Acta Biochimica Polonica
Vol. 40 No. 1 1993

A-Z conformational changes effected in RNA by high pressure

Andrzej Krzyżaniak[1], Jens Peter Fürste[2], Piotr Sałański[3], Janusz Jurczak[3], Volker A. Erdmann[2] and Jan Barciszewski[1]*

[1] *Institute of Bioorganic Chemistry of the Polish Academy of Sciences, Z. Noskowskiego 12, 61-704 Poznań, Poland*
[2] *Institut für Biochemie, Freie Universität Berlin, Thielallee 63, Berlin 33, Germany*
[3] *Institute of Organic Chemistry of the Polish Academy of Sciences, M. Kasprzaka 44, 01-224 Warsaw, Poland*

Since the early studies of Franklin & Gosling, who found solvents influence the helical conformation of nucleic acids [1], various methods have been applied to study the nature of interactions between water, counteranions and nucleotides. The conformation of nucleic acids depends on the extent of their hydration and interaction with counteranions. It is evident that detailed knowledge of hydration of nucleic acids is essential for the understanding of their conformations, their transitions and their interactions and, thus, their function. One of the most striking conformational changes in DNA is the conversion of its right handed B-DNA to its left-handed Z-DNA form [2]. This conversion is strongly influenced by such factors as ionic strength, the nature of cations and anions in solution, concentration of organic solvents, and/or intercalating molecules, temperature or superhelical stress. The transition is a highly cooperative process, suggesting that the interactions between the polymer, the cations and water molecules, differ significantly in these two conformations.

Recently we have shown that poly d(GC) undergoes a reversible B-Z transition upon high pressure [3]. Since this transition occurs in the absence of any salts or alcohol, we reasoned that it is caused by water molecules leaving the polymer due to the aggregation of water under high pressure [3].

We present here a study on the effect of high pressure on the conformation of two oligoribonucleotide duplexes, $r(GC)_6$ and $r(AU)_6$. In this communication we demonstrate that high pressure alone induces some conformational changes in r(PuPy) duplexes, but A to Z transition of those molecules occurs when high pressure is applied in the presence of 5 M NaCl. Figure 1 shows the effect of exposure to the high pressure for 18 h on c.d. spectrum of the duplex of $r(GC)_6$. With increasing pressure two effects were observed. First, the c.d. maximum at 260 nm was shifted to longer wavelengths and there was a change in position of the c.d. band at 280 nm. It is well known that position of bands in the c.d. spectra depends on interaction of a chromofore with solvent molecules. Interestingly, the red shift in the c.d. spectrum was more pronounced at 6 than at 8 kbar. This

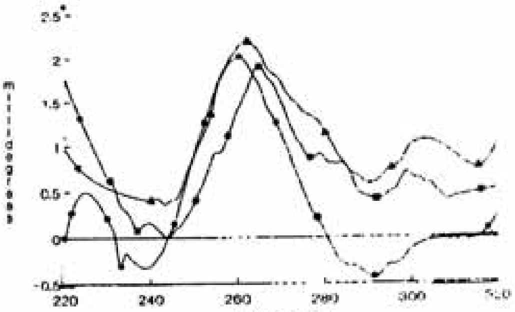

Fig. 1. The circular dichroism (c.d.) spectra of oligoribonucleotide $r(GC)_6$ (0.5 A_{260} units/ml) in the 50 mM Tris/HCl buffer, pH 7.5 containing 150 mM NaCl and 5 mM $MgCl_2$ recorded after high pressure treatment: ■, 6 kbars; ▲, 8 kbars, ● (control), atmospheric pressure.

The samples were exposed to high pressure for 18 h. The spectra were recorded immediately after pressure was released. Thickness of the cell, 0.5 cm.

---

*To whom correspondence should be sent

may indicate that the strongest interactions of water molecules with the polynucleotide chains occur at 6 kbar, where the activity of water is lower. Secondly, a new c.d. band appeared beyond 300 nm. This peak is indicative of light scattering and oligomerization (condensation) of r(GC)$_6$ [4], which is more pronounced at 8 kbar than 6 kbar. However, the spectra of r(GC)$_6$ obtained after the high pressure treatment are not characteristic for the Z-RNA form [5]. Under the high pressure conditions described in the legend to Fig.1, DNA easily goes into the Z-form [3]. This seems to be in agreement with the previous finding that DNA conformation is generally more flexible than that of A-RNA [6].

