SAXS Studies of the Primary Forces Involved in RNA Folding

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Introduction

A comparison of the folding mechanisms of two rather different classes of biopolymer — RNA and proteins — illuminates general physical principles of biopolymer folding. Many small globular proteins have been shown to fold by a two-state mechanism. In contrast, recent biophysical elucidations of the folding pathways of large ribozymes have advanced a description of RNA folding as a quasi-hierarchical process, often hindered by kinetically stable, misfolded intermediates. Thus, from a physical point of view, an outstanding challenge remains: to characterize the fundamental forces that guide RNAs along their folding pathways to their native structures and to distinguish these folding mechanisms from those of proteins.

Methods, Materials, and Results

The thermodynamics and kinetics of RNA folding are dominated by the very large repulsive forces between the RNA secondary structure segments that need to come together in the fully folded molecule. Folding is achieved in the presence of positively charged counterions needed to screen the Coulomb repulsion. In natural systems, the counterions used for this screening tend to be doubly charged Mg^{2+} ions.

In the present studies, we have used small-angle x-ray scattering (SAXS) at the BESSRC beamline at the APS to characterize the role of counterions in RNA folding in two systems: the group I ribozyme from *Tetrahymena thermophila*, and a series of small "double-duplex" model compounds that consist of pairs of DNA duplexes connected by neutral flexible linkers of polyethylene glycol.

SAXS Studies of the Folding of the *Tetrahymena Ribozyme*

The group I ribozyme from *Tetrahymena thermophila* studied by several laboratories as a model RNA is the best characterized large RNA with respect to catalytic function and 3-D structure. The native fold of this 400-nucleotide ribozyme consists of several double-helical segments whose global arrangement is mediated by sequence-specific tertiary contacts between single-stranded loops and bulges. The ribozyme's conformation and catalysis are intimately coupled to metal ions, with folding experiments usually initiated by the addition of divalent magnesium ions. The majority of cations bound to the ribozyme are not localized to specific RNA sites but instead form a highly dynamic counterion "atmosphere" whose properties are a strong focus of the present experimental study.

In order to assess the importance of counterion-induced interactions relative to those involved in tertiary interactions, we made time-resolved SAXS measurements of the folding of the native ribozyme and of a quintuple mutant in which the tertiary hydrogen bonds stabilizing the native fold were eliminated (Fig. 1).

As occurred in previous studies, there was a rapid initial compaction to a partially collapsed state that occurred at a time scale of 10 ms. We have now found this in the folding time-courses for the wild-type tetrahymena ribozyme and for the quintuple knockout mutant of the ribozyme (each initiated by the addition of 10 mM Mg^{2+}). Following this initial partial collapse, a second phase of collapse to a globular state occurs at a time scale on the order of 100 ms for the wild-type ribozyme. Remarkably, however, this second phase is not observed in the folding pathway of the quintuple knockout mutant. This result implies that the second phase is promoted by at least one RNA sequence motif associated with a native long-range tertiary contact, even though all of these contacts appear to be solvent-accessible on the 100-ms timescale in hydroxyl radical footprinting experiments carried out under similar conditions.

The initial collapse of the RNA is also induced by high concentrations of Na+ and the bulkier $[N(CH_3)_4]^+$, suggesting that the data are consistent with the earliest RNA partial collapse being an electrostatic relaxation of the initially extended RNA structure due to enhanced charge screening. In the initial conditions of our experiments (20 mM Na^+), Coulomb repulsion between

