Slipped loop structure of DNA: a specific nucleotide sequence forms only one unique conformer

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Abstract Earlier with some DNA sequences we were able to prove the existence of a new polynucleotide chain folding named slipped loop structure, or SLS [1,2]. However, the possibility of the presence of two SLS isomers in equilibrium was not excluded in the experiments. Here we are dealing with a specially designed structure formed by two short oligonucleotides intended for avoiding such a situation. To minimize the possibility of alternative structure formation and stabilize the conformation under investigation, the oligonucleotide sequences were designed in such a way that the bimolecular structure SLS31 would have two binding sites for antibiotic distamycin A. The sample was exposed to chemical probing both in the presence of distamycin A and without the ligand and the accessible nucleotides were mapped. The results do not suggest the presence in the solution of two isomers with different types of loop slippage without interloop interactions and strongly support the formation of a unique slipped loop conformation stabilized by an additional interloop helix, or slipped loop structure.

Key words: Direct repeat; Slipped loop structure; Chemical probing; Distamycin A binding

1. Introduction

Deoxyribonucleic acids are known to be polymorphic. In addition to the classical A and B double helices, the regions with special sequences can adopt some unusual conformations which can be stabilized both by an appropriate solution environment (high ionic strength or low pH) and superhelical stress. As unusual DNA conformations often contain the nucleotides not involved in base pairing, the presence of such conformations can be detected using chemical probes or single-strand specific nucleases (for a review see [3]).

Short direct repeats are often found in eukaryotic and prokaryotic genomes, including regulatory regions [4]. It was shown that under superhelical stress these regions contain nucleotides sensitive to S1 nuclease [5–7]. The model with two shifted loops, one in each of the opposing strands, was proposed to explain the S1 cleavage data for the 5′-flanking region of *Drosophila* hsp70 gene [5], for adenovirus late promoter [6] and mouse collagen promoter [7]. Furthermore, it has been noticed that the loops themselves have a potential to interact with each other due to the complementarity of their sequences. We shall refer to such a structure as slipped loop structure, or SLS (Fig. 1).

A three-dimensional model of SLS for the B-form of DNA was built using conformational analysis as well as low resolution and imino proton nuclear magnetic resonance (NMR) [8]. The interloop duplex formation was possible for the SL structures with the length of the direct repeats of 6 nucleotides. The scheme on Fig. 1 shows that two types of slipped loops are formed: *isomer I* and *isomer II*, but formation of an interloop helix in *isomer II* is impossible because of stereochemical reasons [1,8].

To study the possibility of SLS existence two deoxyoligonucleotides of 55 and 56 bases potentially capable of folding into SLS were designed and synthesized earlier. In both cases the chemical modification data were in agreement with formation of the slipped loop structure stabilized by the complementary interloop interaction [1,2]. To minimize the occurrence of the conformations alternative to SLS and exclude the structure asymmetry due to the terminal loop influence we dealt with the intermolecular structures formed by two short oligonucleotides.

The **SLS31** sample was designed so that the SL structure could be potentially formed by an interaction of two strands, **S31(1)** and **S31(2)** (Fig. 2). The calculations of permissible secondary conformations for the bimolecular complexes of **31(1)** and **31(2)** revealed the possibility of formation of *isomer I* and *isomer II* without any alternative structures.

The sequences of oligonucleotides were designed so that duplexes III and IV of potentially formed SLS contained sites 5′-TTAA/3′-AATT and 5′-AATT/3′-TTAA for binding antibiotic distamycin A (DstA) specific for AT sequences and binding into the minor groove of B-DNA [9,10]. Thus, the capability of DstA to bind to a bimolecular structure formed by oligonucleotides **31(1)** and **31(2)** would mean the formation of duplexes III and IV (Fig. 2). In this case, evidence for formation of duplex I and interloop duplex II in *isomer I* will testify to the SLS folding. This investigation is aimed to solve the problem connected with possible existence of different isomers.

2. Materials and methods

The deoxyoligonucleotides **S31(1)** 5′-d(GTTAACGCCATGGTTTTTGTGCACCCAATTG)-3′ and **S31(2)** 5′-d(CAATTGGCCATGGTTTTTGTGCACCGTTAAC)-3′ were synthesized by standard phosphoramidite procedure. The calculations of permissible secondary conformations for the bimolecular complexes of **31(1)** and **31(2)** were performed using GCG program [11].

