum for adenosine monophosphate (259 mμ). As seen in Table 1, approximately the same degree of hypochromicity relative to AMP is noted for all the compounds studied.

Discussion.—The following discussion will be facilitated if the conclusions concerning the interaction of the adenine residues in the 5′,5′ compounds are stated first with the supporting arguments following. Our conclusions are: (*1*) The interaction of the adenine residues involves intramolecular stacking: intermolecular interaction does not influence the optical properties to a measurable degree; (*2*) interaction of the adenine chromophores leads to exciton splitting of the circular dichroism band, and the transition contributing in the greatest degree to the circular dichroism and optical rotatory dispersion differs from that in the case of ApA(3′,5′); and *3*) two different arrangements of the adenine residues

with respect to one another account for the shift in the crossover point in the circular dichroism band seen in all of the 5',5' compounds presented here, and for the different signs observed for Ap_2A on the one hand, and Ap_3A and Ap_4A on the other.

These conclusions rest on the following observations and arguments. The optical properties of these compounds are not a function of concentration over a 100-fold range, and estimation of the molecular weight of Ap_4A by equilibrium ultracentrifugation yields a value of 980 which is consistent with no aggregate formation. For the latter measurement, we are indebted to Dr. Michael Young. Hence, the optical properties arise from intramolecular interactions as in the case of ApA(3',5').

Tinoco's[13] treatment of the optical properties of helices in terms of the exciton model leads to the result that the circular dichroism should consist of two bands of equal strength and opposite sign. These bands should, in a dimer such as ApA-(3',5'), be centered about the frequency of the absorption transition which gives rise to the dichroism. When these bands overlap, as they do in the case of ApA-(3',5'), then $\Delta\epsilon$ becomes 0 at the frequency of the absorption transition. The number, frequency, and orientation of transition moments in adenine is still an unsettled matter (cf. Miles *et al.*[14] for a summary discussion). If, for the purpose of this argument, we follow DeVoe and Tinoco[15] and accept the existence and orientation of two in-plane transitions accounting for absorption bands centered at 259 and 267 mμ in adenine, but altering the orientation of the transition moment responsible for the 259 mμ band from 147° to 135°, we can then propose certain models to account for the observed shift in the crossover point and the sign of the circular dichroism in the 5',5' compound.

In the case of the 3',5' compounds, there is considerable evidence[2, 6, 7] which supports an arrangement of the chromophores in which the moments referred to above in one residue are rotated 30° to 45° in the clockwise direction with respect to those in the adjacent residue. Hence, the positive sign of the Cotton effect in such compounds is related to a clockwise displacement of the successive moments. The absorption and rotation due to the transition centered at 259 mμ predominates, and one finds the absorption maximum shifted to shorter wavelengths and a crossover in the circular dichroism near 259 mμ, as predicted by the exciton model.

For the purposes of description of the conformation of the 5',5' compounds, the adenine residue may be considered to have α- and β-faces. Let the α-face be that facing the $C_{2'}$ and $C_{3'}H$ when the C_8 of adenine is near the $C_{5'}$ (i.e., in the *anti* conformation). Thus, in ApA(3',5'), the faces of adenine are in contact $\alpha:\beta$. With the additional flexibility afforded by the longer 5',5' linkage in the compounds studied here, the adenine rings might approach one another with the same faces apposed, i.e., $\alpha:\alpha$ and $\beta:\beta$ (Fig. 2). We propose that in Ap_2A(5',5') the chromophores approach one another with the β-faces apposed and the long axis of the purine rings approximately parallel (Fig. 2*A*). This conformation can be achieved when the C_1'–N_9 torsion angle is a small negative angle (*anti*). In this conformation the 259-mμ transitions lie at right angles and the couple is, therefore, optically inactive,[16] while the 267-mμ transitions are related in the clockwise

