Physical Limits on the Mechanical Measurement of the Secondary Structure of Bio-molecules

1995 Europhys. Lett. 31 335

(http://iopscience.iop.org/0295-5075/31/5-6/015)

EUROPHYSICS LETTERS 10-20 August 1995

Europhys. Lett., **31** (5-6), pp. 335-340 (1995)

Physical Limits on the Mechanical Measurement of the Secondary Structure of Bio-molecules.

R. E. THOMPSON and E. D. SIGGIA

Cornell University Laboratory of Atomic and Solid State Physics
Ithaca, NY 14853-2501, USA

(received 7 March 1995; accepted in final form 5 July 1995)

PACS. 87.15By – Structure, bonding, conformation, configuration, and isomerism of bio-molecules.
PACS. 87.15He – Molecular dynamics and conformational changes.
PACS. 05.40+j – Fluctuations phenomena, random processes, and Brownian motion.

Abstract. – The forces and lengths needed to mechanically denature a protein or nucleic acid are within the capabilities of the Atomic-Force Microscope. However, thermal fluctuations in the filament between the partially unfolded molecule and the force cantilever attenuate any measure of secondary structure on a length $\sim (2/\pi^2)(\varepsilon/k_B T)^{3/2}(aA)^{1/2}$, where ε is the energy required to liberate a length a of filament whose persistence length is A. Hence individual base pairs cannot readily be resolved but the stem-loop structure of RNA should be.

It has recently become possible to measure the force and step size of a single molecular motor [1], the entropic elasticity of λ-phage DNA [2,3], and the rupture force of various intermolecular complexes [4,5], by ingenious micromechanical techniques. Piconewton forces were exerted via optical traps, precisely calibrated hydrodynamic drag, and conventional atomic-force microscopy (AFM); the method of choice depending on the technicalities of attaching molecules to probe and the position sensitivity desired. These and other experiments on single large biomolecules are in a «classical mesoscopic» regime where thermal effects are paramount, yet the entities involved can be treated/parameterized with a few lumped degrees of freedom or by continuum elasticity. Ratchet motors [6] and an analytic treatment of supercoiled DNA [7] are examples of how old physics will find new applications in this regime. Biologists on the other hand will apply these technologies to probe a myriad of intracellular dynamic processes such as vesicular transport, processive enzymes (polymerases), protein-DNA interactions etc. It is hoped that micromechanical techniques will have the same discrimination and selectivity as patch clamping which allowed the study of single-ion channels [8].

In this article we consider the feasibility of descending one level in scale and ask what are the basic physical limitations on the mechanical measurement of the secondary or tertiary structure of large biological molecules. For instance can one pull apart the two strands of double-stranded (ds) DNA and see an oscillation in the measured force as the base pairs unzip and unstack [9]? Might one even sequence DNA this way since the binding free energy of

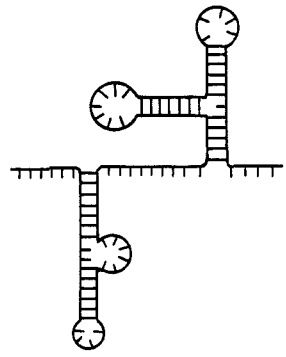

Fig. 1. – Schematic representation of an RNA molecule. The stems subject to the external force can snap in any order.

sequential pairs of base pairs (bp) varies from $\sim 1.5 k_B T$ for AT/TA to $6 k_B T$ for GC/CG [9, 10]?

A both more feasible and interesting system appears to us to be RNA [11, 12]. Biologically it combines the information storage aspects of DNA with the structural and catalytic functions of proteins. Its structure is also intermediate in complexity between DNA and proteins since it is single stranded and undergoes internal base pairing to form its «stem-loop» secondary structure. Finally one can imagine mechanically denaturing a protein.

Since our interest here is merely whether one will get a measurable signal, we model the energy of the DNA/RNA as a generic step-like function of the length, l, of single-stranded (ss) product as

$$E_0 = \varepsilon' \sin^2(\pi l/a) + \varepsilon l/a \,. \tag{1}$$

For DNA, we take $a \sim 0.66$ nm which is twice the height per bp in ds DNA. This underestimates a if both ss filaments are free to fluctuate, since the molecule lengthens as it unstacks, while it is an overestimate if some means can be found to pin one of the strands. For ε we take an average value of $3kT$ and in numerical examples chose $\varepsilon' = \varepsilon/\pi$ to make the minimum of $\partial E_0/\partial l = 0$ (1). For RNA we imagine resolving merely the internally paired stretches (stems), which we proceed to idealize as 10 bp which break as a unit; so we multiply ε and a by 10 [12]. We ignore the complexity of real RNA, which will have stems in both parallel and series, fig. 1, so that two partially denatured stems can coexist.

Our model for the measurement apparatus is shown in fig. 2. The force is measured via the deflection of a linear cantilever with spring constant k which can readily be adjusted in the range 1–100 nm^{-2} ($k_B T = 1$ henceforth) [14]. Displacements below the level of the thermal r.m.s. fluctuations in the cantilever, $1/\sqrt{k}$, can be measured by time averaging the Brownian motion.

