SOME FEATURES OF HYDROLYSIS OF THE HYBRID B-Z-FORM DNA BY SERRATIA MARCESCENS NUCLEASE

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ABSTRACT

Highly polymerized herring testis DNA of the random nucleotide sequence was used as a model of natural substrate to study some features of hydrolysis of the hybrid B-Z form with Serratia marcescens nuclease. The hybrid B-Z form was formed upon addition of 1.15 M MgSO$_4$ and 0.421 mM Co(NH$_3$)$_6$Cl$_3$. The DNA transition from the right handed B-form to the hybrid B-Z-form caused a decrease in Vmax of DNA cleavage with the nuclease. The diminishing Vmax was consistent with diminishing values of Km and Kcat. The binding of Mg$^{2+}$ or Co(NH$_3$)$_6$$^{3+}$ to highly polymerized DNA caused correspondingly about 80-or 7-fold decrease in Km and more than 1600 or 600 decrease in Kcat compared with that of Mg-DNA complex of B-form.

Keywords: Hybrid B-Z-form DNA, Serratia Marcescens Nuclease, Sma Nuc, Mg$^{2+}$, Co(NH$_3$)$_6$$^{3+}$

1. INTRODUCTION

Purine/pyrimidine sequences, poly d(G-C), poly d(A-T) and poly d(A-C) x poly d(G-T), were found to fold into a left-handed helix, Z-type conformation, under reduction of water activity (Pohl and Jovin, 1972; Klump et al., 1993; Leslie et al., 1980; Arnott et al., 1980; Sheardy et al., 1993; Vorlčeková et al., 1982). The B-to Z-helix transition has been mainly studied on short poly- or oligodeoxynucleotide fragments, with strictly alternating purine/pyrimidine sequences. In longer synthetic deoxyoligonucleotides containing both poly d(G-C) stretches and the runs of random nucleotide sequences the helix was converted from the pure right-handed B-form to the hybrid B-Z-form containing regions of both left- and the right-handed helices upon reducing water activity (Sheardy et al., 1993; 1994; Nordheim et al., 1982; Suh et al., 1991). Similar changes we (Filimonova et al., 2008) later found in highly polymerized natural DNA containing random nucleotide sequences.

Current research findings leads to the assumption that within the same molecule of natural DNA radically different geometries coexist and the hybrid B-Z-form of DNA containing left-handed fragments separated by a B-Z junction from right-handed regions really exists in vivo and serves different biological roles. For instance a non random distribution of Z-DNA regions was found across the human genome in neighboring regions of protein-encoding genes (Champ et al., 2004). Also Z-DNA regions are often met near transcription start sites. The transcription, as was reported (Lancillotti et al., 1987), can induce the Z-DNA formation and Z-DNA itself could play a role in transcriptional regulation (Champ et al., 2004; Liu et al., 2001; Oh et al., 2002; Liu et al., 2006; Lukomski and Wells, 1994; Newman and Shull, 1991; Rahmouni and Wells, 1989; Rothenburg et al., 2001). Playing a regulatory role in transcription and in gene expression, or in DNA supercoiling Z-helix segments may act as recognition sites for DNA binding proteins (Sheardy et al.,...
2. MATERIALS AND METHODS

2.1. Materials

Sodium chloride (Ultra), Magnesium sulfate, Hexamminecobalt (III) chloride, Ethylenediaminetetraacetate, highly polymerized DNA from Herring Testes (type XIV) were purchased from Sigma. Tris (Ultra Pure) was purchased from ICN. All the listed reagents were used without further purification.

2.2. Methods

2.2.1. Sample Preparations

The hybrid B-Z-form DNA resembling a combination of left-handed Z-helix embedded into the right-handed B-helix was prepared as previously reported (Filimonova et al., 2008). In particular, water solution of DNA (2.5 mg mL\(^{-1}\)) was extensively dialyzed against 50 mM NaCl containing 100 mM EDTA to remove unwanted divalent ions and then against a 5 mM Tris-HCl buffer, pH 8.0, containing 50 mM NaCl. Then one part of the dialyzed solution was additionally dialyzed against the same buffer containing 1.15 M MgSO\(_4\). Another part of the solution was first 200-fold diluted with a 5 mM Tris-HCl buffer, pH 8.0, containing 50 mM NaCl, then titrated by microliter amounts of 0.14 M Co(NH\(_3\))\(_6\)Cl\(_3\) to Co(NH\(_3\))\(_6\)\(^{2+}\) concentrations of 0.421 mM. For measurement of circular dichroic spectra of the initial (B-form) DNA the water solution of DNA was diluted in 5 mM Tris-HCl buffer, pH 8.0 and then 6 M NaCl was added to 50 mM concentrations.

