SAXS Studies of Counterion-induced Forces in Folding of Nucleic Acids

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Introduction

The massive charge of nucleic acids attracts a condensed layer of mobile counterions, the ion atmosphere, which is intimately involved in their biological and physical processes. However, unlike the site-bonded metals, this ion atmosphere is normally invisible in x-ray crystallographic pictures because it is dynamic. In addition, the many degrees of freedom and long range of electrostatic interactions make the nucleic acid-counterions complex hardly to be modeled by simple stoichiometric descriptions. All these render the understanding of the counterion atmosphere in nucleic acid systems extremely difficult.

Progresses have been made in direct "visualization" of ion distributions around DNA duplexes [1,2]. Nevertheless, the energetic effects of this atmosphere still remain unclear. The widely used non- Linear Poisson-Boltzman (NLPB) model can estimate the electrostatic energies for nucleic acid conformations in different ionic conditions [3,4]. However, this model is incomplete since it leaves out the finite sizes of ions and, of the central importance to this work, the ion-ion correlation effect. The latter, as suggested by recent theoretical work for strongly charged polyelectrolytes and multivalent counterions, can lead to correlation-induced attraction [5,6] owing to the self-rearrangement of the dynamic ion atmosphere. This effect cannot be described in the basic NLPB model, but may be integral to the biological behavior of nucleic acids, including DNA condensation [7] and "electrostatic collapse" in RNA folding [8,9].

To quantitatively probe the fundamental electrostatic forces, in the present studies we have employed a simple tethered DNA in which two DNA duplexes of defined length are connected by a neutral flexible linker (Fig. 1) to isolate the counterion-modulated interactions between two helices. The results provide estimates for repulsive forces in low concentrations of monovalent cations and an upper limit for any attractive force in the presence of multivalent cations.

Materials and Methods:

The tethered DNAs ($12_{/PEG9/}12$), which consist of pairs of duplexes (12base-pair) connected by neutral flexible linkers of polyethylene glycol (PEG9, $-(CH_2-CH_2-O)_3-$), were assembled by the equal-molar oligonucletide components (Qiagen Operon Inc) in desired salt solutions. The final DNA concentration ranged from 0.1 to 0.4 mM.

A control molecule mimicking the "collapsed state" (Fig.1), the circular DNA, was constructed by hybridizing a 32 nt enzymatically cyclized DNA oligonucleotide[10]and its two 12 nt complementary components.

The ion-mediated conformational changes of the tethered-duplexes in different ionic solutions were monitored by small angle x-ray scattering (SAXS) at Stanford Synchrotron Radiation Lab (SSRL) and the BESSRC-CAT 12-ID of the Advanced Photon Source.

SAXS profiles of individual tethered-DNA conformations were calculated as described previously [2]. The predictions of random, collapsed and extended states of the tethered DNA (Fig.1) were obtained by averaging the conformations in the corresponding ensembles described following: for the random ensemble, two duplexes were allowed to take random orientation and positions, with tethered modeled as freely-jointed polymer chain. Conformations causing van der waals surfaces overlap between any pair of atoms were excluded and sampling size was varied from 10^3-10^4; the collapsed state ensemble was generated in the same way above except that only conformations with the distances between the tether attachment points of the

two duplexes at both ends within 22Å (the length of 4 T's) were retained; for low salt extended ensemble, the populations of each conformation in the random ensemble were weighted by a Boltzmann distribution determined by the electrostatic energy calculated for each conformation using NLPB solver Delphi [11].

Results

SAXS is a proper approach to distinguish between the different conformational states of the tethered duplexes. Our control studies showed that different shape molecules, an rod-like 24 bp continous duplex and a cicular DNA that is a mimic of the collapsed state (Fig.1) have clearly distinguishable SAXS profiles that agree with predictions very well. Fig.2B

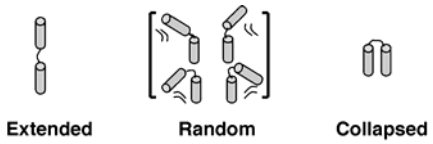

Fig.1 The models of tether-duplex DNA ($12_{/PEG9/}12$) in three conformational states distinguished in this study.

Applying the tethered DNA molecules in different salt conditions, we find that, at low concentrations of monovalent cations (10-20 mM Na^+), the tethered 12 base-pair duplex ($12_{/PEG9/}12$) gives a SAXS profile expected from a model of the extended state (Fig. 2A), thus indicating that screening is not sufficient to allow entropic disordering to overcome the Coulomb repulsion. At high Na^+ concentrations (>1 M), on the other hand, we find a profile that, after correcting for counterion scattering, is consistent with a fully relaxed family of entropically disordered conformations. Cations of higher valence have been predicted to give counterion-induced attraction, so SAXS profiles were collected for the tethered DNA

