Vol. 132, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1985 Pages 95-99

RNA-LIKE CONFORMATIONAL PROPERTIES OF A SYNTHETIC DNA POLY(dA-dU).POLY(dA-dU)

Jaroslav Kypr and Michaela Vorlíčková

Institute of Biophysics, Czechoslovak Academy of Sciences, 612 65 Brno, Czechoslovakia

Received August 19, 1985

Differences in the circular dichroism of poly(dA-dT).poly(dA-dT) and poly(dA-dU).poly(dA-dU) and in its temperature induced changes are reported. A comparison to the data obtained with DNA and RNA indicates that an absence of thymine methyl groups in the polynucleotide results in promoting its RNA-like conformational properties. However, poly(dA-dU).poly(dA-dU) is not an A-DNA type of double helix.

There are two general differences in the chemical structure of DNA and RNA. DNA contains deoxyribose and thymine while the corresponding RNA constituents are ribose and uracil. The sugar moiety significantly affects nucleic acids conformation (1), mainly due to the restriction of ribose geometry in the C3'endo puckering which confers the A-type conformation on RNA. On the other hand, deoxyribose is very flexible (2) and gives DNA much conformational variability (for a review, see 3). Reasons are not, however, known why DNA contains thymine instead of uracil. We address this question in the present communication.

MATERIAL AND METHOD

Poly(dA-dT).poly(dA-dT) was from P-L Biochemicals and poly(dA-dU).poly(dA-dU) from Collaborative Research. Calf thymus DNA and the replicative form of phage f2 RNA were isolated at our Institute and in the laboratory of Dr. J. Doskočil, respectively. The samples were dissolved in 0.02 M Na acetate, pH 6.5, 1×10^{-6} M EDTA at a concentration giving the absorption of about 1 at 260 nm.

Circular dichroism measurements were carried out in thermostated 1 cm pathlength cells using a Jobin-Yvon dichrograph Mark IV calibrated with isoandrosterone.

0006-291X/85 $1.50

RESULTS

Though circular dichroism is rather unreliable in an absolute identification of the left-handed Z-DNA conformation (4), it is respectable in distinguishing B-DNA from A-DNA or A-RNA double helices (ref. 5, Fig. 1). Tilted base pairs in the A-type double helices appear to be the most probable cause of the difference in the circular dichroism of the two basic conformations (6,7).

In Figure 1 we present the CD spectra of poly(dA-dT).poly(dA-dT) and poly(dA-dU).poly(dA-dU). They differ both in the short and long wavelength region, which is a consequence of their different conformations because the single-stranded polynucleotides have very similar CD spectra (not shown). The long wavelength CD band of poly(dA-dT).poly(dA-dT) has a remarkable appearance. Its maximum is strong and lies at 262 nm, which is not usual with DNA (5, Fig. 1). However, there remains a characteristic shoulder superimposed

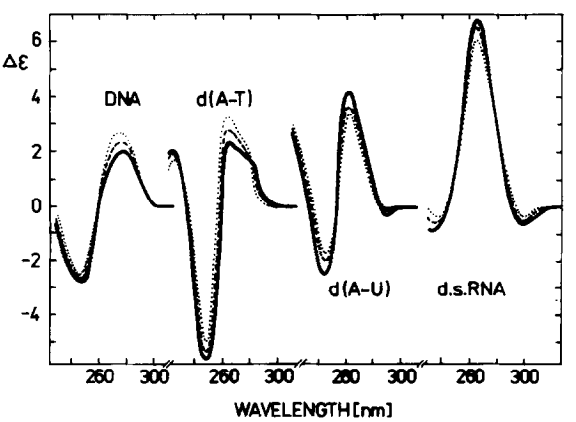

FIGURE 1: CD spectra of calf thymus DNA at ——— 0.5 °C, - - - 22.5 °C, ····· 56.5 °C; poly(dA-dT).poly(dA-dT) ——— 0 °C, - - - 10 °C, ····· 27 °C; poly(dA-dU).poly(dA-dU) ——— 1 °C, - - - 25 °C, ····· 32 °C; replicative form of f2 phage RNA ——— 2 °C, - - - 40 °C, ····· 62 °C.

on the main long wavelength band of poly(dA-dT).poly(dA-dT) in the position where DNA has the positive maximum. The shoulder is absent with poly(dA-dU).poly(dA-dU) while the maximum around 260 nm is, especially at low temperatures (Fig. 1), much more expressed. In addition, a new small band appears in the CD spectrum of poly(dA-dU).poly(dA-dU) at 290 nm while the negative band at 245 nm is much smaller than with poly(dA-dT).poly(dA-dT). By all these differences the CD spectrum of poly(dA-dU).poly(dA-dU) becomes remarkably similar to the CD spectra of A-type double helices (note the tendency in the series poly(dA-dT).poly(dA-dT), poly(dA-dU). .poly(dA-dU) and double-stranded RNA, Fig. 1).

