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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 $Co(NH_3)_6Cl_3$. 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; Vorlíčková *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 helixes 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*.,

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1993; 1994; Jaworski *et al*., 1987; Lancillotti *et al*., 1987; Jimenez-Ruiz *et al*., 1991; Mazur *et al*., 2003; Rich *et al*., 1984; Rich, 1994). These multiple roles of Z-helix in DNA, including some roles in carcinogenesis (Sunderman, 1989) and disease susceptibility (Blackwell and Searle, 1999; Takahashi *et al*., 2004), led us to study the cleavage of the hybrid B-Z-form of natural DNA with the sensitive to DNA conformation nuclease that was a main goal of the undertaken research. For this purpose we chose the extracellular endonuclease of Gram negative bacterium *Serratia marcescens* (EC 3.1.30.2), Sma nuc.

Although Sma nuc, which is potently degrading both DNA and RNA, is known as nuclease with broad specificity (SCOP, 2009; RCSB PDB, 2014) it shows some sensitivity to the secondary structure of the substrate. We demonstrated (Filimonova *et al*., 2003) that the nuclease responds on the changes in secondary structure of nucleic acids upon addition of metal cations. This enzyme is well studied (Biedermann *et al*., 1989; Miller *et al*., 1994; Meiss *et al*., 1995; Friedhoff *et al*., 1996; Romanova and Filimonova, 2012). Sma nuc represents a large group of homologous nucleases, including the apoptotic Endo G, which are widely found in nature and share functionally important amino-acid residues (SCOP, 2009; RCSB PDB, 2014; Brenda, 2014). Additionally it displays some antiviral activity and has demonstrated some repressive effects on several cancer tumors (Benedik and Strych, 1998).

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-helixes embedded into the right-handed B-helixes 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 $Co(NH_3)_6Cl_3$ to $Co(NH_3)_6^{3+}$ 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 $M^{-1} \cdot cm^{-1}$.

To study the direct influence of the metal cations (Mg^{2+}, $Co(NH_3)_6^{3+}$) on enzyme conformation, first the traces of multivalent metal cations from the nuclease 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 $Co(NH_3)_6Cl_3$ 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 nuclease 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 $MgSO_4$ 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 $MgSO_4$ 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 $Co(NH_3)_6Cl_3$ (**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 $MgSO_4$ or $Co(NH_3)_6Cl_3$ are shown in **Fig. 2**. The resulted aromatic CD spectra of the nuclease after preincubation with $MgSO_4$ or $Co(NH_3)_6Cl_3$ 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 Mg^{2+} 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^{2+}	6800/1	0.0004	0.99	0.44
B-Z-hybrid	$Co(NH_3)_6^{3+}$	280/1	0.0045	2.69	1.20
B-helix [a)]	Mg^{2+}	30 /1	0.0310	1655.30	737.60

[a)]-Filimonova *et al*. (2003)

