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Effect of elastic energy on the folding of an RNA hairpin

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HIGHLIGHTS

- We use a zipping model to analyse the effect of elastic energy on the folding of an RNA hairpin.
- Isotonic unfolding occurs at a well-defined critical load.
- Isometric unfolding produces a series of sharp tension peaks as the hairpin unzips base by base.
- Series compliance broadens the tension peaks and produces hysteresis.
- A stiff force transducer and handles could improve the resolution of single-molecule experiments.

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ABSTRACT

We analyse the folding and unfolding of an RNA hairpin using a conventional zipping model that includes both the free energy for RNA binding and the elastic free energy of the system. Unfolding under isotonic conditions (where we control the applied load) is known to occur at a well-defined critical load. In marked contrast, we find that unfolding under isometric conditions (where we control the extension of the hairpin) produces a series of sharp peaks in the average load as the stem of the hairpin starts to unzip base by base. A peak occurs when the elastic energy stored in the unzipped arms of the hairpin becomes so large that it is energetically favourable for the next base pair to unzip: the consequent increase in the contour length of the unzipped arms reduces their elastic energy and causes the average load to fall abruptly. However, as the contour length of the unzipped arms increases, the peaks become less distinct. Moreover, when we include the long DNA/RNA handles that have been used in single-molecule experiments, the unzipping of individual base pairs cannot be resolved at all. Instead, with the hairpin in the folded state, the average load increases with extension until the elastic energy stored in the handles makes it energetically favourable for the hairpin to unzip over a narrow range of extensions. The resultant yield point produces a mechanical hysteresis loop with a negative slope, as observed experimentally. Unfolding of the hairpin is also affected by the elastic energy stored in a compliant force transducer. We find that short, stiff handles and a stiff force transducer could improve the resolution of mechanical experiments on single molecules.

1. Introduction

In recent years, single-molecule experiments have made it possible to study the folding and unfolding of individual RNA hairpins (Liphardt et al., 2001; Collin et al., 2005). These experiments provide a model system for exploring how a macromolecule folds into the three-dimensional structure that is required for its correct biological function. RNA hairpins therefore allow us to study the detailed dynamics of macromolecular folding. Moreover, one might wonder if the conformational change produced by hairpin formation could usefully be exploited in a molecular nanomachine. If so, then understanding the folding and unfolding of RNA hairpins might contribute to the development of nanotechnology.

Prior to the advent of single-molecule experiments, folding was generally studied using macromolecules in solution (Fersht, 1999). In its unfolded state, a macromolecule forms a disordered random coil with a high entropy. In contrast, the folded state is more ordered and has a lower entropy. Hence, for the folded state to be thermodynamically stable, it must have a binding energy that is large enough to offset its lower entropy. Unfolding of a macromolecule in solution is therefore a transition from an ordered to a disordered state that is akin to the melting of a crystal.

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In single-molecule experiments, the macromolecule (such as an RNA hairpin) is held under tension by optical tweezers or an atomic-force microscope (AFM). Hence, in addition to the entropy and binding energy, we must also take account of the elastic energy of the system. In this paper, we demonstrate the effect of that elastic energy on the folding and unfolding of an RNA hairpin. We find that the effect is strikingly different, depending on whether we fix the applied load (isotonic conditions) or the length of the sample (isometric conditions). This is important in understanding the origin of the hysteresis observed by Liphardt et al. (2001) and Collin et al. (2005). Furthermore, since elastic energy is fundamental to the operation of molecular motors like actomyosin (Thomas and Thornhill, 1998) and kinesin (Thomas et al., 2002), it is clear that we must understand its effect on folding before we can design or analyse folding-based nanomachines.

Our theoretical analysis of the folding and unfolding of an RNA hairpin uses a conventional 'zipping' model that includes both the free energy for RNA binding and the elastic free energy of the system. This model, which is described in the following section, was originally introduced by (Bockelmann et al., (1997; 2002)) and further developed by Cocco et al. (2003), Gerland et al. (2003), Liu and Ou-Yang (2005) and Vieregg and Tinoco (2006). Our contribution here is to demonstrate the crucial role played by elastic energy in this zipping model.

2. Zipping model

Fig. 1a illustrates states in the zipping model for the folding of an RNA hairpin that consists of a tetra-loop and a stem containing N complementary base pairs. State 0 represents the unzipped RNA hairpin, which at zero tension forms a random coil. The first step in folding the hairpin is assumed to be loop formation (Zhang and Chen, 2006), which Bonnet et al. (1998) found to be the rate-limiting step for the folding of DNA hairpin-loops. In state 1, the RNA tetra-loop that has formed is closed by binding together the first base pair on the stem. The remaining base pairs along the stem then bind sequentially to form states 2 to N. Unfolding the hairpin reverses the sequence of transitions, as indicated in Fig. 1a. Hence, the RNA hairpin opens and closes like a zip fastener.

