The Restriction Enzyme BamHI is Poised for Sliding along DNA in the Nonspecific Complex

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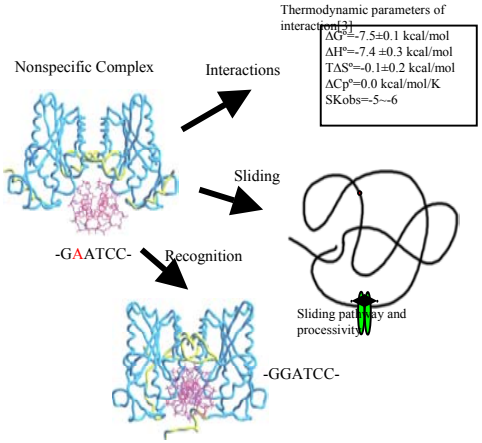
Introduction

How does the specific DNA-binding protein quickly find its cognate site within a long stretch of DNA? A "sliding" model has been proposed and tested in some type II restriction endonuclease enzymes, including BamHI [1,2]. According to this model, the long-range Coulombic interaction dominated in the nonspecific protein-DNA interaction makes protein stay close to DNA, where the protein performs one-dimensional random walk along the DNA. When the protein slides to its cognate site, the specific interaction between the protein and the DNA leads to the final tight specific complex, usually accompanied with structural changes in both protein and DNA.

The mechanism and the details of sliding are still unclear. For the BamHI-DNA system, the thermodynamic parameters of the interactions have been measured in the lab of Jen-Jacobson[3], but the structural basis for the nonspecific interaction in solution, and for sliding, remains unknown.

The breakthrough work in structural biology comes from Aggarwal's Lab[4]. The high resolution crystal structure of the nonspecific BamHI-DNA complex solved recently, in which BamHI binds to a 11 base-pair DNA with only one base pair different from its cognate site (GGATCC->GAATCC), reveals many features of the nonspecific interactions[4]. This structure fills in the gap between free BamHI and the specific BamHI-DNA complex.



Based on these experimental findings, we were interested in answering the following **questions**:

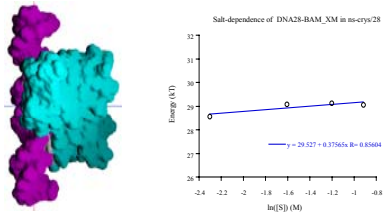
•Could the nonspecific structure account for the measured thermodynamic parameters of the nonspecific interactions?

•Could this structure constitute the structural basis for BamHI sliding along DNA?

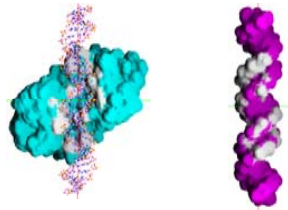
The **answer** to these questions is **No**!

Reasons for the Negative answer:

1. *Unfavorable electrostatic interaction and no salt-dependence for the nonspecific complex in the crystal structure:*
[calculated salt-dependence of electrostatic interaction in the model with 28mer DNA based on the crystal structure]



2. *Heat capacity change upon complexation is too large (expected to be negligible for the nonspecific complex):*
[estimation based on the calculated buried surface in the model]



ΔCp=0.32(±0.04)ΔA(np) - 0.14(±0.04)ΔA(pol)
ΔA=ΔA(np) + ΔA(pol) = 1600Å^2(np) + 1400Å^2(pol) = 3000Å^2
ΔCp≈-0.3 (kcal · Mol^{-1}K^{-1}) << 0.0 (kcal · Mol^{-1} K^{-1})

Questions:

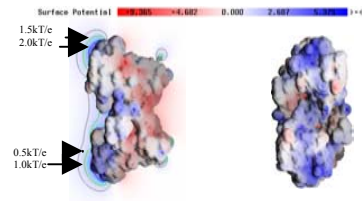
Q1. Is there an electrostatically favorable nonspecific complex with a different structure?

Q2. If so, what does the crystal structure of the nonspecific complex represent?

Answers:

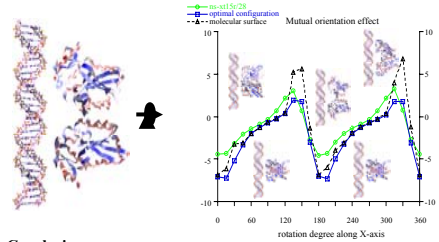
A1. Analysis of electrostatic (ES) potential surface of BamHI indicates that the most positive part of BamHI is located at the C-terminus. This is shown below.

