Volume 225, number 1,2, 11–15 FEB 05342 December 1987

Slow exchanging protons in the Z-form of G-C and A-C alternating polymers by using a rapid dialysis method

B. Hartmann, J. Ramstein and M. Leng

Centre de Biophysique Moléculaire, 1A, Avenue de la Recherche Scientifique, 45071 Orleans Cedex 2, France

Received 2 October 1987

Using a dialysis method we have measured the hydrogen exchange (HX) kinetics in poly(dG-dC)·poly(dG-dC), poly(dG-m^{5}dC)·poly(dG-m^{5}dC), poly(dG-br^{5}dC)·poly(dG-br^{5}dC) and platinated poly(dA-br^{5}dC)·poly(dG-dT) under experimental conditions in which these polymers adopt the Z-conformation. The latter polymer has one slow exchanging proton with a half-time of about 2 h, whereas the other G-C alternating polymers display a slow class of two protons with exchange half-time of about 6 h. These exchange half-times are independent of ionic strength and of the nature of the salt for all these polymers in the Z-form. The slow proton exchange appears to be strongly correlated to the Z-conformation but rather independent of the Z-DNA sequence. The comparison of the proton exchange rates with the corresponding B⇌Z transition rates is not in favour of the same rate limiting step for both processes.

Hydrogen exchange; Z-DNA

1. INTRODUCTION

The hydrogen exchange (HX) kinetics of the protons involved in double-stranded nucleic acid base pairing is widely accepted to be related to internal base movements leading to the disruption of the hydrogen bonds [1]. These characteristic fluctuations of the base pair position are likely to play an important role in recognition processes between biological macromolecules.

Using the hydrogen-tritium exchange with gel filtration we had shown that the overall HX kinetics in poly(dG-dC)·poly(dG-dC) Z-DNA is much slower than in all A-DNA and B-DNA double helices so far measured [2,3]. Two protons were found to be especially slow with an exchange half-time of 7 h at 0°C. Such a slow class of protons has also been found in poly(dI-br^{5}dC)·poly(dI-br^{5}dC) (Z-form) by using IR spectroscopy to measure hydrogen-deuterium exchange; however with this technique the number of slow protons could not be determined [4]. The comparison of the poly(dG-dC)·poly(dG-dC) and poly(dI-br^{5}dC)·poly(dI-br^{5}dC) (both in the Z-form) HX kinetics led us to identify these two slow protons as the cytosine amino protons [4]. Mainly because of the unusually slow exchange rate of these two protons, NMR (which has been widely used in the field of HX kinetics) failed to measure their exchange rate.

Since the discovery of the Z-DNA conformation in double-stranded nucleic acids with alternating G-C sequences [5,6], it appeared that this conformation is not restricted solely to this polymer. In particular, it was shown that, in circular plasmid DNA, stretches of alternating A-C sequences, which are the most abundant purine-pyrimidine dinucleotide repeats in eukaryotic genomes, can undergo the Z transition in response to topological

Correspondence address: B. Hartmann, Centre de Biophysique Moléculaire, 1A, Avenue de la Recherche Scientifique, 45071 Orleans Cedex 2, France

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

constrains [7–11]. In linear DNA a similar B⇌Z transition was observed for poly(dA-br^{5}dC)·poly(dG-dT) at high ionic strength and high temperature [12]. Indeed bromuration [13,14], as well as methylation [15] on C5 of cytosine, is very efficient in stabilizing the Z-form of DNA.

It was thus interesting to investigate further the generality of the presence in Z-DNA of these two slow exchanging protons in the case of poly(dG-m^{5}dC)·poly(dG-m^{5}dC), poly(dG-br^{5}dC)·poly(dG-br^{5}dC) and poly(dA-dC)·poly(dG-dT) polymer.

We here show that all the examined polymers display exchanging protons with much slower rates than in B-DNA.

Moreover, these proton exchange rates are compared to the corresponding B⇌Z transition rate and the existence of a possible common rate limiting step for both processes is discussed.

2. MATERIALS AND METHODS

Poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) were purchased from PL Biochemical. Poly(dG-br^{5}dC)·poly(dG-br^{5}dC) was synthesized according to Malfoy et al. [13] and poly(dA-br^{5}dC)·poly(dG-dT) according to Morgan et al. [16]. All polymers were treated with phenol, precipitated with ethanol and then extensively dialyzed against the buffer. The quantitative fixation of chlorodiethylenetriaminoplatinum(II) chloride on the N7 of the guanine of poly(dA-br^{5}dC)·poly(dG-dT) was performed by the method described by Macquet and Butour [17].

The absorption spectra were recorded on a Uvikon Bio/820 (Kontron) spectrophotometer and the circular dichroism spectra on a Jouan-Roussel dichrograph connected to a microcomputer (Apple II).

