The distribution of angles in DNA arcs of 32 base pairs

Alexander Vologodskii

New York University, New York, NY 10003

To address the probability of appearance of strongly bent and hooked DNA segments in circular DNA molecules we investigated the corresponding local conformational properties by the computer simulation. The equilibrium conformational ensembles were sampled by Monte Carlo simulation for the discrete wormlike chain as was described earlier (1). Each 8 bp of the double helix were modeled by one straight segment of the model chain. We simulated the conformational sets of unknotted chains and trefoils and analyzed each conformation in the sets to construct the distribution of angles, $P(\varphi)$, between the ends of arcs formed by 5 consecutive straight segments (Fig.1). The arcs correspond to DNA segments of 32 bp in length, a probable

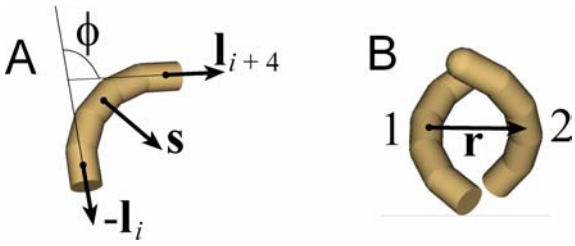

Figure 1S. Definitions of angles used in the analysis of the arc juxtaposition. (A) Each arc is specified by five segments $\mathbf{l}_i$,... $\mathbf{l}_{i+4}$. The direction of the arc is specified by vector $\mathbf{s} = \mathbf{l}_{i+4} - \mathbf{l}_i$. (B) Two arcs were considered to be juxtaposed if the distance between their middle segments, *r*, is smaller than 8 nm. Angles $\vartheta_1 = \arccos(\mathbf{s}_1\mathbf{r}/s_1 r)$ and $\vartheta_2 = \arccos(-\mathbf{s}_2\mathbf{r}/s_2 r)$ specify the mutual orientation and positions of two juxtaposed arcs. Two juxtaposed arcs were considered to be hooked if both ϑ_1 and ϑ_2 do not exceed 60º.

size of DNA segments interacting with topo II (2,3). The distributions $P(\phi)$ were constructed for all arcs of 5 segments, and for pairs of juxtaposed arcs in the unknotted chains and trefoils. The calculated distributions are shown in Fig. 2S. We see from the figure that in the scale of this plot the distributions are identical for all arcs and for pairs of juxtaposed arcs, regardless of the topology of the circular chains. This means that knotting does not affect the bending of the juxtaposed arcs substantially. The bend angles larger than π/2 always have very small probability of appearance.

Sometimes, however, two juxtaposed arcs are substantially bent. We investigated mutual orientation of such juxtaposed arcs. If arcs *i* and *j* are hooked, the angles between their directions, specified by vectors $\mathbf{s}_i$ and $\mathbf{s}_j$, should be close to 180º (This is a necessary but not a sufficient condition that two juxtaposed arcs are hooked). We found, however, that the distributions of angles between the directions of substantially bent juxtaposed arcs with good accuracy correspond to the angle distribution between two random independent vectors (Fig. 3S). The distributions for the unknotted chains and for trefoils were not distinguishable within accuracy of the simulations. Thus, regardless of the topology, the juxtaposed arcs have only regular, rather moderate bending and their mutual orientations are absolutely random.

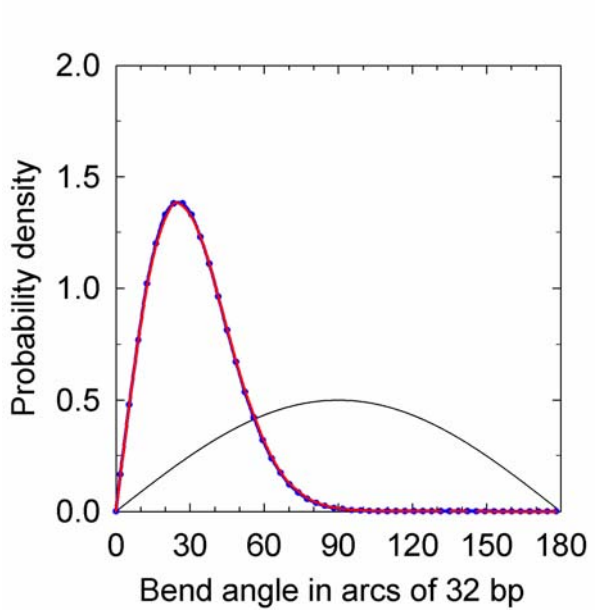

Figure 2S. The simulated distributions of bend angles, $P(\phi)$, in DNA segments of 32 bp (arcs). The data were obtained for all arcs of 32 bp in length (red line and circles) and for pairs of juxtaposed arcs for unknotted chains (green line and circles) and trefois (blue line and circles). The simulation was performed for DNA molecules 7 kb in length. For comparison, the dot line shows the angle distribution between adjacent segments of the freely-jointed chain.