Surface potential and iso-potential contours of BamHI

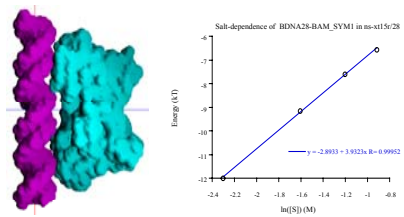


Consequently, a possible ES-favorable complex would position these two parts of BamHI dimer in close contact to DNA.

This hypothesis was tested by exploring the different mutual orientations of the nonspecific complex.

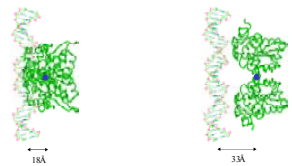


Conclusion:
An ES-favorable complex could be formed with the BamHI dimer axis parallel to the DNA axis, with the two C-termini pointing towards DNA. The calculated salt-dependence and SASA-based ΔCp calculations are in good agreement with experiments for such a complex.

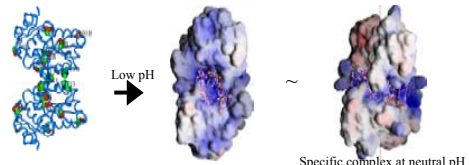


ΔA ≈ 20Å^2 ΔCp ≈ 0.0 (kcal/mol/K): Electrostatic interaction is favorable.

A2. The crystal structure model differs from the proposed ES-favorable model in two transformation steps. The BamHI dimer is moved away from the original position by 15Å, and rotated to be parallel to the DNA axis:

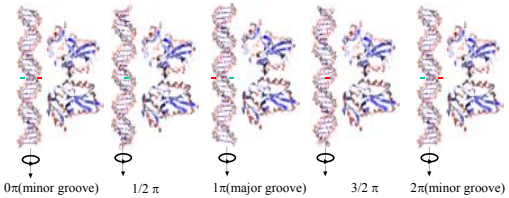


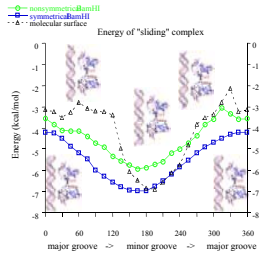
We find that the difference between these complexes is due to the low pH in the crystallization conditions.
Possible change of protonation state at low pH(4.8) from pKa calculation[7]:
His121/133, Asp196, Glu2/6/77/86/101/113



Conclusion:
The low pH shifts the most positive part of BamHI dimer from the C-termini to the center, where the DNA is located in the crystal structure of the nonspecific complex. In the specific BamHI-DNA complex (at neutral pH), the ES potential is maximally positive exactly where DNA is positioned in the crystal structure. Together these examples indicate the role of ES in the process of complexation.

Does the ES-favorable nonspecific complex model explain how BamHI slides along DNA?
Simulated parallel movement:



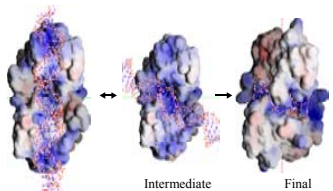


BamHI prefers to slide along DNA helical pitch smoothly.

Conclusions from this study:

• An ES favorable model of the nonspecific BamHI-DNA complex is proposed to represent the nonspecific interaction at neutral pH (as opposed to the low pH crystallization conditions). BamHI is positioned parallel to DNA with its center close to the minor groove. The model also accounts for the sliding pathway, in which BamHI follows the DNA helix pitch.

• In the search for its cognate site, BamHI may use an intermediate state with similar surface property (e.g., ES potential) as in the nonspecific crystal structure (see below), before the transition to the final specific complex.



The question still remains:
•How does one base-pair change in the DNA sequence lead to such a dramatic structural change of the complex (from the nonspecific to the specific)?

Method:

Electrostatic calculations with the Nonlinear Poisson-Boltzmann method in UHBD [5] based on the nonspecific crystal structure[4]. Atomic parameters are from CHARMM27 [6], dielectric constant is 4 (solute) and 78 (solvent), boundary between solute and solvent defined by vdW and solvent accessible surface.

References:

[1]Nardone, G, et al.(1986): JBC, 261(26), 12128-33
[2]Pingoud & Jeltsch (1997): Eur. J. Biochem. 246(1), 1-22
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[6]Foloppe & Mackerell (2000): J. Comp. Chem. 21(2), 86-104
[7]Mehler, EL(1996): J. Phy. Chem. 100(39), 16006-18