**ACCURATE PREDICTION OF DNA OPENING PROFILES BY PEYRARD-BISHOP NONLINEAR DYNAMIC SIMULATIONS**


1 Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA; 2Los Alamos National Laboratory, Los Alamos, NM, USA

* Corresponding author: e-mail: ausheva@bidmc.harvard.edu

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**Summary**

**Motivation:** The Peyrard-Bishop nonlinear model has proven to be an accurate predictor of the elastic properties of DNA. Through dynamical simulations, it is possible to gather statistical data on the local opening propensity of a limited sequence of double-stranded DNA. It is important to compare these computational results with experiment, to evaluate the usefulness of the Peyrard-Bishop model in a predictive capacity.

**Results:** Simulation and analysis of three linear DNA duplexes yields three distinct opening profiles by the Peyrard-Bishop model. Controlled digestion of radioactively-labeled templates by S1 nuclease, which selectively cleaves single-stranded DNA, shows an excellent correlation of the predicted openings with experimental data.

**Introduction**

It has long been known that double-stranded DNA is subject to temporary, localized openings of its two strands (Levitt, 1983). The available thermal energy in the system serves to destabilize the double helix structure at specific positions on the DNA sequence. These localized openings are significant enough in both size and duration to allow for chemical reactions to occur. NMR proton exchange measurements show that these openings may have lifetimes as long as 1 ms (Gueron, Leroy, 1995; Leroy et al., 1988). Theoretical studies have also supported this data, predicting significant distortions in the equilibrium structure (Giudice, Lavery, 2003; Levitt, 1983). These openings have been described in the physical literature as solitons or $\alpha$-premeltons, and it has been speculated that they may play a role in the sequence-dependent opening of double-stranded DNA and transcription (Banerjee, Sobell, 1983; Prohofsky, 1988). As these dynamic openings may be functionally important sites in DNA, it is advantageous to develop experimental assays and computer models which can recreate “opening profiles” for DNA fragments. Here we report the close correspondence of S1 nuclease cleavage assay results with highly accurate Peyrard-Bishop model dynamic simulations (Peyrard, Bishop, 1989).

**Model and Methods**

**S1 nuclease cleavage assays.** S1 nuclease is a member of a family of glycoprotein nucleases which has been shown to selectively cleave single-stranded DNA (Wiegand et al., 1975). It has been widely employed in studies of transcriptional systems. The bulkiness of S1 nuclease aids in selectively cleaving larger temporary openings over small openings, but it results in a very weak signal. We overcame this issue of sensitivity by incubating the dsDNA with S1 nuclease for a longer period of time than in usual assays (45 mins). The noncoding (lower) strand for the non-promoter control and the F5 promoter, and the coding (upper) strand for the AdMLP was [32P]-labeled at the 5'-terminus with T4 polynucleotide kinase (Invitrogen). The labeled strand and the unlabeled strand were then annealed by temperature cycling, and the dsDNA was used as a substrate for S1 nuclease cleavage. 0.2 nM dsDNA was incubated with S1 nuclease (50 units of enzyme...
per reaction) at 28 °C for 45 minutes in buffer containing 10 mM HEPES (pH 7.2), 50 mM NaCl and 4 mM Zn(C$_2$H$_3$O$_2$)$_2$, as recommended by the supplier (Roche). The reaction was stopped with 20 mM EDTA. After ethanol precipitation, the DNA digestion products were electrophoretically separated on a 10 % sequencing gel (National Diagnostics). A Molecular Dynamics Phosphorimager 400-B was used to document the results.

**Model.** A modified Peyrard-Bishop model (Dauxois et al., 1993) with optimized parameters was applied in Langevin dynamical simulations. The sequence was repeated on both ends of the fragment to avoid terminal base pair effects, effectively circularizing the DNA sequence without any torsional effects. Simulations were run on several Sun Blade workstations. 100 separate PB model realizations were simulated over a 1 ns timescale using 1 femtosecond intervals, yielding 10$^8$ data points per sequence. Instances of a 10 bp opening over a threshold value of 2.1 Angstroms were recorded, and the values at the central base pair are reported.

**Implementation and Results**

We have recently demonstrated that destabilized regions of double-stranded DNA fragments are sensitive to S1 nuclease digestion under mild conditions, and that opening profiles can be derived from these assays (Choi et al., 2004). We chose four DNA sequences to examine for our studies (Table). The S1 nuclease assay results vary with the DNA sequences tested (Fig. 1).

![Fig. 1. S1 nuclease cleavage assays of four tested DNA sequences. (a) 62 bp sequence randomly selected from the cDNA of human transcription factor IIB; (b) 86 bp sequence of the adenovirus major late (AdML) promoter; (c) 69 bp sequence of the adeno-associated viral (AAV) P5 promoter and an associated mutant sequence. Numbered base pair positions are labeled to the left of each panel, and the associated DNA marker ladders are shown in lane M.](image)

<table>
<thead>
<tr>
<th><strong>Table.</strong> Experimental sequences tested</th>
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<tr>
<td><strong>Name</strong></td>
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<tr>
<td>62 bp sequence from cDNA of hTFIIB</td>
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<tr>
<td>86 bp AdML promoter</td>
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<tr>
<td>69 bp AAV P5 promoter</td>
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<td>69 bp AAV P5 mutant promoter</td>
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Molecular dynamics simulations were run with these same DNA sequences, and the results compiled into a sequence-dependent opening profile. Comparison of the Peyrard-Bishop (PB) model-predicted opening profiles of the DNA fragments with the density profiles from these experimental assays shows a clear correspondence (Fig. 2).

**Fig. 2.** Close correspondence between PB model predictions and S1 nuclease experimental assays. PB model opening profiles plot base pair position versus recorded instances of large opening formation in simulations. (a) 62 bp non-promoter sequence; (b) 86 bp AdML promoter; (c) 69 bp AAV P5 promoter; (d) 69 bp AAV P5 mutant promoter.

**Discussion**

The Peyrard-Bishop model has been shown to accurately describe the denaturation of DNA, and experimental results support its predictions (Campa, Giansanti, 1998). We report that even for oligonucleotides of around 100 bp in length, the method precisely simulates DNA dynamical motion, recreating thermal fluctuational openings which are also observed through experiments with S1 nuclease. We conclude that the Peyrard-Bishop model stands as a valuable tool for the prediction of these phenomena in double-stranded DNA.
Acknowledgements

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References