The overall pressure-induced changes in the c.d. spectra of r(AU)$_6$ (Fig. 2) are very similar to those observed for r(GC)$_6$, though in the present case the differences between the spectra recorded after exposure to various pressures are smaller. The Cotton effects were observed for r(AU)$_6$ at 230 and 285 nm. In addition, the spectra shown in Figs. 1 and 2 resemble very closely those of r(GC)$_3$ in the buffer containing either up to 5 M NaCl [5], or 6 M $NaClO_4$ [5] or at elevated temperature [7]. They differ however, from the Z-RNA spectra of (GC)$_3$ taken in 2.85 M $MgCl_2$ [5] and 6 M $NaClO_4$ at 42°C [8]. This may indicate that concentrated magnesium chloride is a much stronger dehydration agent than either sodium chloride or perchlorate. For r(AU)$_6$ we also observed a c.d. band above 300 nm which probably corresponds to the condensed form of RNA [4], however of lower intensity than in the case of r(GC)$_6$.

We checked also the effect of the high pressure on native 5S rRNA purified from wheat germ. At 6 kbar, the c.d. maxima of 5S rRNA were shifted to a higher wavelength and a new band appeared at 300 nm (Fig. 3). Generally, the spectra of 5S rRNA resemble very much those of r(GC)$_6$ (Fig. 1).

Since neither high salt conditions [6] nor high pressure alone induce the A-Z RNA transition in oligoribonucleotide duplexes, we decided to check the combined effect of these two factors. After exposure of r(GC)$_6$ to high pressure in the presence of 5 M NaCl the positive Cotton effect, diagnostic for Z-RNA, appeared at about 295 nm (Fig. 4), while there was no band above 300 nm. In addition, the new band showing a positive Cotton effect appeared at 235 nm. The c.d. spectra for r(AU)$_6$ are similar to those for r(GC)$_6$ (Fig. 5). However, the Cotton effect is not as pronounced as in the case of r(GC)$_6$. It has been shown for poly d(GC) that the sodium or magnesium ion induced transition between B and Z-DNA can be perturbed by hydrostatic pressure [9]. At variance with those observations we noticed clearly a synergetic effect of high salt concentrations and elevated pressure.

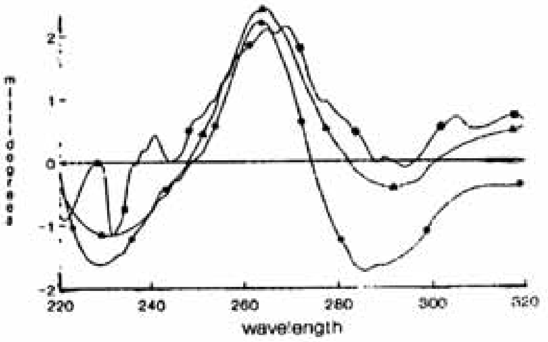

Fig. 2. The c.d. spectra of oligoribonucleotide r(AT)$_6$ exposed to the high pressure.
Conditions and designations as in Fig. 1

It is well known that the left handed Z-conformation in nucleic acids can be induced by altering such parameters as ionic strength, content of organic solvents, temperature and pressure. It is conceivable that changes in all these parameters affect in a similar way the molecular interactions underlying conformational transition. Our experiments were focused on the hydration-dehydration process and one can envisage that the common mechanism consists of elimination of water molecules from the hydration shell of macromolecules. In the B form of DNA, the phosphate groups are far apart and are hydrated independently. The distance be-

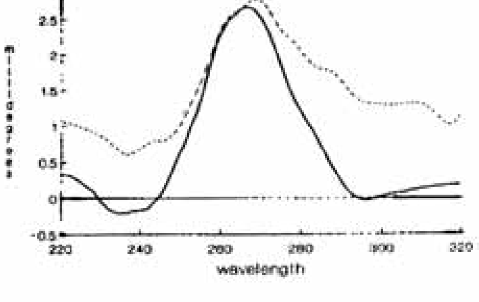

Fig. 3. The c.d. spectra of 5S rRNA from wheat germ exposed to the: atmospheric pressure (thick solid line), and 6 kbar (dashed line) during 18 hours.
The spectra were recorded in the 50 mM Tris/HCl buffer, pH 7.5, containing 150 mM NaCl and 5 mM $MgCl_2$ immediately after pressure was released