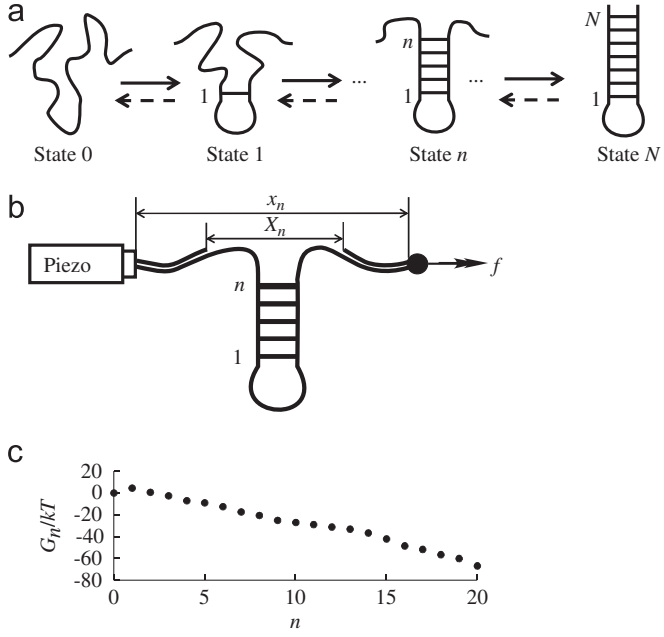

Fig. 1. (a) Schematic representation of states 0 to N in the zipping model for the folding of an RNA hairpin. (b) An RNA hairpin (not drawn to scale) with extension X_n in the partially folded macrostate n is attached by DNA/RNA handles to a piezoelectric actuator and a force transducer that measures the applied load f. The total extension of the hairpin and handles is x_n. (c) Standard free energies G_n at 25 °C for successive states of the CD4-siRNA hairpin studied by Collin et al. (2005), calculated as described in the appendix.

Note that each 'state' in the zipping model actually represents a macrostate in which the unzipped arms of the hairpin make extremely rapid transitions between many different conformations. The unzipped arms therefore possess conformational entropy. However, as the hairpin folds, the unzipped arms become shorter and their conformational entropy decreases. For the folded hairpin to be thermodynamically stable, the increase in free-energy due to the loss of conformational entropy during folding must be offset by a decrease in free-energy from forming chemical bonds between base pairs in the stem.

Fig. 1b shows the RNA hairpin in the partially folded macrostate n, where the tetra-loop has formed and the first n base pairs are bound. As in single-molecule experiments (Liphardt et al., 2001; Collin et al., 2005), we assume that complementary strands of DNA have been used to attach one arm of the RNA hairpin to a force transducer and the other arm to a piezoelectric actuator. One can therefore control either the applied force f or the overall length x of the hairpin and the DNA/RNA handles.

To analyse RNA folding, we must take account of both the free energy for binding the hairpin and the elastic free energy of the system (Bockelmann et al., 1997; Bockelmann et al., 2002; Vieregg and Tinoco, 2006). The partition function for the system at temperature T at constant pressure may therefore be written as

$$Z = \sum_{n=0}^{N} z_n \exp[-G_n/kT] \tag{2.1}$$

where G_n is the Gibbs free energy of the partially folded hairpin in macrostate n and z_n is the partition function for the handles and the unzipped arms of the hairpin, which we may write as

$$z_n = \exp[-G_{el,n}/kT] \tag{2.2}$$

where $G_{el,n}$ is the elastic Gibbs free energy of the handles and the unzipped arms. Both G_n and $G_{el,n}$ are measured relative to the free energy of a random coil in solution at zero tension. The experimentally determined G_n, which we refer to here as the "binding energy", therefore includes a $T\Delta S$ term arising from the conformational entropy of the random coil. This positive physical contribution to G_n counteracts the negative chemical contribution due to bonding of the base pairs. For an RNA hairpin, other workers use term "folding energy" rather than "binding energy" for G_n. However, the elastic free energy $G_{el,n}$ may also be regarded as a form of "folding energy" for an unzipped chain. We therefore prefer to use the terms "binding energy" (which is independent of the applied load) and "elastic energy" (which depends on the applied load) to distinguish between the two important energies in the zipping model.

Note that we use Gibbs (rather than Helmholtz) free energy in Eqs. (2.1) and (2.2). The partition functions Z and z_n are therefore 'isobaric–isothermal' partition functions (Tuckerman, 2010), which are appropriate for a system at constant pressure p and constant temperature T. They take account of any $p\Delta V$ work done by the system on the surroundings during folding, which may entail a small volume increase ΔV.

It follows from Eqs. (2.1) and (2.2) that the equilibrium probability p_n for finding the system in macrostate n at temperature T is

$$p_n = \exp[-(G_n + G_{el,n})/kT]/Z \tag{2.3}$$