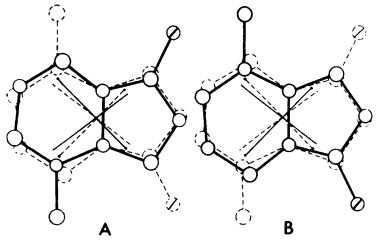

FIG. 2.—Proposed stacking arrangement for the adenine rings of the 5′,5′ set of compounds. The ring lying above the plane of the page is in solid lines; that below, in dotted lines. The lines within the rings indicate the transition moment directions referred to in the text; that responsible for the 259-mμ band is terminated by a dot ($C_{1'}$ is indicated by a barred circle). $C_{5'}$ lies to the right of the ring in all cases.

sense. Hence, the sign of the circular dichroism remains positive but the crossover shifts to the vicinity of 267 mμ. In the case of Ap_3A and Ap_4A, we propose that the chromophores approach one another as described above, but with the α-faces apposed (Fig. 2*B*). Again, the 259 mμ transition moments may lie at right angles while the 267 mμ moments are now related in the counterclockwise sense. The crossover does not change, but the sign of the circular dichroism is inverted.

If the change in shape of the absorption bands, as presented in Figure 1, is interpreted in terms of the exciton model of chromophore interaction, then the shift of the absorption maximum to 259 mμ is consistent with a right angle relation in the dinucleoside of the transition moments responsible for this band.[16] One would expect to see little evidence of exciton splitting of the longer wavelength transition (centered at 267 mμ) because it is a considerably weaker absorber.

Manipulation of space-filling models suggests that while the conformations described above are easily made they are not the only ones possible. It is also possible to stack the adenine rings when the $C_{1'}$–N_9 torsion angles are both in the *syn* quadrant and when one is *syn* and the other *anti*. It is difficult to visualize why one conformation might be preferred over an alternate. In this connection, however, it appears that the *anti* conformation is preferred in purine nucleosides (see review of Yang and Samejima[17] for a summary of the evidence). Furthermore, the conformation described for Ap_2A is the least strained of the possible stacking arrangements for that compound. In the conformations suggested for Ap_3A and Ap_4A, it is possible to form a hydrogen bond between the amino nitrogen of each adenine ring and the $C_{2'}$-OH of the opposite ribose residue.

The change in the orientation of the transition moment for the 259-mμ absorption band from 147° to 135° which we proposed above is not necessary in this argument. It is only necessary that this moment in one chromophore be related to the same moment in the neighbor by 90°. The long axes of the adenine residues may be rotated to produce this result without the proposed alteration in the orientation of the moment. However, the most ready fit of the space-filling models is achieved when the long axes of the chromophores are parallel.

Bush and Scheraga[18] have recently described optical activity in higher oligomers and polymers of AMP which they interpret as likely to be due to an n-π^* transition centered at 278 mμ. Neither we nor they find any evidence for such an optically active transition in adenine dinucleoside phosphates. Cantor *et al.*[19] have found an inversion of the circular dichroism spectra of cyclic compared with linear deoxythymidine oligonucleotides. The inversion of the circular dichroism spectra which we have reported above presents a somewhat different problem,

since the conformational mobility of the 5′,5′ compounds is greater than that of ApA(3′,5′), owing to the greater length of the phosphate bridge in the former group of compounds. Thus, whatever the actual conformation which gives rise to the optical activity we have described, it is clear that it is different from that of ApA(3′,5′) and of comparable stability.

In relating the sign of the circular dichroism of an exciton band to the molecular geometry of dinucleoside monophosphates, it must be remembered that it is the angular relation of the transition moments of the interacting chromophores which determines the sign of the optical activity arising from the exciton interaction. Zavil'gel'skii and Li[20] have noted that the sign of the circular dichroism curve for Apψ is negative, while that for ApU is positive. From this they have inferred that the helical sense of stacking of the dinucleoside monophosphates is opposite in the two cases. It appears more likely that it is the angular relation of the transition moments which has changed, due to the rotation of the uracil residue in changing the riboside linkage from N_1 to C_5. It is interesting to speculate whether a stacking of bases in unpaired portions of the tRNA molecule might occur between bases in noncontiguous regions of the molecule. While there is no direct evidence for the occurrence of such an interaction, it is pertinent to note the results of Seno *et al.*[21] in which, contrary to the behavior of all other purified species of tRNA studied by them, $tRNA^{met}$ exhibited a hyperchromicity as salt was added to a distilled water solution of that species of tRNA. Thus, provided there is no hindrance due to stacking with nearest neighbors, a base in a single-stranded area might engage in stacking with a noncontiguous base in another single-stranded region. Such an arrangement is of comparable energy to that due to hydrogen bonding of two such bases.[22]

The authors thank Dr. I. Tinoco, Jr., for his helpful comments on this manuscript. We wish to acknowledge the careful and thoughtful participation of Miss Patricia Crawford in these studies.