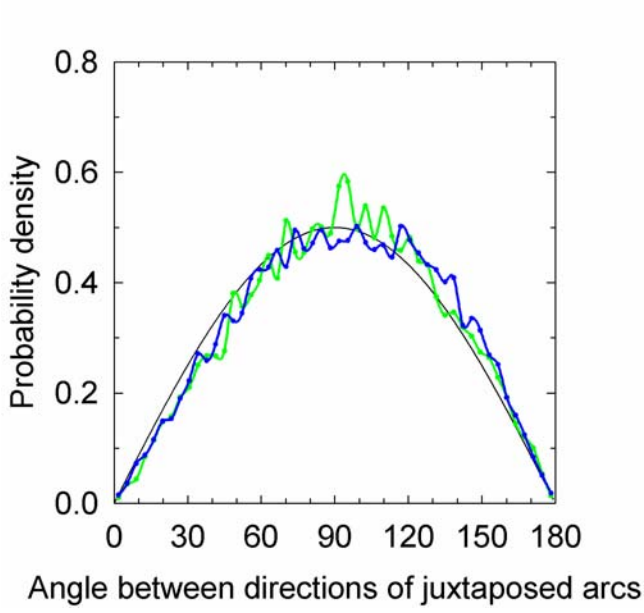

Figure 3S. The simulated distributions of angles between the directions of the juxtaposed arcs. The direction of the arc and the juxtaposition of two arcs are defined in Fig. 1S. A pair of juxtaposed arcs contributed into the distribution only if they were substantially bent, so that $\phi_1 + \phi_2 \geq 150^{\circ}$. The simulation was performed for the unknotted chains (green line and circles) and for trefoils (blue line and circles). For comparison, the distribution of angles between the directions of two randomly oriented independent vectors is shown by the black line.

Finely, we calculated the probability that two juxtaposed arcs are hooked. Two arcs were considered to be hooked if both ϑ_1 and ϑ_2 are smaller than 60º (see Fig. 1S). Since strongly bent arcs are so rare in the simulated conformations, we were able to perform the analysis for hooked arcs which were only moderately bent. Such pairs of arcs were specified by condition that $\phi_1 + \phi_2 \geq 150º$ or $\phi_1 + \phi_2 \geq 180º$. The results of this analysis show that the probabilities of hooked juxtapositions are extremely small for both trefoils and unknotted DNA molecules (Table 1).

Table 1. Simulated probabilities of the hooked juxtapositions in unknotted DNA and DNA forming a trefoil knot. The probabilities were calculated as the number of hooked juxtapositions divided by the total number of juxtapositions. The simulation was performed for DNA molecules 7 kb in length.

	Probability, $\phi_1 + \phi_2 \geq 150º$	Probability, $\phi_1 + \phi_2 \geq 180º$
Unknotted chains	$(1.7 \pm 0.3) \cdot 10^{-5}$	$(0.4 \pm 0.1) \cdot 10^{-6}$
Trefoils	$(5.0 \pm 0.5) \cdot 10^{-5}$	$(1.9 \pm 0.2) \cdot 10^{-6}$

One can also see from the Table 1 that the probabilities of hooked juxtapositions are different in the knotted and unknotted chains. This difference corresponds to the difference in the juxtaposition probabilities obtained for the hairpin-like G segment model (5). It is not surprising, since the probability that a segment is located inside a hairpin formed by a strongly bent segment (so it is hooked with bent segment) should be the same for transient hairpins and ones stabilized by the protein binding. The effect of higher probability of hooked juxtapositions in trefoils was also obtained in the simulations for the freely-jointed chain model (4), which catches the effect but cannot be used to estimate the probability of hooked juxtapositions formed by the small arcs. The effect is due to the entropic localization of knots (5).

REFERENCES

1. Vologodskii, A. (2007) In Monastryrsky, M. (ed.), *Topology in molecular biology*. Springer, Berlin - Heidelberg - New York, pp. 23-41.
2. Peng, H. and Marians, K.J. (1995) The interaction of Escherichia coli topoisomerase IV with DNA. *J. Biol. Chem.*, **270**, 25286-25290.
3. Dong, K.C. and Berger, J.M. (2007) Structural basis for gate-DNA recognition and bending by type IIA topoisomerases. *Nature*, **450**, 1201-1205.
4. Burnier, Y., Weber, C., Flammini, A. and Stasiak, A. (2007) Local selection rules that can determine specific pathways of DNA unknotting by type II DNA topoisomerases. *Nucl. Acids Res.*, **35**, 5223-5231.
5. Vologodskii, A.V., Zhang, W., Rybenkov, V.V., Podtelezhnikov, A.A., Subramanian, D., Griffith, J.D. and Cozzarelli, N.R. (2001) Mechanism of topology simplification by type II DNA topoisomerases. *Proc. Natl. Acad. Sci. USA*, **98**, 3045-3049.
6. Cantor, C.R. and Schimmel, P.R. (1980) *Biophysical chemistry*. W. H. Freeman and Company, New York.