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B–Z DNA reversible conformation changes effected by high pressure

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There are numerous data showing that a DNA molecule with alternate pirymidine–purine sequence can adopt a left-handed, double-helical Z-DNA conformation. Such structural changes of DNA occur as a consequence of environmental conditions (e.g. 4 M NaCl) or chemical modification (e.g. methylation or bromination of bases). In this paper, we found for the first time that high pressure (several kilobars) can change the DNA conformation from the B to the Z form. When the pressure is reduced to an atmospheric one, DNA conformation returns back to the B-form. The Z-DNA structure formation was confirmed by circular dichroism (CD) and ultraviolet (UV) measurements. However, we found, that the values of the ratio of absorbance at the wavelengths 295 and 257 nm in the range of 0.3–0.4 is not a fully conclusive proof for the Z-DNA conformation. Although the ratio is typical for Z-DNA form, it is not obvious that the negative band in CD spectrum will be observed. On the other hand, methylated DNA does not undergo B→Z DNA transitions at the high pressure. These conformational changes of DNA molecules could be interpreted as the effect a of different hydration of various DNA forms.

Conformational change; B–Z DNA; High pressure

1. INTRODUCTION

The phenomena of conformational changes of biological molecules are well known in molecular biology. One can suspect that these changes play an important role in many cellular processes [1–4]. However, for example, the precise role of the well-documented B→Z DNA transition is still only very little recognized [1]. There are many experiments which have brought evidence that a piece of DNA with an alternate pirymidine-purine sequence can adopt a left-handed double-helical structure (Z-DNA) under specific conditions [5,6]. Poly(dGdC)·poly(dGdC) changes its conformation from B- to Z-DNA in 4 M NaCl at room temperature [1], but poly(dGm^{5}dC)·poly(dGm^{5}dC) does the same under similar, but much closer to physiological conditions [7]. The conformational changes are unambiguously monitored by circular dichroism (CD) spectra which show a negative Compton effect at 295 nm very diagnostic for Z-DNA. The same conclusion has so far been obtained with UV measurements of the ratio of absorbance at 295 and 257 nm. The values of the ratio of A_{295}:A_{254} around 0.4 usually suggested the presence of a Z-DNA structure [8]. The exact mechanism of B–Z DNA changes and a precise effect of milieu on it is still obscure.

Trying to understand this process, we analyzed a new phenomenon – the effect of high pressure on the structural changes of DNA. It is well known from organic chemistry that high pressure is one of the factors which influences molecular conformation and the mechanism of reaction [9]. Therefore, it was interesting to probe the structural changes of biological molecules [10], for example nucleic acids, under high pressure.

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2. MATERIALS AND METHODS

2.1. *Nucleic acids*

Poly(dGdC)·poly(dGdC) and poly(dGm^{5}dC)·poly(dGm^{5}dC) (Pharmacia) were dissolved in 50 mM Tris-HCl, pH 7.5, buffer containing different amounts of NaCl as indicated in the legend to the figures.

2.2. *High pressure experiments*

1 A_{260} of the polymer in a total volume of 1 ml was loaded into high pressure apparatus. Details concerning this method have been published earlier by one of us [11].

2.3. *UV and CD spectra*

All CD spectra were recorded on Jasco J-20 spectropolarimeter and UV spectra on Beckman spectrophotometer.

3. RESULTS AND DISCUSSION

Two polymers, poly(dGdC)·poly(dGdC) and its methylated analog poly(dGm^{5}dC)·poly(dGm^{5}dC), were used as an example to check the influence of high pressure on DNA conformation. As one can see from Fig. 1, there is no pressure effect on poly(dGdC)·poly(dGdC) structure after 1 h exposure to 6 kbars or a 19-h experiment with a pressure of 10 kbars. On the other hand, in the experiment carried out under 6 kbars for 19 h conformational transition of B-DNA to its Z-form is evidently complete. The observed effect of

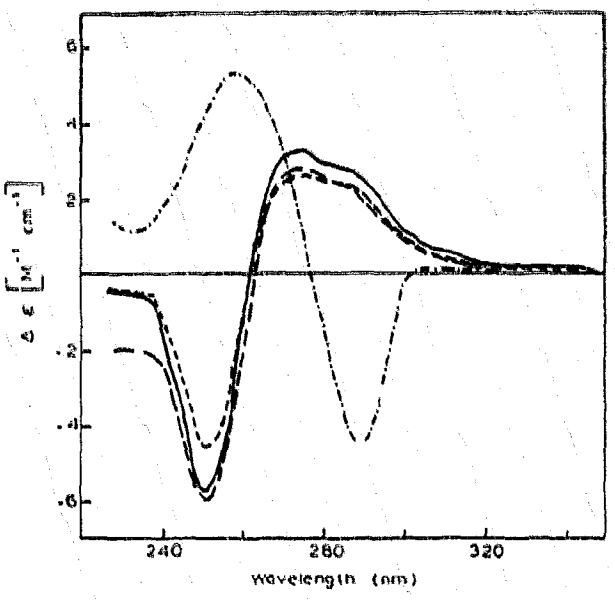

Fig. 1. Circular dichroism (CD) spectra of poly(dGdC)·poly(dGdC) after different treatment with high pressure (several kilobars); (——) atmospheric pressure; (– – –) 6 kbar, 1 h; (·–·) 6 kbar, 19 h; (·+·+·) 10 kbar, 19 h. The samples of 1 A_{260}/ml were prepared in 50 mM Tris-HCl, pH 7.5, buffer containing 150 mM NaCl. 1 A_{260} unit is the amount of DNA which dissolved in 1 ml and measured on 1 cm path length at 260 nm gave an absorbance of 1; 1 kbar is the pressure equal to 1013 atm.