For native gel electrophoresis and chemical probing the oligonucleotide 5′-ends were labelled with [γ-^{32}P] using T4 polynucleotide kinase (Amersham).

The quality of synthesized oligonucleotides was tested by polyacrylamide gel electrophoresis under denaturing conditions: 20% polyacrylamide gel (19:1 acrylamide/bis-acrylamide), 7 M urea in 1×TBE, pH 8.3.

In order to estimate possible depurinisation of the nucleotides the oligonucleotides treated for 30 min in hot piperidine (90°C) were used.

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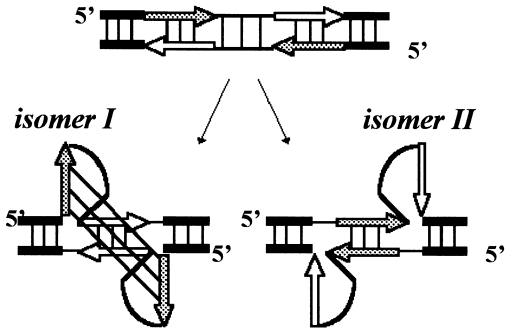


Fig. 1. Scheme of Slipped Loop Structure formation in a duplex DNA.

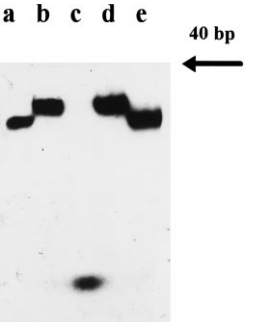


Fig. 3. Electrophoretic analysis of complexes formed by oligonucleotides **S31(1)** and **S31(2)** in native polyacrylamide gel in buffer containing 10 mM $MgCl_2$. Concentrations of strands are: a: oligonucleotide **dS31(1)***, 10^{-4} M(N); b: equimolar mixture of oligonucleotides **dS31(1)*** and **dS31(2)**, 10^{-4} M(N) of each strand; c: equimolar mixture of oligonucleotides **S31(1)*** and **S31(2)**, 10^{-5} M(N) of each strand; d: equimolar mixture of oligonucleotides **dS31(1)** and **dS31(2)***, 10^{-4} M(N) of each strand; e: oligonucleotide **S31(2)***, 10^{-4} M(N). The labelled oligonucleotides are marked '*'.

The correctness of the synthesized sequences was confirmed by the Maxam-Gilbert procedure [12].

Concentrations of oligonucleotides were determined as follows. Optical density at 260 nm, 90°C, was increased by 20% to account for residual hypochromicity [13]. Molar concentrations in nucleotide units were computed using a mean molar extinction coefficient per residue [13]. Aqueous solutions of distamycin A hydrochloride (Sigma) were prepared daily. The molar extinction coefficient was taken as 30 mM/cm at 303 nm [9].

The sample **SLS31** was prepared by heating the equimolar mixture of strands **S31(1)** and **S31(2)** till 95°C followed by cooling down to 3–5°C for 2 h in an appropriate buffer.

The presence of bimolecular complexes was checked by native polyacrylamide gel electrophoresis: 15% polyacrylamide gel (29:1 acrylamide/bis-acrylamide), 0.5×TBE, pH 8.3, in the presence of 10 mM $MgCl_2$ of 5°C and 5 V/cm.

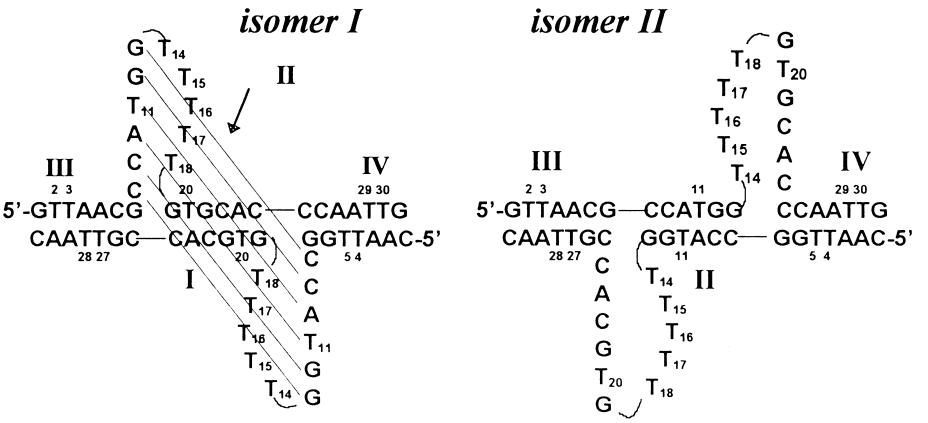
Circular dichroism spectra were obtained with Jasco-715 spectropolarimeter with a thermostatic cuvette with optical path length of 1 mm at 4°C in 10 mM sodium cacodylate (pH 7.0), 10 mM $MgCl_2$. DNA concentration was 10^{-4} M(N) of **S31(1)** and **S31(2)** each. The experimental data were analyzed with the Microcal Origin computer program (Microsoft).