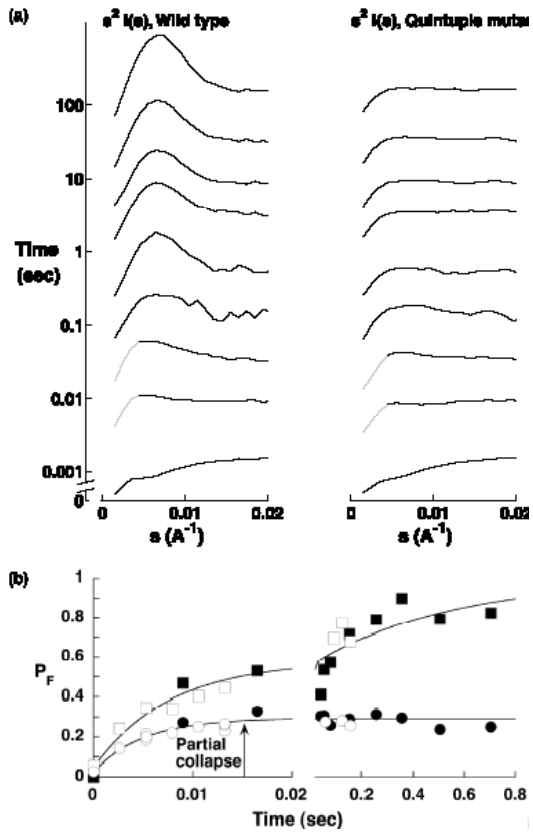

FIG. 1. Global shape changes during folding of the Tetrahymena ribozyme initiated by the addition of $Mg2^{+}$. SAXS profiles obtained for the wild-type molecule (a) and for the tertiary-contact-knockout mutant (b) are displayed as Kratky plots.

RNA double helices will not be screened effectively by the monovalent atmosphere. The initial RNA structure should therefore consist of double helices splayed away from each other. Such a structural model is in agreement with the SAXS profile of the unfolded state. The addition of either multivalent ions or ~1 M monovalent ions is expected to effectively screen interhelical repulsion, thereby allowing the initial conformation to relax to a more compact dynamic conformational ensemble. The observed 15 ms time points from each of these time-courses are indeed consistent with the SAXS profile predicted for a random conformational ensemble. These results have been published [1].

SAXS Studies of Counterion Shielding of a Simplified Model DNA System

To quantitatively probe the fundamental forces that can stabilize the folded state of RNA molecules, we have performed equilibrium measurements on a set of "double-duplexes." They consist of pairs of DNA duplexes connected by neutral flexible linkers of polyethylene glycol.

The models were designed to isolate electrostatic forces. As these model nucleic acids do not contain specific ion-binding sites or tertiary interaction motifs, the dependence of their thermodynamic behavior on counterion charge and concentration provides a direct measure of the effects of counterions on the electrostatic forces between the duplexes.

Theoretically, we can think of the system as having three distinct thermodynamic states: a fully extended conformation, a fully collapsed state (where the two DNA duplexes are in close contact), and a disordered state (where the two duplexes are oriented at an arbitrary relative angle). The occupancy of these states in equilibrium will depend on the balance between the interduplex force and the entropy of disordering the relative angle between the duplexes (see Fig. 1). In addition, counterion-induced *attractive* forces — in which the spatial correlation between counterions yields a net attraction (which may also be thought of in terms of salt bridges, where, for instance, a Mg^{2+} ion may link two phosphates) — have been demonstrated theoretically and observed in more complex systems. Thus, the basic goals of this project are to quantitatively evaluate the attractive and repulsive electrostatic forces in nucleic acid systems and to describe these forces computationally.

From our measurements, we find that at low concentrations of monovalent cations (10-20 mM Na^{+}), the double 12-base-pair duplex with a short PEG linker gives a SAXS profile expected from a model of the extended state, 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 is, after correcting for counterion scattering, 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 double duplex in the presence of high concentrations of a series of divalent, trivalent, and tetravalent cations. In all cases, we found the SAXS profile to be identical to that expected for a fully relaxed molecule, as in Na^{+}, and distinct from the collapsed spectrum. Extending these measurements by using a double duplex with 80-base-pair helices gives the same result. This measurement thus provides an upper bound on the strength of any counterion-induced attractive forces. (A manuscript on this work is in preparation.)

Acknowledgments

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