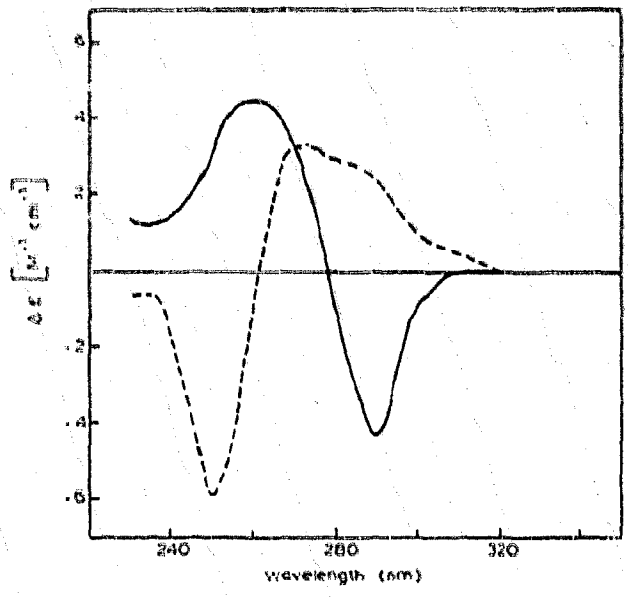

Fig. 2. Circular dichroism (CD) spectra of poly(dGdC)·poly(dGdC) in the presence (——) and absence (– – –) of 4 M NaCl at room temperature. 1 A_{260} unit of poly(dGdC)·poly(dGdC) (Pharmacia) was dissolved in 1 ml of 50 mM Tris-HCl, pH 7.5, buffer containing 4 M NaCl or 150 mM NaCl, respectively. CD spectra were recorded after 10 min sample incubation at room temperature.

high pressure on poly(dGdC)·poly(dGdC) is identical to this one induced by concentrated sodium chloride (Fig. 2 and [12]). The lack of DNA transition observed under 10 kbars is interesting and surprising. It could be explained by a rise of viscosity of the solvent resulting from increasing pressure. In such conditions water solutions are 'almost' frozen and any conformational changes of the biomolecule are probably significantly restricted. The viscosity of water at 10 kbars is about twice that at 1 atm [13]. It is known that some proteins and enzymes are more active upon exposure to high pressure because they have no possibility of conformational changes [10]. It should be added that the conformation of the DNA which was previously exposed to high pressure is changed completely from the Z-form to the B-form after being kept for 5 h under atmospheric pressure.

On the other hand, poly(dGm^{5}dC)·poly(dGm^{5}dC) showed almost no changes in CD spectra after high pressure treatment, neither after 1 h nor in the 19 h experiments (Fig. 3A). Although there is no CD diagnostic band for Z-DNA, one can easily notice that in these experiments (1 and 19 h) some changes still occur in the CD spectrum, especially in the short wavelength region (250 nm) (Fig. 3A). However, the UV spectra of poly(dGm^{5}dC)·poly(dGm^{5}dC) exposed to various pressures are similar to those published earlier for Z-DNA [7]. This means that out of two parameters (CD negative band at 295 nm and an A_{295}/A_{257} ratio in the range 0.25–0.41) specific and characteristic for Z-DNA, only the first one really proves B-Z DNA conformational transition (Fig. 3B). The question still remains open as to what kind of changes really occur in modified DNA which are visualized by CD spectra. It is well known that the addition of 5 mM Mg^{2+} to poly(dGm^{5}dC)·poly(dGm^{5}dC) gives a stable Z-DNA conformation after heating of the DNA up to 60°C followed by quick cooling. The presence of a methyl group on the cytosines in B-DNA increases the solvent-accessible area, while adding the same methyl group to Z-DNA results in a decrease in the area accessible to the solvent molecule [14]. In our experiment we did not observe B-Z DNA transition in methylated DNA under a pressure of either 6 or 10 kbars at room temperature. One can suppose that the steric hindrance caused by the methyl group is probably too big for structural transition, especially under conditions of a higher viscosity of water [14]. In comparing our data with those of others [15], one can suppose that under high pressure the modified analog of poly(dGdC)·poly(dGdC) adopts an A-DNA-like conformation.

