New insights on protein/DNA non specific interaction: the DNase I/DNA model

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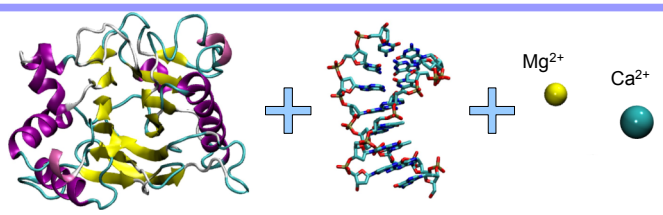
The molecular basis of protein/DNA non-specific interactions are often elusive.
DNase I/DNA system was chosen as a representative and rather simple model of non-specific complex.
DNase I is an enzyme that cleaves the phosphodiester backbone of the DNA double helix **in presence of Ca^{2+} and Mg^{2+}**
DNase I at low concentration cleaves the DNA phosphate linkages with **variable probabilities**.

Where are located the counterions? What is their role? Which DNA properties influence the cleavage efficiency?



DNase I and counterions

Identification of 4 counterion binding sites by Molecular Dynamics

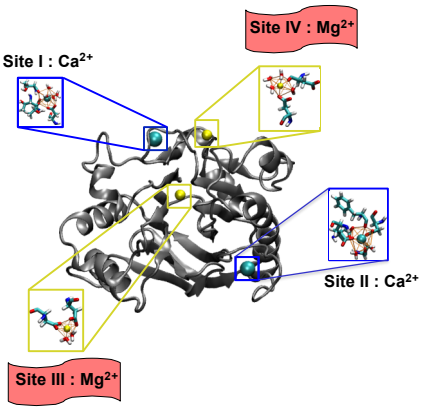
6 MD simulations
AMBER/Parm99
300K, 25 ns with

- ►Na^+
- ► Ca^{2+} and Na^+
- ► Ca^{2+} and Mg^{2+}

In all cases, 4 counterion binding sites were identified.

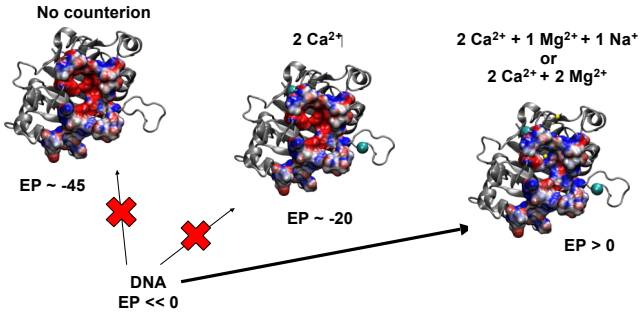
The best coordinations were obtained for

2 Ca^{2+} (sites I and II) and 2 Mg^{2+} (sites III and IV)



Electrostatic effect of counterions

Electrostatic potential (EP), Poisson Boltzmann : APBS methods [1] of the DNase I region corresponding to the DNase I / DNA interface.



Conclusion

DNase I activity requires both Ca^{2+} and Mg^{2+}. Two Ca^{2+} sites were previously observed by X-ray [2]. **MD simulations allowed to identify two additional sites coordinating Mg^{2+}.**

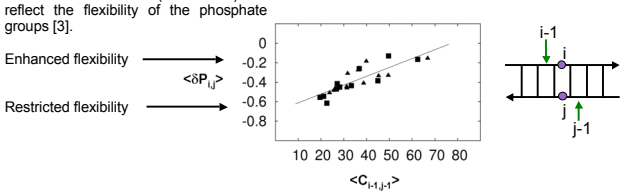
DNA is negatively charged. Thus, interaction with Dnase I needs a positive protein interface. The Dnase I interface is negative without counterion and remains repulsive in presence of two Ca^{2+}. A positive interface requires 4 counterions. **The presence of 4 counterions bound to Dnase I allows the interaction with the DNA**.

DNase I activity and DNA flexibility

DNase I cleavages and DNA flexibility

Two oligomers were
i) studied by NMR
ii) submitted to DNase I digestion,
cleavage activity being limited by the binding step.

The ^{31}P chemical shifts (δP in NMR) reflect the flexibility of the phosphate groups [3].

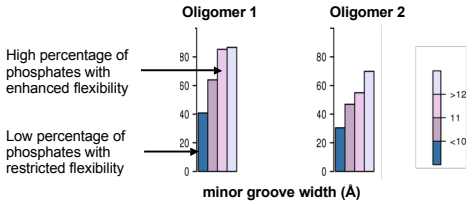


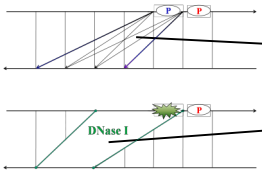
The δP of facing phosphates i and j are correlated to the cleavage intensities (C) of the phosphate linkages i-1 and j-1.

DNA flexibility and minor groove dimensions

DNase I fills the DNA minor groove, generating an enlarged minor groove [2].

Minor groove width and phosphate group flexibility are correlated in the NMR structures of free oligomers.





Flexible phophate linkages **P** enlarges the minor groove (width > 11 Å)

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in a large part of the region filled by the **DNase I** (minor groove width ~13 Å)

Conclusion

DNAse I activity is sensitive to the flexibility of the DNA: more flexible i,j dinucleotides make better i-1,j-1 substrates.

Flexible phosphates allow the minor groove to vary towards a shape favoring the binding of Dnase I.

[1] Baker NA et al. **PNAS**, 2001
[2] Oefner C and Suck D. **JMB**, 1996
[3] Heddi B et al, JACS, 2006