B-Z transition in poly(dG-dC)·poly(dG-dC) in the presence of formaldehyde amino derivatives

A.M Poverenny, V.I. Kiseleva*, B.V. Tyaglov+ and V.I. Permogorov+

Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk, Kaluga Region, and +*All-Union Research Institute of Genetics and Selection of Industrial Microorganism, Moscow, USSR*

Received 18 March 1985, revised version received 11 April 1985

It was shown by circular dichroism that the B-Z transition of poly(dG-dC) poly(dG-dC) in high NaCl concentrations occurred more rapidly in the presence of formaldehyde and Tris The product of formaldehyde and glycine interaction induces changes in the poly(dG-dC) poly(dG-dC) CD spectral characteristics of a 'B-like' conformation It is supposed that the B-Z transition occurs without large-scale hydrogen bond breakage

B-Z transition *Circular dichroism* *Poly(dG-dC) poly(dG-dC)* *Formaldehyde* *Aminomethylolic compound* *Amino derivative* *Glycine*

1. INTRODUCTION

In 1972 Pohl and Jovin [1] described the conformational changes in poly(dG-dC)·poly(dG-dC) caused by high-salt concentration. These changes were tentatively interpreted as a cooperative transition of poly(dG-dC)·poly(dG-dC) from the right-handed (B) to left-handed (Z) double-helical structure. From experimental studies of the equilibrium and kinetics of this transition two detailed quantitative models for its mechanism were derived by Pohl and Jovin: (i) breakage of the Watson-Crick hydrogen bonds; (ii) disorder of the stacking interaction between nitrogen bases in the DNA chains without dissociation into single strands. Pohl and Jovin gave preference to the first model [2]. Other authors suggested that the B-Z transition occurred without breakage of the hydrogen bonds [3,4].

For a more complete understanding of this problem, it was of interest to investigate the effect of formaldehyde amino derivatives on the B-Z transition in poly(dG-dC)·poly(dG-dC). These compounds were shown to be capable of fixing the denaturation changes in DNA [5,6] thus preventing formation of hydrogen bonds between nitrogen bases. Formaldehyde amino derivatives can therefore be assumed to interfere with the B-Z transition that is preceded by breakage of the Watson-Crick hydrogen bonds along the whole length of polynucleotide chains.

2. MATERIALS AND METHODS

Poly(dG-dC)·poly(dG-dC) was purchased from P-L Biochemicals (USA) (lot no.569-112), formaldehyde and Tris from Merck (FRG), glycine from Reanal (Hungary), and sarcosine from INC Pharmaceuticals (USA). Circular dichroism (CD) spectra and kinetics of the CD changes were recorded with a Jouan dichrograph in a 1.0 cm cell. The concentration of poly(dG-dC)·poly(dG-dC) solution was measured by the ultraviolet absorption on an SP 8000 spectrophotometer (Pay Unicam, England) at a wavelength of 254 nm; the molar extinction coefficient was 8400. All the determinations were carried out with 7.5 mM cacodylate buffer; the pH was controlled.

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B V (Biomedical Division)
00145793/85/$3 30

3. RESULTS

Fig.1 shows the time-dependent CD ellipticity changes of poly(dG-dC)·poly(dG-dC) under different conditions (CD intensity changes were observed at fixed wavelengths of 265 and 290 nm). In 3.5 M NaCl and 7.5 mM sodium cacodylate buffer with 0.03 M Tris (pH 7.2), the B-Z transition is more extended in time (monitoring period 220 min). Formaldehyde accelerates the transition, the rate being strongly dependent on solution pH. The acceleration is slight at pH 6.6 but is pronounced at pH 3.4 (the half-times of the B-Z transition under the above-mentioned conditions are given in table 1).

Under the same conditions (pH 3.4), but without formaldehyde, the B-Z transition in poly(dG-dC)·poly(dG-dC) was not accelerated, and even slowed down (fig.2).

Fig.3 shows the CD spectra of poly(dG-dC)·poly(dG-dC) in 0.15 M NaCl with 0 33 M formaldehyde and different concentrations of glycine in the mixture (pH 6.7). It clearly indicates the negative band at 285 nm and insignificant changes in the short-wavelength range (250 nm). Initially, the CD spectrum has the same characteristic features at high salt concentrations. When glycine is replaced by sarcosine (the secondary amine – glycine derivative) or methylamine, the poly(dG-dC) poly(dG-dC) conformational changes mentioned above were not observed under the same conditions (0.15 M NaCl, 0.33 M formaldehyde, pH 6.7); the polynucleotide evidently maintains the B-form.