What is the general interpretation of the high pressure effect on DNA conformational changes? It can be seen that the B→Z DNA structural transition is fully reversible and does not occur for methylated DNA and for poly(dGdC)·poly(dGdC) under very high pressure (10 kbars). One can suggest that the most important

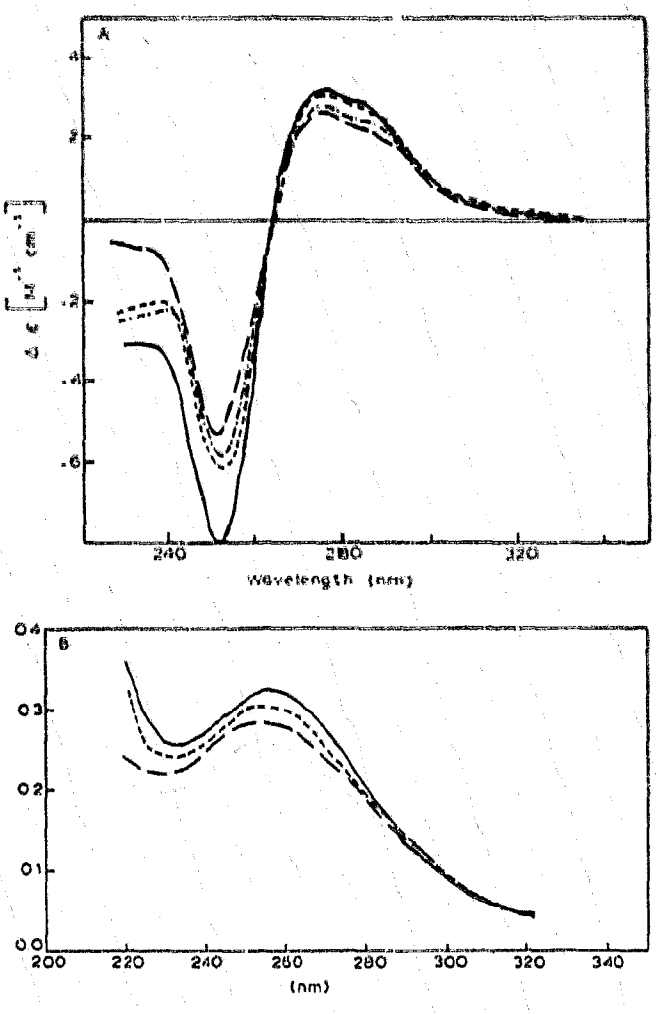

Fig. 3. (A) Circular dichroism (CD) spectra of poly(dGm^{5}dC)·poly(dGm^{5}dC) after different treatment with high pressure: (——) atmospheric pressure; (– – –) 6 kbars 1 h; (–·–) 6 kbars, 19 h; (+ + +) 10 kbars, 19 h. Sample was dissolved in Tris-HCl, pH 7.5, buffer containing 150 mM NaCl. (B) UV spectra of poly(dGm^{5}dC)·poly(dGm^{5}dC) after different treatment with high pressure; (——) atmospheric pressure; (– – –) 1 kbar; (–·–) 3 kbar; (+ + +) 9 kbar. The experiment was carried out for 19 h.

factor for a conformational switch of DNA is the solvent effect, e.g. a hydration of DNA. As shown in Fig. 1, the same type of B→Z DNA structural transition is observed under high pressure as well as with 4 M NaCl. We suspect that in both types of experiments there is a strong competition for water molecules between DNA and sodium chloride. DNA undergoes structural transitions upon a change in water activity due to [16]. The similar changes in DNA have been observed due to increase in temperature [17,18]. The B-form prevails at high water activity but at its reduced activity, DNA adopts the A-form or, in the sequences with alternating *syn* and *anti* conformations of the bases, the Z-form [16]. It has been determined in solution and evaluated theoretically that there are at least twice as many water molecules per B-DNA nucleotide as per A-DNA nucleotide (4–9 for the latter) [19]. On average, in crystals of A-DNA and Z-DNA oligomers at most 6–7 water molecules per residue have been detected, while only 4 water molecules per residue have been found in crystals of B-DNA oligomers. The energetically favorable phosphate hydration observed in the A-DNA and Z-DNA forms, which have water bridges between phosphates, led to the suggestion that phosphate hydration is less economical in B-DNA, where no such bridges could be formed [19]. Although such intrastrand water bridges between phosphate groups have been implicated in nucleic acid transitions, their role as the main driving force of such changes is debatable [16,19]. A very similar situation is effected by high pressure. Under the high pressure conditions, the order of water molecules is changed and aggregates or crystals could be formed. It means that active water is present in limited amounts. On the other hand, the DNA molecule needs a solvent to form its final structure and has to compete for water molecules. A limited amount of active water could induce conformational changes of DNA on the basis of hydration economy [16,19]. From crystallization experiments it is already known [16] that A- and Z-DNA forms are more economic in hydration than the B-DNA form. As one can see, such changes are not possible in poly(dGm^{5}dC) because methylated DNA has some steric hindrance which does not allow for change in its conformation. The energy of hydration is too high to change the conformation of methylated DNA.

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Note added in proof

When this work was finished two other papers concerning effects of high pressure on the biomolecules had been published. In one of them changes in stability of ribosomes can be followed [20]. Also the specificity of restriction enzyme is effected by high pressure [21].