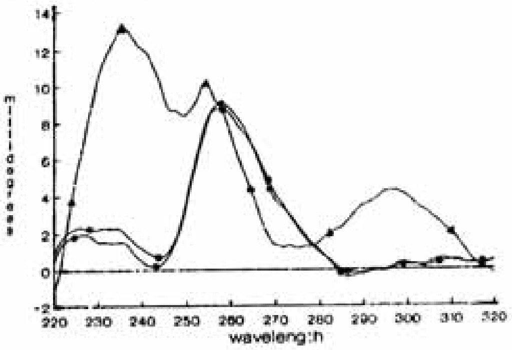

Fig. 4. The c.d. spectra of oligoribonucleotide r(GC)₆ in the buffer (see the legend to Fig. 1) non-exposed and exposed to high pressure for 18 h: ●, 150 mM NaCl, atmospheric pressure; ■, 5 M NaCl, atmospheric pressure; ▲, 5 M NaCl, 6 kbar.
The spectra were recorded immediately after pressure was released

tween free oxygen atoms of phosphate groups in B-DNA is 6.6 Å, but these oxygen atoms in A-DNA and Z-DNA are at a distance of 5.3 Å and 5.1 Å, respectively, and thus can be bridged by water molecules [10]. High pressure enhances interaction of water molecules, even to a point where water starts to crystallize [11]. This means that under the high pressure conditions, the interactions between water molecules become dominant over their interaction with the macromolecules. Thus, our data are consistent with the model based on the assumption that the Z-form formation is driven by the dehydration mechanism [12].

The B to Z DNA transition induced by high pressure occurs at lower salt concentration than A to Z RNA transition. These differences arise from various hydration mechanisms of these nucleic acids. Because of the presence of 2′-hydroxyl group, RNA structures are to a greater extent stabilised by water mediated hydrogen-bond bridges than are DNA structures. The hydrogen bond network provides a higher stabilisation energy for RNA than for DNA. Therefore, DNA more readily undergoes conformational changes caused by dehydration than does RNA [6, 10]. Upon reducing the water activity in RNA solution, the hydrogen bonding interaction between the cytidine hydroxyl oxygen and NH_2 group of the 5′-adjacent guanosine in Z-RNA can be expected to twist the amino group out of plane of the base. This effect could be responsible for the observed difference in the c.d. spectra of Z-RNA and Z-DNA [13].

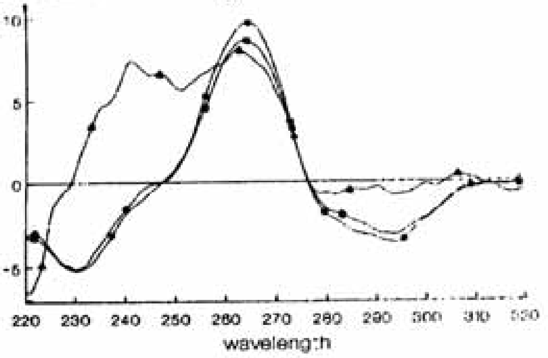

Fig. 5. The c.d. spectra of oligoribonucleotide r(AU)₆ in the buffer (see the legend to Fig. 1) treated as in the legend to Fig. 4: ●, 150 mM NaCl, atmospheric pressure; ■, 5 M NaCl, atmospheric pressure; ▲, 5 M NaCl, 6 kbar

REFERENCES

1. Franklin, R.E. & Gosling, R.G. (1953) *Acta Crystallogr.* **6**, 673 - 676.
2. Rich, A., Nordheim, A. & Wang, A.H.J. (1984) *Annu. Rev. Biochem.* **53**, 791 - 846.
3. Krzyżaniak, A., Sałański, P., Jurczak, J. & Barciszewski, J. (1991) *FEBS Lett.* **279**, 1 - 4.
4. Vorlickova, M., Kypr, J., Jovin, T.M. & Planck, M. (1990) *Biopolymers* **29**, 385 - 392.
5. Adamiak, R., Gałat, A. & Skalski, B. (1985) *Biochim. Biophys. Acta* **825**, 345 - 352.
6. Sundaralingam, M. & Westhof, E. (1987) in *Biomolecular Stereodynamics I* (Saarma, R.H., ed.) pp. 301 - 326, Adenine Press, Guilderland, New York.
7. Behe, M.J., Felsenfeld, G., Szu, S.C. & Charney, E. (1985) *Biopolymers* **24**, 289 - 300.
8. Hall, H., Cruz, P., Tinoco, I., Jr., Jovin, T.M. & van de Sande, J.H. (1984) *Nature (London)* **311**, 584 - 586.
9. Macgregor, R.B. (1988) *Biopolymers* **27**, 1687 - 1690.
10. Saenger, W., Hunter, N.N. & Kennard, O. (1986) *Nature (London)* **324**, 385 - 388.
11. Bett, K.E. & Cappi, J.B. (1965) *Nature (London)* **207**, 620 - 622.
12 Gueron, H. & Demaret, J.-P. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5740 - 5743.
13. Ross, W.S., Hardin, C.C., Tinoco, I., Jr., Rao, N., Pearlman, D.A. & Kollman, P.A. (1989) *Biopolymers* **28**, 1937 - 1957.