Chemical modifications of nucleotides by $KMnO_4$ were carried out at 4°C as previously described [1,2]. The reactions were performed in 50 μl of the reaction mixture based on the standard buffer: 10 mM sodium cacodylate (pH 7.0), 1 mM EDTA (pH 7.0), 10 mM $MgCl_2$. Concentration of bimolecular complexes **SLS31** in the reaction solution was $3{\cdot}10^{-6}$ M (10^{-4} M(N) of each strand **S31(1)** and **S31(2)**). $KMnO_4$ was added up to final concentration of 10–50 μg/ml. The incubation time was 1–3 min. Reactions were stopped by Hz-stop solution: 0.3 M sodium acetate, 0.1 mM EDTA, 25 μg/ml tRNA. Gel electrophoresis was carried out in 20% polyacrylamide gel (19:1 acrylamide/bis-acrylamide), 7 M urea in 1×TBE, pH 8.3.

The radioautographs were scanned using 300A Computing Densitometer (Molecular Dynamics, USA).

3. Results

To determine whether the low temperature complexes are intermolecular we applied the electrophoresis under native conditions. No bimolecular complexes were observed at 5°C in the equimolar mixture of 10^{-5} M(N) oligonucleotides **S31(1)** and **S31(2)** (Fig. 3c). A unique bimolecular structure exists when oligonucleotides **S31(1)** and **S31(2)** are mixed at a concentration of 10^{-4} M(N) of each strand (Fig. 3b,d). The structures formed by identical strands (duplexes **S31(1)/S31(1)** and **S31(2)/S31(2)**) have higher electrophoretic mobilities (Fig. 3a,e) and were not observed in equimolar mixture of oligonucleotides **S31(1)** and **S31(2)**. So, as follows from the electro-



S31(1): 5'-GTTAACGCCATGGTTTTTGTGCACCCAATTG

S31(2): 5'-CAATTGGCCATGGTTTTTGTGCACCGTTAAC

Fig. 2. Two-dimensional representation of **SLS31** formed as bimolecular complex by oligonucleotides **S31(1)** and **S31(2)**.

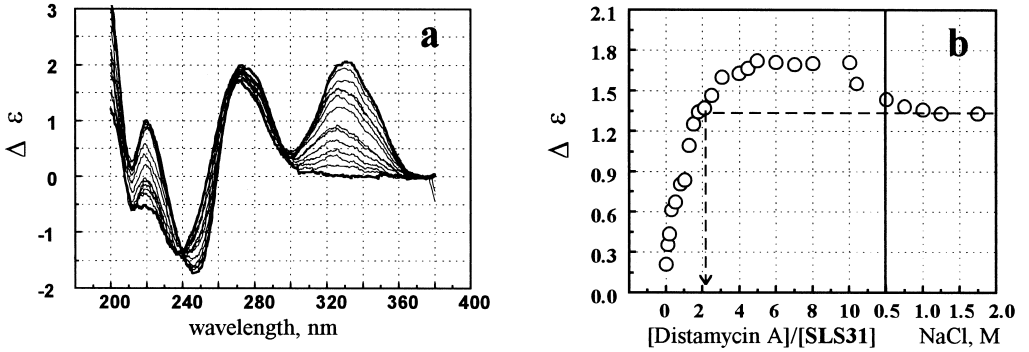


Fig. 4. a: CD spectra of sample **SLS31** at a ratio DstA/**SLS31** concentrations from 0 to 4. b: Titration of **SLS31** with DstA at 320 nm and effect of added NaCl.

phoresis data, the bimolecular structure **S31(1)/S31(2)** early designated as **SLS31** can only be formed at equal concentrations 10^{-4} M(N) of each strand.