The DNA concentration (in nucleotides) was determined by absorption spectroscopy using a molar extinction coefficient of 6,500 M\(^{-1}\) cm\(^{-1}\) at the UV maximum of 258 nm.

Sma nuc endonuclease was isolated and characterized as previously described (Filimonova et al., 2003). Concentration of the enzyme was calculated as previously (Romanova and Filimonova, 2012) using the molecular mass and molar extinction coefficient of 47,292 M\(^{-1}\) cm\(^{-1}\).

To study the direct influence of the metal cations (Mg\(^{2+}\), Co(NH\(_3\))\(_6\)\(^{2+}\)) on enzyme conformation, first the traces of multivalent metal cations from the enzyme preparation were removed as was reported (Filimonova et al., 2003). Then the enzyme solution was diluted with 1.15 M MgSO\(_4\) or 0.14 M Co(NH\(_3\))\(_6\)Cl\(_3\) containing 50 mM Tris-HCl buffer, pH 8.0, to the cation concentrations 0.1, 1.0, 10.0 mM or 0.29, 1.49, 2.90 mM respectively. After a 15 min preincubation at room temperature, the CD spectra of the enzyme preparation in the presence or in the absence of the metal cations were recorded.

2.2.2. Circular Dichroism Spectrometry (CD)

CD spectra of 5.02 µM Sma nuc nuclese or 1.44 mM (in a nucleotide equivalent) DNA were recorded at room temperature in a 10 mm or 2 mm path length cuvette at 210-310 nm using a Jasco-J 500 A spectrometer (Jasco, Japan). The spectral data were normalized by subtraction of the base line that was a spectrum of the solution without DNA.

2.2.3. Kinetic Study

The nuclease activity was determined by the hyperchromic effect of hydrolysis of DNA preparations using a λ-35 Perkin Elmer spectrophotometer (Perkin Elmer, USA). Rates of the reaction were recorded at 260 nm until the progress curves became non-linear. These rates were calculated from the linear part of the reaction progress curves (initial velocities) using the applied Rate Analysis software package.
Experiments were carried out in 10 mm cuvettes at 30°C. After adding 0.51 µM Sma nuc solution (13.63 µg mL⁻¹) to 125-fold volume (1000 µL) of pre-warmed (3-5 min) appropriately prepared and diluted DNA solution, the measurements were done for 15-60 min. The Km and Kcat values were determined from Lineweaver-Burk double reciprocal plot. Kcat was calculated as Vmax per 1 mM of Sma nuc.

3. RESULTS

CD spectra of the DNA in the absence (taken as a control) and in the presence of 1.15 M MgSO₄ are shown in Fig. 1A (dark and grey lines respectively). The control spectrum was characterized by a deep trough at 243 nm and a peak at 273 nm. CD spectrum of the DNA in the presence of 1.15 M MgSO₄ had both decreased towards the control line peak around 275 nm and a trough at 243 nm. In the spectrum upon the addition of 0.421 mM Co(NH₃)₆Cl₃ (Fig. 1B, grey line) the high peak at 275 nm also decreased relative to the control (dark line) and the trough at 243 nm became shallower.

CD spectra of the Sma nuc preparations at a wide range concentrations of MgSO₄ or Co(NH₃)₆Cl₃ are shown in Fig. 2. The resulted aromatic CD spectra of the nuclease after preincubation with MgSO₄ or Co(NH₃)₆Cl₃ were identical between themselves and with the spectrum recorded in the absence of inspected metal cations.

Kinetic analysis of hydrolysis of the hybrid B-Z-form of DNA with the nuclease revealed a sufficient decrease in Vmax independently on the type of binding cations. As shown in Table 1, upon DNA transition to the hybrid B-Z-form there is a drastic decrease of both Km and Kcat regardless of Mg²⁺ presence. Therefore the transition affects both the strength of enzyme binding to the substrate and the rate of productive dissociation of the enzyme-substrate complex.

Table 1. Kinetic parameters for the cleavage of DNA in the presence of metal cations

| The form of DNA | Type of the binding metal cation | Ratio of the metal ions per phosphate in DNA | Km (mg/mL) | Vmax (mM/min) | Kcat (sec⁻¹) |
|----------------|----------------------------------|---------------------------------------------|------------|---------------|--------------|
| B-Z-hybrid Mg⁺ | 6800/1                           | 0.0004                                      | 0.99       | 0.44          |
| B-Z-hybrid Co(NH₃)₆³⁺ | 280/1                            | 0.0045                                      | 2.69       | 1.20          |
| B-helix Mg⁺ | 30/1                             | 0.0310                                      | 1655.30    | 737.60        |