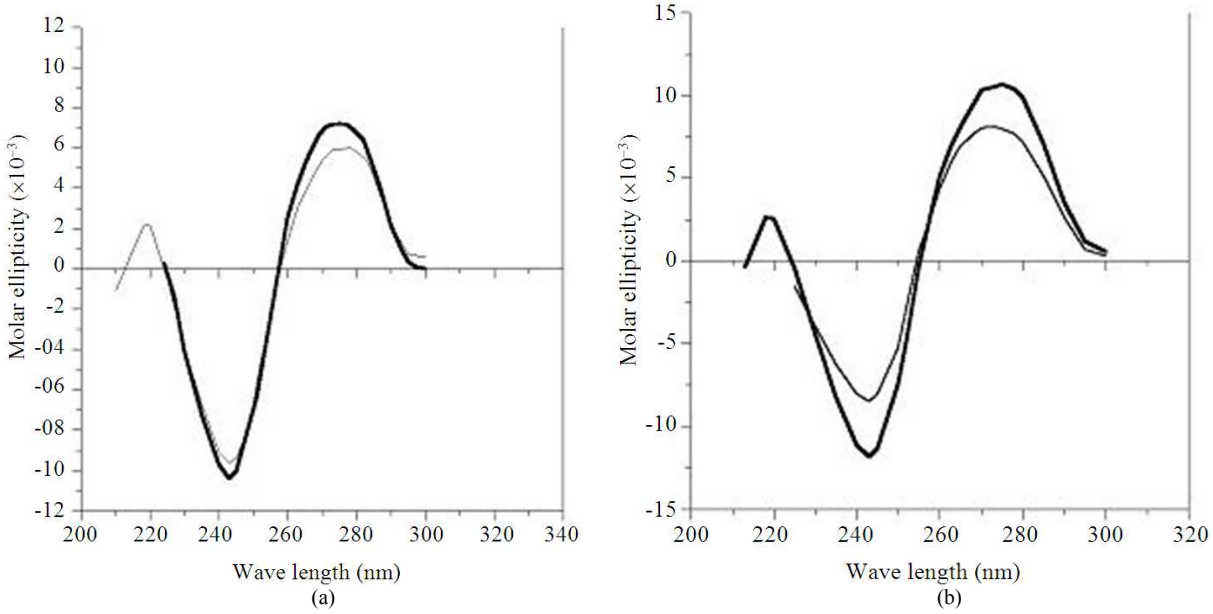

Fig. 1. Circular dichroic spectra of the DNA in 0.05 M NaCl (dark line) and in the presence of 1.15 M $MgSO_4$ (A) or after titration with $Co(NH_3)_6Cl_3$ to 0.421 mM (B) at 21°C (grey lines)

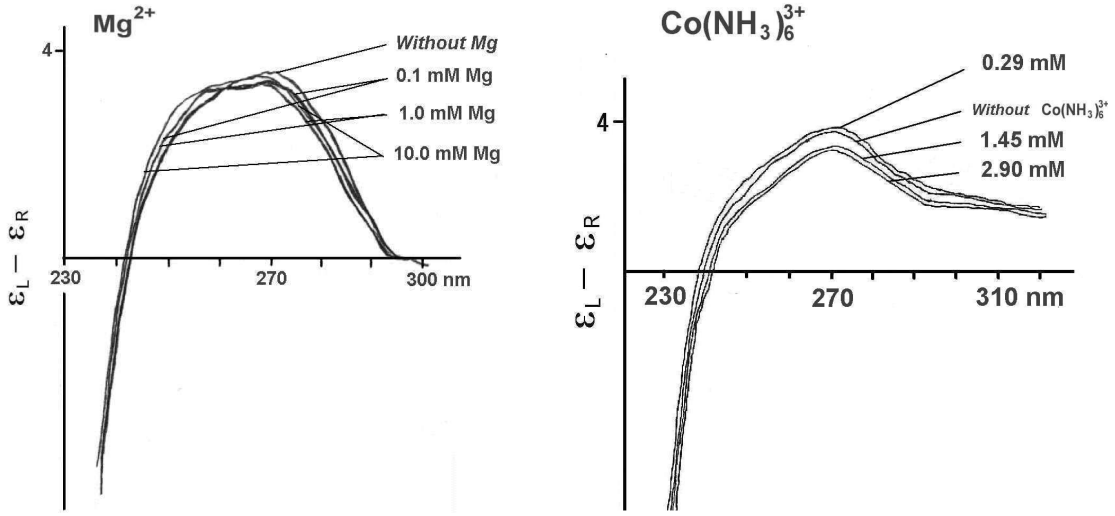

Fig. 2. Circular dichroic spectra of Sma nuc preparation in the absence (without) and in the presence of Mg^{2+} or $Co(NH_3)_6^{3+}$ at metal cation to the nuclease ratio of 19.92 (0.1 mM), 199.20 (1 mM) and 1992.00 (10 mM) or 57.8 (0.29 mM), 288.8 (1.45 mM) and 577.7(2.9 mM), correspondently

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 spectrums upon the addition of 1.15 M $MgSO_4$ or 0.421 mM $Co(NH_3)_6Cl_3$ (**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 $Co(NH_3)_6Cl_3$. 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*.,

2003) the aromatic CD spectra of Sma nuc preparation after preincubation with vast excess of $MgSO_4$ or $Co(NH_3)_6Cl_3$. 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 $MgSO_4$ or $Co(NH_3)_6Cl_3$ 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 $Co(NH_3)_6Cl_3$ 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 cations binding to DNA significantly repressed the catalytic function of Sma nuc and increased the enzyme's affinity to the substrate. Third, the mechanism of Mg^{2+} and $Co(NH_3)_6^{3+}$ action on the activity of *S. marcescens* nuclease towards the hybrid B-Z-form DNA was obviously identical with the previously discovered mechanisms of Mg^{2+}, $C_7H_5O_2Hg^+$ and $Co(NH_3)_6^{3+}$ (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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