For concentration determinations, the following extinction coefficients at 260 nm were used: 7100 $M^{-1}\cdot cm^{-1}$ for B-poly(dG-dC)·poly(dG-dC), 7000 $M^{-1}\cdot cm^{-1}$ for B-poly(dG-m^{5}dC)·poly(dG-m^{5}dC) and 6000 $M^{-1}\cdot cm^{-1}$ for Z-poly(dG-br^{5}dC)·poly(dG-br^{5}dC) and B-poly(dA-br^{5}dC)·poly(dG-dT).

Tritiated water was obtained from CEA (Saclay, France). All other chemicals were of the best grade commercially available.

The radioactivity was measured with an LKB 1216 liquid scintillation spectrometer. Rapid dialysis was achieved in a system described by Englander and Crowe [18]. This method needs about one order of magnitude less material than the gel filtration method and is thus particularly well suited to measure HX kinetics of polymers which are only available in tiny amounts. The number of still unexchanged hydrogens per nucleotide pair (H/M) as function of time was calculated as:

$$H/M = \frac{111 \times 2 \times \epsilon_{260}}{\mathrm{cpm}_0} \times \frac{\mathrm{cpm}_t}{A}$$

ϵ_{260} is the molecular extinction coefficient at 260 nm for the polymer and A is the absorbance. cpm_0 and cpm_t are the radioactivity level measured in counts per minute, of the labelling solution and of the polymer at time t during the hydrogen exchange process, respectively.

3. RESULTS AND DISCUSSION

The HX curves of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) and poly(dG-br^{5}dC)·poly(dG-br^{5}dC) under experimental conditions in which the polymers adopt the Z-conformation are presented in fig.1. For comparison we have shown on the same figure the exchange curve of poly(dG-dC)·poly(dG-dC). Both polymers are characterized by a slow class of two protons with the same exchange time ($t_{1/2}$ = 5.7 h) as for poly(dG-dC)·poly(dG-dC). This slow class has been found previously on polymer films by IR spectroscopy [21,22]. Varying the ionic strength and the type of ions does not affect the exchange half-time of these two protons.

To identify the exchanging protons let us compare the HX exchange curves of fig.1 to the poly(dG-dC)·poly(dG-dC) HX curves obtained previously with the gel filtration method [2,3]. In the latter case, between 0°C and 35°C two classes of protons were measured: a fast class of 3 protons (guanosine amino and imino proton) and a slow class of 2 protons (cytosine amino protons) whose measured exchange half-times at 0°C are 20 min and 7 h, respectively (for B-DNA the slowest protons have an exchange half-time of about 10 min). The activation energy of the slow class is equal to

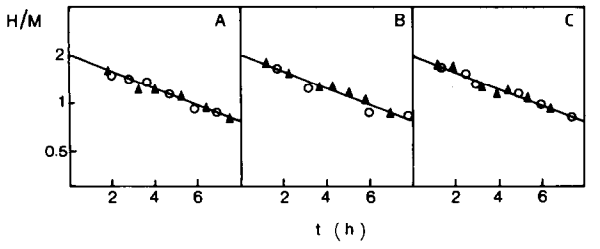

Fig.1. Semi-logarithmic plot of hydrogen-exchange curve at 4°C of: (A) Z-poly(dG-dC)·poly(dG-dC) in 3 M $NaClO_4$, 10 mM Tris, pH 7.1 (▲); in 4.5 M NaCl, 10 mM Tris, pH 7.1 (○); (B) Z-poly(dG-m^5dC)·poly(dG-m^5dC) in 3 M $NaClO_4$, 10 mM Tris, pH 7.1 (▲); in 3 M NaCl, 10 mM Tris, pH 7.1 (○); (C) Z-poly(dG-br^5dC)·poly(dG-br^5dC) in 3 M NaCl, 10 mM Tris, pH 7.1 (▲); in 0.2 M NaCl, 2 mM Tris, pH 7.1 (○). Nucleotide concentration, 1.8×10^{-4} M.

20 kcal which gives a corresponding calculated exchange half-time of 5.5 h at 4°C. From this it is clear that the two protons of poly(dG-dC)·poly(dG-dC) measured here by dialysis correspond to the two protons of the slow class evidenced previously by the gel filtration method. Because of the greater dead-time of the dialysis method as compared to gel filtration, the three protons of the fast class escaped detection.

In the case of poly(dG-m^5dC)·poly(dG-m^5dC) and poly(dG-br^5dC)·poly(dG-br^5dC), as the HX kinetics of the two slow protons are identical to the one obtained with poly(dG-dC)·poly(dG-dC) it seems very reasonable to assign the protons to the cytosine amino protons.

Thus the Z-conformation of all three polymers is characterized by a slow class of 2 protons exhibiting a remarkably constant exchange half-time. Although it is not yet understood why these protons are so slow in Z-DNA (the slowest protons of B-DNA or A-DNA exchange are in the minute time range), it is likely that the unusual slow exchange rate of these protons reflects some characteristic feature of the internal base pair motion in Z-DNA.