This work was supported by the Atomic Energy Commission (Contract AT(30-1)-2643), the American Cancer Society (grant E-102), and the National Institutes of Health (grant CA-00558). This is publication no. 1358 of the Harvard Cancer Commission.

[1] Massoulié, J., and A. M. Michelson, *C. R. Acad. Sci., Paris*, **259**, 2923 (1964).

[2] Van Holde, K. E., J. Brahms, and A. M. Michelson, *J. Mol. Biol.*, **12**, 726 (1965).

[3] Holcomb, D. N., and I. Tinoco, Jr., *Biopolymers*, **3**, 121 (1965).

[4] Michelson, A. M., T. L. V. Ulbricht, T. R. Emerson, and R. J. Swan, *Nature*, **209**, 873 (1966).

[5] Brahms, J., A. M. Michelson, and K. E. Van Holde, *J. Mol. Biol.*, **15**, 467 (1966).

[6] Rich, A., D. R. Davies, F. H. C. Crick, and J. D. Watson, *J. Mol. Biol.*, **3**, 71 (1961).

[7] Bradley, D. F., I. Tinoco, Jr., and R. W. Woody, *Biopolymers*, **1**, 239 (1963).

[8] Zamecnik, P. C., M. L. Stephenson, C. L. Janeway, and K. Randerath, *Biochem. Biophys. Res. Commun.*, **24**, 91 (1966).

[9] Randerath, K., C. L. Janeway, M. L. Stephenson, and P. C. Zamecnik, *Biochem. Biophys. Res. Commun.*, **24**, 98 (1966).

[10] Zamecnik, P. C., C. L. Janeway, K. Randerath, and M. L. Stephenson, in *Regulation of Nucleic Acid and Protein Biosynthesis*, ed. U. V. Koningsberger and L. Bosch (Amsterdam: Elsevier, 1967), p. 169.

[11] Lowry, O. H., N. R. Roberts, K. Y. Leiner, M. L. Wu, and A. L. Farr, *J. Biol. Chem.*, **207**, 1 (1954).

[12] Tinoco, I., Jr., R. W. Woody, and D. F. Bradley, *J. Chem. Phys.*, **38**, 1317 (1963).

[13] Tinoco, I., Jr., *J. Amer. Chem. Soc.*, **86**, 297 (1964).

[14] Miles, D. W., M. J. Robins, R. K. Robins and H. Eyring, these PROCEEDINGS, **62**, 22 (1969).
[15] DeVoe, H., and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 518 (1962).
[16] Tinoco, I., Jr., *Radiation Res.*, **20**, 133 (1963).
[17] Yang, J. T., and T. Samejima, in *Progress in Nucleic Acid Research and Molecular Biology*, ed. J. N. Davidson and W. E. Cohn (New York: Academic Press, 1969), vol. 9, p. 223.
[18] Bush, C. A., and H. A. Scheraga, *Biopolymers*, **7**, 395 (1969).
[19] Cantor, C. R., R. H. Fairclough, and R. A. Newmark, *Biochem.*, **8**, 3610 (1969).
[20] Zavil'gel'skii, G. B., and L. Li, *Molekulyarnaya Biologiya*, **1**, 323 (1967).
[21] Seno, T., M. Kobayashi, and S. Nishimura, *Biochem. Biophys. Acta*, **174**, 71 (1969).
[22] Pullman, B., and A. Pullman, in *Progress in Nucleic Acid Research and Molecular Biology*, ed. J. N. Davidson and W. E. Cohn (New York: Academic Press, 1969), vol. 9, p. 327.