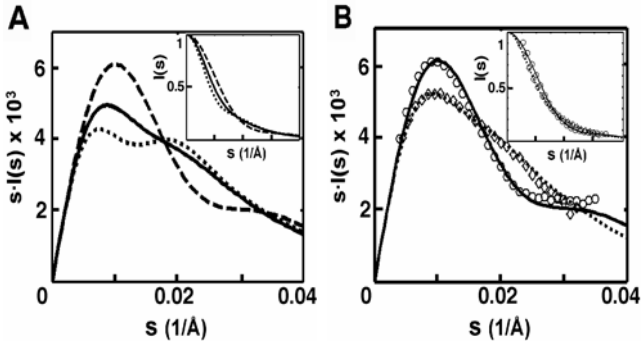

Fig.2 Monitoring conformational states of the tethered DNA duplexes by SAXS. (A) Predicted profiles of extended (···), random (—) and collapsed (---) states of 12bp tethered DNA ($12_{/PEG9/}12$). Scattering intensity *I(s)* has been multiplied by the scattering angle *s* to help illustrate profile differences; the inset shows the unweighted profile with intensity normalized to unity at *s*=0. (B) Experimental SAXS profiles of 24 bp duplex DNA (◊) and circular DNA(o) compared to the predicted profiles of duplex (···) and circular DNA (—). SAXS data were obtained with 0.2 mM DNA in 1.2 M NaCl, 100 mM MOPS, pH=7.0

in the presence of high concentrations of Mg^{2+}, Putresine^{2+} (data not shown), spermidine^{3+}, Co[NH3]6^{3+}(data not shown) and spermine^{4+} (data not shown). In all cases we find the SAXS profile is identical to that expected for a fully relaxed molecule, as in Na^+, and distinct from the collapsed spectrum (Fig.3). Extending these measurements using a double duplex with 80 base-pair helices gives the same result.

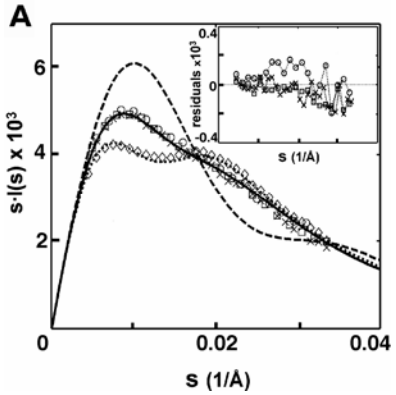

Fig.3: Experimental SAXS profiles of the tethered DNA construct ($12_{/PEG9/}12$) in different salt conditions: 20 mM Na^+ (◊), 1.2 M Na^+ (□), 0.6 M Mg^{2+} (o), and 75 mM spermidine^{3+} (x), compared to the predicted SAXS profiles for the extended (···), random (—) and collapsed (---)states. Inset: Residuals of experimental SAXS profiles compared to the random state prediction, plotted against *s* from 0-0.04 (1/ Å).

These results can provides quantitative estimates and limits for repulsive and attractive electrostatic forces between the two DNA helices, because the SAXS profile for the tethered DNA can be readily calculated by applying Boltzmann weighting (from the assumed inter-helical potentials) to the random ensemble (Materials and Methods). We employed two models to describe the inter-helical potentials:

1) a pairwise Yukawa potential was used as a general functional form for the repulsion, or the effective attraction mediated by ions, between two phosphates:

$$\Delta G(r)=\Delta G^{0}_{p\text{-}p}\cdot a\cdot e^{-(r-a)/\lambda}/r \qquad [1]$$

where r is phosphate-phosphate distance, a is the minimal approach between phosphates (4 Å), and λ is the scale of the potential. The overall inter-helical potential of a given tethered DNA conformation is then the sum of potentials of all pairs of phosphates:

$$\Delta G_{DNA\text{-}DNA}=\Sigma_{ij}\Delta G_{ij}=\Sigma_{ij}\,\Delta G^{0}_{p\text{-}p}\cdot a\cdot e^{-(rij-a)/\lambda}/r_{ij} \qquad [2]$$

2) As the ion-induced attractive forces have been suggested to be highly orientation-dependent [12-13], an "orientation constrained model" was applied to model the geometrical constraints of the hypothetical attractive forces and to obtain estimates of the upper limit of attraction in high salt. In this model, we classified the molecules that have the dihedral angle and center-mass distance between two helices smaller than defined cutoffs as "collapsed"; the remainders are "non-collapsed". We then assumed a constant attractive potential existing between the helices for "collapsed" molecules

With Yukawa potential model we estimated the repulsion in low salt (10-20 mM Na^+) to be 2.1-4.6 kT/bp. Upper limits for the putative attractive force of 0.1-0.2 kT/bp were calculated using both Yukawa and "orientation constrained model"[14].

Discussion

The results thus far indicate that SAXS of model systems can be adopted as a powerful probe of nucleic acid electrostatic forces, that repulsive forces dominate at low monovalent concentrations (concentrations used in many RNA folding studies), and that counterion-induced attractive forces are not strong enough to overcome the thermal entropy in our test samples such that it's unlikely to be the "driving force" of nucleic acid collapse. The question of their importance in stabilizing the natively folded states of structured RNAs remains a subject for further study. This work has been written up and accepted for publication in PNAS [14].

Acknowledgments

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