Table 1

Half-times of poly(dG-dC) poly(dG-dC) B-Z transition under different conditions

Conditions	Half-time of B-Z transition (min)	
	265 nm	290 nm
3.5 M NaCl, 7 5 mM sodium cacodylate, 0 03 M Tris, pH 7 2	35	25
As above, but pH 6 6 plus 0.33 M formaldehyde	25	18
As the latter, but pH 3 4	3	2

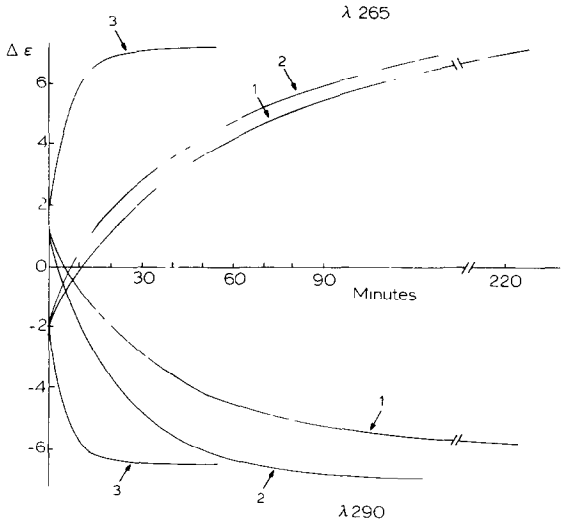

Fig 1. Kinetics of the poly(dG-dC) poly(dG-dC) CD changes at 265 and 290 nm Polynucleotide was dissolved in 7 5 mM cacodylate buffer plus 3 5 mM NaCl and (1) 0.03 M Tris, pH 7 2, (2) 0.03 M Tris, 0 33 M formaldehyde, pH 6 6; (3) 0 03 M Tris, 0 33 M formaldehyde, pH 3 4

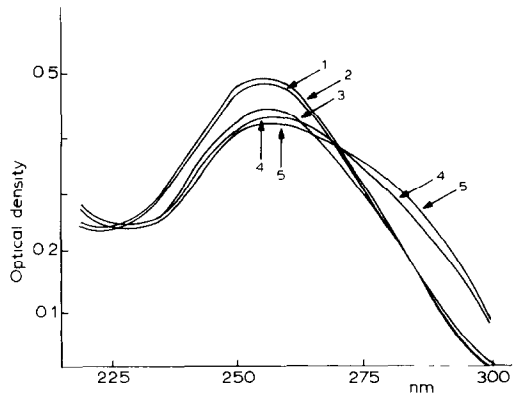

Fig.2 Time-dependent UV absorption in the structural B-Z transition of poly(dG-dC)·poly(dG-dC) Polynucleotide was dissolved in 7 5 mM cacodylate buffer plus 3.5 M NaCl and 0.03 M Tris, pH 3 4· (I) without formaldehyde (1, 0 min, 2, 45 min); (II) with 0.33 M formaldehyde (3, 0 min, 4, 5 min; 5, 15 min)

