Pulling apart DNA and RNA

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1 Introduction

Measurements of individual molecules are necessary for an understanding of many biological processes. The properties of DNA and RNA strands are particularly important, since these molecules are central to the function of all organisms. In the past five years, several experiments have begun to measure the forces holding these molecules together — by pulling them apart, using optical tweezers, piezoelectric actuators, or atomic force microscopy. These experiments demonstrate various properties of single-stranded DNA [3], single-stranded RNA [2], and double-stranded DNA [1, 3]. As it improves, the technique will be used more and more for its ability to quickly reveal information about specific DNA and RNA sequences.

2 Features of double-stranded DNA

As discussed in class, Bockelmann et al [1] performed an experiment in which the two strands of a large double-helix of DNA were repeatedly pulled apart and allowed to join together again. One strand is connected to a movable microscope slide, and the other to the end of a flexible lever, and a precise measurement of the deflection of the lever allows the tension applied to the DNA to be determined. The measurements agree very well with a simple theoretical calculation similar to the one described in class: if the local densities of G-C and A-T pairs is known, the force profile can be roughly predicted. This analysis is based on two assumptions:

1. There are just two relevant binding energies: $E_{\text{A-T}}$ and $E_{\text{G-C}}$ — interactions of neighboring strands are unimportant.

2. The total free energy in the system can be analyzed as a sum of independent contributions: the base pair binding energy, the single-stranded DNA coiling energy, and the lever deflection energy. There must be no significant interactions between these separate parts.

The experiments on single-stranded DNA by Rief et al [3] (described in Section 3) reveal a little more about the validity of these assumptions. Before discussing their results in this area, however, we will introduce the first part of their experiment, which is a completely different way of melting the two strands of a DNA double-helix. They attached *both* strands of one end of the helix to a substrate, and pulled on *one* strand of the other end with the tip of an atomic force microscope. This one strand gradually straightened out under tension, stretching the bonds between it and the second, unattached strand. A sharp transition was seen at force of 30–70pN, interpreted as the breaking of individual H-bonds holding the two strands together. Beyond this transition, the second strand does not, however, detach from the first: it is assumed to relax into a less-stretched helix where it can again form H-bonds with the first strand. At forces of 150-350pN, another phase transition is usually observed: *this* is interpreted as the melting of the double-helix.

The most important thing shown by this different method of pulling on double-stranded DNA is that the forces required for both transitions are sequence dependent. The wide range of critical forces mentioned above corresponds to the difference between $\begin{matrix}\mathrm{GCGCGC}\\ \mathrm{CGCGCG}\end{matrix}$ and $\begin{matrix}\mathrm{ATATAT}\\ \mathrm{TATATA}\end{matrix}$ DNA, respectively the most strongly and most weakly bound DNA used in the experiment. Since the strands do not unzip gradually, however, the required forces can probably only be compared with the average concentrations of the two types of base pairs across the whole segment of DNA. The critical forces will be mostly independent of the order of the G-C and A-T pairs.

3 Single-stranded RNA and DNA

Single-stranded DNA can also form helices, by binding to itself like RNA. In the study by Rief et al, one strand of a double-stranded $\begin{matrix}\mathrm{GCGCGC}\\ \mathrm{CGCGCG}\end{matrix}$ or $\begin{matrix}\mathrm{ATATAT}\\ \mathrm{TATATA}\end{matrix}$ DNA molecule was stretched until the tension induced on the other strand was sufficient to partially melt the double-helix. Because of the self-complementarity of its sequence, the free strand then bound to itself, folding into a helical hairpin structure like that of RNA. Releasing the tension

on the first strand then caused it to also form a helical hairpin, and the researchers could measure unzipping forces for this single strand of DNA. Their measurements of the forces holding base pairs together agreed well with [1], despite the very different approach. This is very strong evidence that the two experiments are actually measuring the local unzipping force and are not corrupted by the tertiary structure of the DNA or the experimental apparatus, confirming the assumptions mentioned in Section 2.

Liphardt et al [2] performed a similar study on short sequences of single-stranded RNA. Since RNA comes naturally in single-stranded units, no melting of strands was necessary, and the researchers could simply attach opposite ends of one RNA strand to glass beads, and stretch it out between an optical trap and an piezoelectric actuator. The three different RNA sequences used all held together up to a force of 10–20pN, similarly to DNA. However, differences between RNA and DNA were seen in two main ways: First, through measurements of hysteresis, a junction of three separate helices was shown to fold more slowly than a similar single helix. The extra zipping time is probably required for the RNA strand to "discover" this more complicated conformation. Second, in a more complicated strand of RNA, tertiary interactions with magnesium ions caused the structure to become more stable, but "brittle", and created an extra, fairly stable intermediate state between the zipped and unzipped conformations. When a constant near-critical force was applied to this strand, it was measured to hop continuously between the zipped, unzipped, and intermediate states. The experiments on these two strands of RNA clearly show that its complicated structure leads to a much richer response to applied forces.

4 Future uses for pulling

Pulling apart single molecules is a simple technique that may become very inexpensive and widely used, as its applications are demonstrated.

In the near future, the most important use for DNA pulling will be in DNA sequencing, since pulling enables hundreds of thousands of base pairs to be quickly surveyed. While this survey does not reveal the precise sequence of the DNA, it gives a view of the sequence on a much larger scale than can be seen with any other method, and will allow local, precise sections of the sequence to be accurately joined together. Experiments similar to [1] and [3] will be necessary for refining estimates of base-pair binding energies, so that

the technique can become accurate enough for this application.

Pulling apart RNA can give us information about its actual secondary and tertiary structure, which is important because it can't be easily found any other way. As discussed in class, various methods have been applied to the problem of predicting RNA structure, but the most successful technique remains X-ray crystallography. It is clear that any easily obtained bits of evidence about the structure would be welcome here; experimental measurements of single RNA molecules similar to [2], once refined, will provide a unique and helpful viewpoint. It is possible that the combined attack of this and other inexpensive techniques will become the most widely used method of RNA structure determination, and a similar attack may soon be applied to proteins.

References

[1] U. Bockelmann, B. Essevaz-Roulet, and F. Heslot. DNA strand separation studied by single molecule force measurements. *Phys. Rev. E*, 58(2):2386–2394, August 1998.

[2] Jan Liphardt, Bibiana Onoa, Steven B. Smith, Ignacio Tinoco Jr., and Carlos Bustamante. Reversible unfolding of single RNA molecules by mechanical force. *Science*, 292:733–737, April 2001.

[3] Matthias Rief, Hauke Clausen-Schaumann, and Hermann E. Gaub. Sequence-dependent mechanics of single DNA molecules. *Nature Struct. Biol.*, 6(4):346–349, April 1999.