To study the HX kinetics in Z-DNA with the $(dA\text{-}dC)_n$ sequence under relatively moderate conditions (3.5 M NaCl at 4°C) we used poly(dA-br^5dC)·poly(dG-dT) with 5% of the guanine substituted on the N7 by chlorodiethylenetriaminoplatinum (II) chloride. This reaction stabilizes greatly the Z-form [19,20] and has been shown not to perturb the HX kinetics of poly(dG-dC)·poly(dG-dC) [3]. According to ultraviolet and dichroism spectra the low salt form of this polymer belongs to the B-family and the high salt form to the Z-family. The transition mid-point is 2.4 M NaCl at 0°C and the transition half-time is 3 min at 0°C (not shown). The HX kinetics presented in fig.2 displays a slow class comprising one proton with an exchange half-time of 1.7 h.

The Z-form of platinated poly(dA-br^5dC)·poly(dG-dT) has a slow class of one proton per base pair whose exchange half-time ($t_{1/2}$ = 1.7 h) although smaller than those obtained with the previous G-C alternating polymers ($t_{1/2}$ = 5.7 h) is still longer than in B-DNA (under the same experimental conditions it has been checked that B-DNA protons are too fast to be measured by this method). The identification of this proton is not straightforward as before and several possibilities exist. For example, it may represent the sum of the guanine and thymine imino protons or the amino protons of either adenine, guanine or cytosine (because of the alternating A-br^5C sequence all these identifications could lead to an amplitude of one proton per base pair). Although none of these identifications can be ruled out at this stage, by analogy with poly(dG-dC)·poly(dG-dC) the cytosine amino protons seem to us the most attractive candidates. Mirau et al. [23] found with NMR that the guanine and thymine imino protons of poly(dA-br^5dC)·poly(dG-dT) in the Z-form are

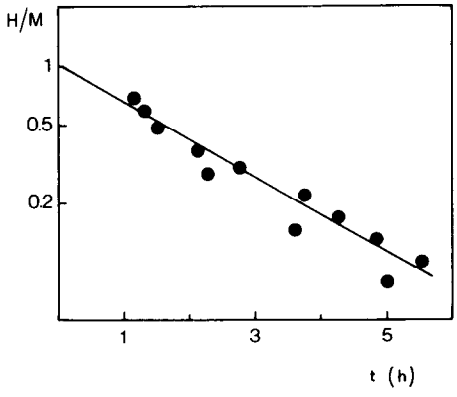

Fig.2. Semi-logarithmic plot of hydrogen-exchange curve at 4°C. Semi-logarithmic plot of the slow class of Z-platinated poly(dA-br^5dC)·poly(dG-dT) in 3.5 M NaCl, 10 mM Tris, pH 7.1. Nucleotide concentration, 5×10^{-5} M.

much slower than in the B-form and have an exchange half-time with a lower limit at 0°C of about 30 min. According to these results our measured protons could well be the two imino protons but we do not favour this identification because in all cases so far studied (B-DNA, A-DNA, Z-DNA) the slowest protons are always amino protons, the imino proton belonging to the fast class. Concerning the smaller value of the exchange half-time of the measured proton as compared to poly(dG-dC)·poly(dG-dC) Z-DNA, it is tempting to relate this difference to some peculiarity of the Z-DNA conformation of this polymer. Such slight difference in Z-DNA conformation of poly(dA-br^{5}dC)·poly(dG-dT) has already been suggested by Jovin et al. [12].

In summary, this study shows that DNAs belonging to the Z family are all characterized by the presence of an unusual class of slow protons (in particular, A-C sequences display a slow class of one proton per base pair with an exchange half-time of about 2 h).

In conclusion it is worthwhile to discuss our results in the context of the B⇌Z transition mechanism.

The base pair flipping during the B⇌Z conformational transition is compatible with a mechanism involving a breakage of the hydrogen bonds with opening of the base pair [24]. Mirau and Kearns [25] noticing in the case of poly(dG-dC)·poly(dG-dC) the similar unusual slow rates both of the imino proton exchange rate in Z-DNA and of the B⇌Z transition, suggested that both processes might have the same limiting step. The results here reported show that three polymers present a slow exchanging class of amino protons with the same exchange half time, despite the fact that they have grossly different transition rates (at 20°C, the transition rate of poly(dG-dC)·poly(dG-dC) is in the hour time range, of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in the minute time range whereas poly(dG-br^{5}dC)·poly(dG-br^{5}dC) adopts only the Z-conformation).

Furthermore, as it is known that the B⇌Z transition rates are dependent upon the ionic strength and upon the nature of the salt, we have varied the nature of the salt in the case of poly(dG-dC)·poly(dG-dC) and the ionic strength in the case of poly(dG-br^{5}dC)·poly(dG-br^{5}dC). As already pointed out, there is no change in the corresponding proton exchange half-time. Thus as far as the slow amino proton exchange rates are concerned our results do not support a B⇌Z transition mechanism having the same limiting step as the Z-DNA proton exchange mechanism.

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