Filimonova et al. (2003)

![Circular dichroic spectra of the DNA in 0.05 M NaCl (dark line) and in the presence of 1.15 M MgSO₄ (A) or after titration with Co(NH₃)₆Cl₃ to 0.421 mM (B) at 21°C (grey lines)
4. DISCUSSION

In this study we used highly polymerized DNA of random nucleotide sequence to mimic naturally occurring biological molecules, suggesting that any molecules of native DNA contain potentially Z-DNA-forming sequences. To induce a transition from pure B- to the hybrid B-Z-form resembling a combination of left-handed Z-helixes embedded into the right-handed B-helixes, we chose, as previously stated (Filimonova et al., 2008), Mg$^{2+}$ and Co(NH$_3$)$_6^{3+}$ at appropriate amounts. The expected changes were controlled with CD spectrometry. The low-salt spectrum at 50 mM NaCl, taken as a control, was identical with the previously reported one for the B-helix DNA in solution (Filimonova et al., 2008).

The diminishing, relatively the control line, peak around 275 nm and a trough at 243 nm in the spectrum upon the addition of 1.15 M MgSO$_4$ or 0.421 mM Co(NH$_3$)$_6$Cl$_3$ (Fig. 1A and B) served us a signal of the DNA conversion from the pure B-form to the hybrid B-Z-form polymer. The CD spectra of the Mg -DNA and Co(NH$_3$)$_6$ -DNA preparations (Fig. 1, grey lines) were fully identical with the previously published spectra (Filimonova et al., 2008) evidencing a transition from the B- to the hybrid B-Z-form of DNA upon addition of 1.15 M MgSO$_4$ or 0.421 mM Co(NH$_3$)$_6$Cl$_3$. By this reason the other steps of earlier undertaken analysis (Filimonova et al., 2008) on further confirmation of the DNA conversion from the pure B- to the hybrid B-Z-form were not here submitted and discussed.

As the kinetic analysis of hydrolysis of the hybrid B-Z-form DNA with the nuclease revealed a sufficient decrease in Vmax independently on the type of binding cations we concluded that the DNA transition from pure B-form to the hybrid B-Z-form depressed the enzymatic activity. As upon DNA transition to the hybrid B-Z-form there is a drastic decrease of both Km and Kcat (Table 1) the transition affects both the strength of enzyme binding to the substrate and the rate of productive dissociation of the enzyme-substrate complex, regardless of Mg$^{2+}$ presence that is known as activating cation of Sma nuc. A significant decrease of Kcat for all conformers compared to that of the B-DNA suggests that the substrate secondary structure plays the main or rather important role in productive dissociation of the enzyme-substrate complex. Although the inspected metal cations vary in physics-chemical properties, including the binding sites in DNA (Behe and Felsenfeld, 1981; Harder and Johnson, 1990), their effect on hydrolysis is very similar, obviously due to their similar ability to induce the transition of DNA from pure right handed helix to the hybrid form with enclosed left-handed fragments.

To be sure that the inspected metal cations at high concentration do not directly influence the nuclease activity we recorded as previously (Filimonova et al., 2008)
2003) the aromatic CD spectra of Sma nuc preparation after preincubation with vast excess of MgSO₄ or Co(NH₃)₆Cl₃. The similarity of resulted spectra with the spectrum of nuclease in the absence of metal cations indicated a lack of metal influence on the tertiary structure of the enzyme at wide range of metal cations/Sma nuc ratio. This led us to conclusion that excess of MgSO₄ or Co(NH₃)₆Cl₃ in the surroundings of the nuclease exerts influence on the nuclease activity if they were bound with DNA and transformed the DNA secondary structure from the pure B- to the hybrid B-Z-form.

5. CONCLUSION

First, this study demonstrates the ability of Serratia marcescens nuclease to digest the hybrid B-Z form of DNA. Using MgSO₄ and Co(NH₃)₆Cl₃ the transition of highly polymerized DNA to the hybrid B-Z-form was induced. Second, the transition was followed by a decreased Vmax of the nuclease hydrolysis of the hybrid DNA in comparison with the pure B-DNA. The diminished activity was consistent with drastically diminished values of Km and Kcat. Both mechanisms of Mg²⁺ and Co(NH₃)₆³⁺ action on the activity of S. marcescens nuclease towards the hybrid B-Z-form DNA was obviously identical with the previously discovered mechanisms of Mg²⁺, C₆H₅O₂Hg²⁺ and Co(NH₃)₆³⁺ (Filimonova et al., 2003). The inspected metal cations similarly bound to the substrate (DNA), which induced changes in the secondary structure of the substrate that was responsible for the altered enzymatic activity of S. marcescens nuclease.

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