Formation of duplexes III and IV of **SLS31** was tested using distamycin A binding with the sample. Fig. 4a shows the changes in the circular dichroism (CD) spectrum for **SLS31** upon increasing the ligand concentrations. The binding phenomenon is characterised by increasing dichroism in the 310–360-nm and 220–240-nm regions. The presence of two isodichroic points is in agreement with the presence of two species in the solution: free DNA and DNA–DstA complex. The increase in the CD signal at the drug/DNA stoichiometries higher than 2 most probably reflected non-specific weak complexes with the sites containing GC pairs (Fig. 4b) [9]. The complex with stoichiometry of 2:1 does not dissociate upon increasing salt concentration up to 2 M NaCl (Fig. 4b). This property is inherent to the specific complexes of DstA with AT-sites in B-DNA [9]. As follows from the form of CD spectra, the ligand binds to DNA as a monomer [10]. These facts indicate the presence in the sample **SLS31** of two duplexes containing no less than three AT pairs [9]. So, formation of duplexes III and IV as the components of **SLS31** folding has been proved.

To study formation of the interloop helix we performed chemical probing. In case of SLS folding of the sample **SLS31** thymines $T_{14–18}$ are unique unpaired bases in every strand **S31(1)** and **S31(2)**. Since only these nucleotides may be used as a control of the extent of modification on unpaired bases ('negative' control) we applied $KMnO_4$ which is specific to unpaired thymines.

If the interloop duplex II in *isomer I* of **SLS31** could not be formed it is reasonable to expect the presence of both *isomer I* and *isomer II* in equal concentrations in buffer solution since the energies of their formation are nearly the same. In this case almost half of thymines T_{11} in each strand **S31(1)** and **S31(2)** would be protected from the modification (duplex II in *isomer II*), but at the same time these bases in *isomer I* would be modified by the reagent. The average modification extent of T_{11} would be nearly half of that for thymines $T_{14–18}$. The same is true for thymines T_{11}. Thus, the mere difference in modification extent of thymines T_{11}, T_{20} on the one hand and thymines $T_{14–18}$ on the other does not prove the formation of interloop helix and a 'positive' control is required. The modification extent of the thymidines which must be involved in base pairing both in *isomer I* and *isomer II* can be used for this purpose. Thymines T_2, T_3 and T_{29}, T_{30} of oligonucleotide **S31(1)** and thymines T_4, T_5 and T_{27}, T_{28} of oligonucleotide **S31(2)** meet this requirement.

Oligonucleotide **S31(2)** was selected for radio-labelling because thymines T_4, T_5 and T_{27}, T_{28}, that may be used as the positive control, are more distant from 5′- and 3′-ends, respectively, as compared with thymines T_2, T_3 and T_{29}, T_{30} of oligonucleotide **S31(1)** (Fig. 2).

The radioautograph with the results of the $KMnO_4$ modification reactions of **SLS31** in the presence of DstA is given in Fig. 5. Densitograms of the gel showing $KMnO_4$ reactions without ligand and in the presence of DstA are shown in Fig. 6a and b.

According to these data, T-stretch $T_{14–18}$ of oligonucleotide **S31(2)*** in **SLS31** is sensitive to $KMnO_4$ attack. One can see that the modification extent of thymine T_{20} and thymines T_4, T_5, T_{27}, T_{28} both in the presence of DstA and in its absence is nearly the same. This fact does not allow the existence of the equilibrium of *isomers I* and *II* without the interloop interactions and is in agreement with the duplex I formation in *isomer I* (Fig. 2). On the other hand, the sensitivity of thymine

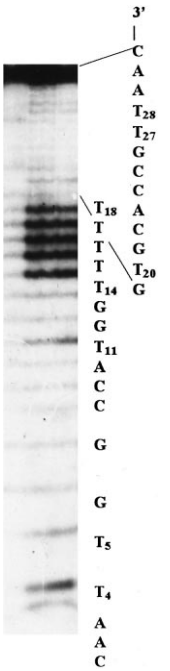


Fig. 5. The radioautograph with $KMnO_4$ probing of **SLS31** in the presence of DstA.