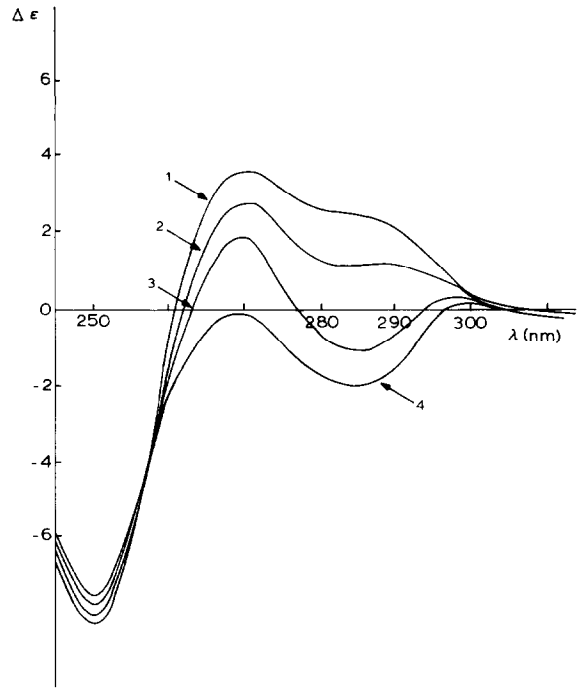

Fig 3 Poly(dG-dC)·poly(dG-dC) CD spectra in 0.15 M NaCl in the presence of formaldehyde and glycine. Polynucleotide was dissolved in 7.5 mM cacodylate buffer plus 0.15 M NaCl (pH 6.7). (1) without formaldehyde and glycine (B-form); (2) with 0.33 M formaldehyde and 0.004 M glycine, (3) with 0.33 M formaldehyde and 0.008 M glycine; (4) with 0.33 M formaldehyde and 0.04 M glycine

4. DISCUSSION

The presence of formaldehyde and the primary amine Tris in the reaction mixture at high salt concentrations accelerates rather than hampers the B-Z transition in poly(dG-dC)·poly(dG-dC). This result and the ability of aminomethylolic compounds to fix denaturation changes in the DNA secondary structure also enable one to conclude that the above transition is not accompanied by a large-scale dissociation of the double helix into single strands. In the opposite case, formaldehyde amino derivatives would slow down or block the transition completely.

Significant dependence of the B-Z transition rate in the presence of formaldehyde and Tris on solution pH perhaps accounts for carbocation formation (between formaldehyde and this primary amine) catalysed by H^+ [7]. The striking carbocation reactivity leads to rapid interaction with the guanine amino group. This is probably the reason for the pronounced acceleration of the poly(dG-dC)·poly(dG-dC) B-Z transition induced by aminomethylolic compounds at high salt concentrations.

The CD spectrum of poly(dG-dC)·poly(dG-dC) at low salt concentration (0.15 M NaCl, pH 6.7) with formaldehyde and glycine acquires some features characteristic of a 'B-like' conformation under high salt conditions. These are negative maxima in the wavelength range 280–290 nm and negligible optical activity changes at 250 nm. Such CD spectra were observed by Goto [8], when he studied the kinetics of the poly(dG-dC)·poly(dG-dC) B-Z transition in 3.5 M NaCl. It was suggested that these CD spectral changes are associated with a subtle change in polynucleotide hydration. We observed similar CD spectra in our experiments immediately after polynucleotide and 3.5 M NaCl were mixed.

Thus, one may conclude that the modification of poly(dG-dC)·poly(dG-dC) by glycine methylolic derivatives causes formation of a conformational structure in 0.15 M NaCl, of which the CD spectrum is identical with that obtained immediately for this polynucleotide in a high salt concentration.

As noted previously, formaldehyde amino derivatives may modify the bases in the DNA double helix due to the interaction with guanosine [5,6]. It is assumed that triazine ring formation may occur between the nitrogen atoms of the guanosine nucleus and an amino group and aminomethylolic compound [9]. Perhaps this is the modification of poly(dG-dC)·poly(dG-dC) which is one of the reasons for its conformational changes, registered nearly instantly at high salt concentrations [8]. This supposition is confirmed by experiments with sarcosine. This secondary amine does not form triazine rings with guanosine in the DNA double helix [9], nor does it form the B-like structure of poly(dG-dC)·poly(dG-dC). However, not all primary amines that can form triazine rings with guanosine in the presence of formaldehyde cause the polynucleotide transition into a 'B-like' form. For example, methylamine does not undergo this conversion. It is proposed that the presence of amino acid carboxy and amino

groups is needed for a conformational transition of poly(dG-dC)·poly(dG-dC) under the above conditions.

Thus, firm evidence of the B-Z transition proceeding without large-scale hydrogen bond breakage was obtained. It is proposed that the B-Z transition results from stacking interaction disturbance facilitated by formaldehyde amine derivative modification of poly(dG-dC)·poly(dG-dC).

Moreover, it was shown that the formation of B-like structures, immediately observable under high salt conditions, can occur at physiological ionic strength in the presence of such normal metabolites as formaldehyde and amino acids.

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