Poly(dA-dU).poly(dA-dU) and poly(dA-dT).poly(dA-dT) also differ as far as their temperature-induced changes in CD are concerned. While the positive band of poly(dA-dT).poly(dA-dT) increases with an increasing temperature as it is usual with DNA (8) the positive band of poly(dA-dU).poly(dA-dU) surprisingly decreases (Fig. 1). A similar depression is displayed by RNA (Fig. 1).

DISCUSSION

This communication demonstrates differences between poly(dA-dT).poly(dA-dT) and poly(dA-dU).poly(dA-dU) in their circular dichroism and its changes with temperature. A comparison to RNA indicates that poly(dA-dU).poly(dA-dU) shares some conformational properties with an A-type conformation. However, the polynucleotide is not an A-DNA double helix because it undergoes the normal highly cooperative B-A conformational transition in ethanol solutions (M.V. and J.K., unpublished data). The suggestion that arrangement of base pairs in poly(dA-dU).poly(dA-dU) has something in common with

the base pair disposition in RNA is corroborated by the polynucleotide interaction with dipyrandium (9).

Poly(dA-dT).poly(dA-dT) seems to have a peculiar alternating conformation with an A-type geometry of the purine residues (10-13). We believe that it is the A-type geometry that gives rise to the unusual positive CD band of poly(dA--dT).poly(dA-dT) shifted to 260 nm, where RNA normally has the maximum ellipticity (Fig. 1). On the other hand, phosphorus NMR suggests that the double helix of poly(dA-dU).poly (dA-dU) is regular rather than alternating (14). An absence of the thymine methyl groups thus obliterates the alternating character of the structure. Rather surprisingly, the obliteration results in a strengthening of its A-type conformational properties, though a global B-type character of the double helix is preserved. This is not a contradiction but only a hint that we have not yet properly understood all conformational properties of DNA. An illustration that base pair steps with an A-DNA character can exist within the B-DNA sugar-phosphate backbone can be found in the Dickerson's dodecamer (15). It thus appears that not only ribose but also uracil promotes RNA-like conformational properties in the double helices of nucleic acids and that the thymine methyl group may significantly participate in the physical phenomena that permit DNA perform its diverse biological functions.

After finishing this work, we encountered a closely relevant paper of Greve et al. (16) who as early as 1977 conclude in a close accordance with the present results that poly(dA-dT).poly(dA-dT) increases A-type nature of the conformation prior to denaturation (Fig. 1). This conclusion was done prior to the knowledge of the crystal structure of

the tetranucleotide d(ATAT) (10) and the concept of the alternating B conformation (11), which demonstrates the potential of CD spectroscopy to reveal subtle details of nucleic acids conformation.

ACKNOWLEDGEMENT

We appreciate the secretarial assistance of Mrs. Irina Hebelková and Mrs. Marcela Přerovská with the manuscript preparation.

REFERENCES

1. Leslie, A.G.W., Arnott, S., Chandrasekaran, R., and Ratliff, R.L. (1980) J. Mol. Biol. 143, 49-72.
2. Levitt, M., and Warshel, A. (1978) J. Am. Chem. Soc. 100, 2607-2613.
3. Cold Spring Harbor Symposia on Quantitative Biology (1983) Volume XLVII.
4. Tomasz, M., Barton, J.K., Magliozzo, C.C., Tucker, D., Lafer, E.M., and Stollar, B.D. (1983) Proc. Natl. Acad. Sci. USA 80, 2874-2878.
5. Ivanov, V.I., Minchenkova, L.E., Minyat, E.E., Frank-Kamenetskii, M.D., and Schyolkina, A.K. (1974) J. Mol. Biol. 87, 817-833.
6. Yang, J.T., and Samejima, T. (1968) Biochem. Biophys. Res. Commun. 33, 739-745.
7. Johnson, B.B., Dahl, K.S., Tinoco, I., Jr., Ivanov, V.I., and Zhurkin, V.B. (1981) Biochemistry 20, 73-78.
8. Gennis, R.B., and Cantor, C.R. (1972) J. Mol. Biol. 65, 381-399.
9. Vorličková, M., Kypr, J., and Kleinwächter, V. (1985) Biochim. Biophys. Acta 838, 236-243.
10. Viswamitra, M.A., Kennard, O., Jones, P.G., Sheldrick, G.M., Salisbury, S., Falvello, L., and Shakked, Z. (1978) Nature 273, 687-688.
11. Klug, A., Jack, A., Viswamitra, M.A., Kennard, O., Shakked, Z., and Steitz, T.A. (1979) J. Mol. Biol. 131, 669-680.
12. Shindo, H., Simpson, R.T., and Cohen, J.S. (1979) J. Biol. Chem. 254, 8125-8128.
13. Cohen, J.S., Wooten, J.B., and Chatterjee, C.L. (1981) Biochemistry 20, 3049-3055.
14. Shindo, H. (1981) Eur. J. Biochem. 120, 309-312.
15. Dickerson, R.E., and Drew, H.R. (1981) J. Mol. Biol. 149, 761-786.
16. Greve, J., Maestre, M.F., and Levin, A. (1977) Biopolymers 16, 1489-1504.