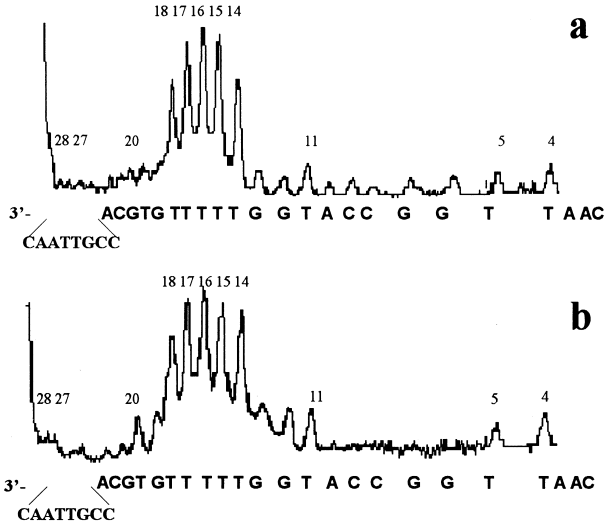


Fig. 6. The densitograms of $KMnO_4$ probing of **SLS31** in the presence of DstA (a) and without ligand (b).

T_{11} to the reagent is significantly lower than that of thymines $T_{14–18}$. This proves the involvement of this nucleotide in the base pairing and thus the formation of interloop duplex II in **SLS31**. Since we have already proved the formation of duplexes I, II, III and IV, formation of the slipped loop structure stabilized by the complementary interloop interaction is established. By the way, the results of this study exclude the so-called 'kissing' SLS conformer as well [14].

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References

[1] Gorgoshidze, M.Z., Minyat, E.E., Gorin, A.A., Demchuk, E.Ya., Farutin, V.A. and Ivanov, V.I. (1992) Mol. Biol. (English translation) 26, 832–838.
[2] Minyat, E.E., Khomyakova, E.B., Petrova, M.V., Zdobnov, E.M. and Ivanov, V.I. (1995) J. Biomol. Struct. Dynam. 13, 523–527.
[3] Wells, R.D., Amirhaeri, S., Blaho, J.A., Collier, D.A., Hanvey, J.C., Hsieh, W., Jaworski, A., Klysik, J., Larson, J.E., McLean, M.J., Wohlrab, F. and Zacharias, W. (1988) in: Unusual DNA Structures (Wells, R.D. and Harvey, S.C., Eds.) pp. 1–21, Adenine Press.
[4] Yagil, G. (1991) Crit. Rev. Biochem. Mol. Biol. 26, 475–559.
[5] Mace, H.A., Pelman, H.R.B. and Travers, A. (1983) Nature 304, 555–557.
[6] Ju, Y.-T. and Manley, J.L. (1986) Cell 45, 743–751.
[7] McKreon, C., Schimidt, A. and de Crombrugghe, C. (1984) J. Biol. Chem. 259, 6636–6640.
[8] Ulyanov, N.B., Bishop, K.D., Ivanov, V.I. and James, T.L. (1994) Nucleic Acids Res. 22, 4242–4249.
[9] Zasedatelev, A.S., Gursky, G.V., Zimer, Ch. and Thrum, H. (1974) Mol. Biol. Rep. 1, 337–342.
[10] Wemmer, D.E., Geierstanger, B.H., Fagan, P.A., Dwyer, T.J., Jacobsen, J.P., Pelton, J.G., Ball, G.E., Leheny, A.R., Chang, W.-H., Bathini, Y., Lown, J.W., Rentzeperis, D., Marky, L.A., Singh, S. and Kollman, P. (1994) in: Struct. Biology, The State of Art, Proc. of the Eight Conversation, SUNYNY (Sarma, R.H. and Sarma, M.H., Eds.) pp. 301–323, Adenine Press, San Diego.
[11] Zuker, M. (1989) in: RNA processing (Dahlberg, J.E. and Ableson, J.N., Eds.) Vol. 180, pp. 262–288, Methods of Enzymology, Academic Press, San Diego.
[12] Maxam, A.M. and Gilbert, W. (1980) in: Nucleic Acids (Grossman, L. and Moldave, K., Eds.) Vol. 65, pp. 499–559, Methods of Enzymology, Academic Press, New York.
[13] Minchenkova, L.E., Shchyolkina, A.K., Chernov, B.K. and Ivanov, V.I. (1986) J. Biol. Struct. Dynam. 4, 463–475.
[14] Ulyanov, N.B., Ivanov, V.I., Minyat, E.E., Khomyakova, E.B., Petrova, M.V., Lesiak, K. and James, T.L. (1997) Immobile Slipped-Loop Structures of DNA in Solution (Sarma, M.H. and Sarma, R.H., Eds.) Proceedings of the Tenth Conversations in the Discipline Biomolecular Stereodynamics.