The most serious impediment to the mechanical denaturation experiments under discussion is not the AFM technology *per se* but the extreme floppiness of the ss filament linking the cantilever to the intact molecule. The entropic elasticity of ds DNA is accurately modeled [3] as an inextensible tube with a bending energy $E_b = (1/2)A \int \kappa^2 \mathrm{d}s$, where κ is

(1) Experimental support for our model parameters is provided by Smith *et al.* [13], who report an abrupt unstacking transition for DNA along with a length change by a factor of 1.85.

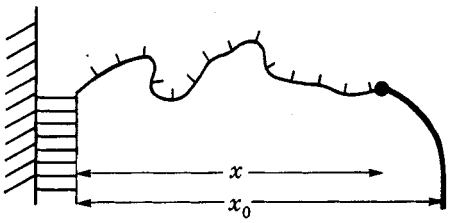

Fig. 2. – Schematic of the force experiment computed in eq. (3). The «stair case» energy (1) is represented by the ladder on the left. The bases on the ss filament, ticks, are spaced by a, and the base of the cantilever (heavy line) is positioned at x_0. Its deflection is $(x - x_0)$.

the local curvature, s is the arclength, and the correlation length $A \sim 50$ nm under normal ionic conditions [14]. Much less is known, quantitatively about ss DNA but because the backbone zig-zags, «bending» occurs via bond rotation, a freedom not present in ds DNA. A force measurement, fit to the freely jointed chain model, gave a segment size, $2A$, ~ 0.3, below the monomer size of ~ 0.5 nm [5]. However, small internal RNA loops seem to be disfavored by more than the absence of the usual base pairing would account for, which argues for a larger A [15], as do numerical simulations [16]. The mobility of ss DNA in gels can also be interpreted as favoring a many bp long persistence length (2). We use a compromise value $A_{ss} = 0.5$ nm in numerical estimates that follow. Use of the elastic energy E_b with A_{ss} so small requires some justification in these circumstances. We feel it is a better guess than the freely jointed chain model since it may parameterize some of the effects of the nucleotide overlap [16], and in practice we only use E_b for moderately stretched chains.

To calculate the force f as a function of x_0 in fig. 2 in thermal equilibrium, we need the

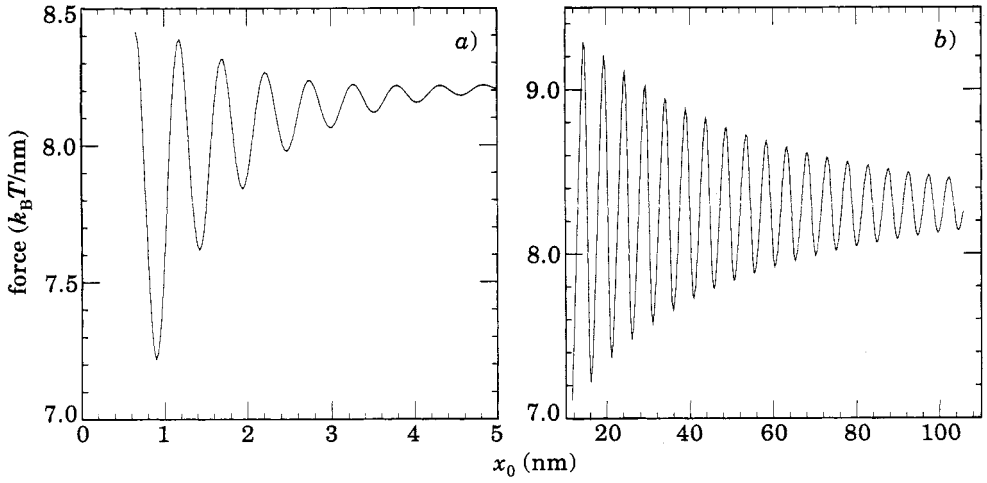

Fig. 3. – *a*), *b*) Force *vs.* x_0 for the DNA (RNA) parameters $(a, \varepsilon) = (0.66\text{ nm}, 3k_BT)$ and $(6.6\text{ nm}, 30k_BT)$. The values of the spring constant k are 100, $1k_BT/\text{nm}^2$, respectively, which maximizes the oscillations in $f/\sqrt{k_BTk}$ with x_0.

(2) Mayer, Slater and Drouin [17] infer from experiments the amount of DNA per gel pore from which a Kuhn length can be deduced, given a pore size.

work W required to extend a polymer of arclength l a distance x. It suffices to integrate the interpolation formula for $f(x)$ in ref. [3] which is exact for small f, where the polymer behaves like a linear spring with $f/x = 1.5/Al$; and for large f where the polymer undergoes small fluctuations about a straight line. The maximum error in f *vs.* x is 16% at fixed x and 4% at fixed f:

$$W(x, l) = \frac{x^2}{4A_{ss}(l - x)} + \frac{x^2}{2lA_{ss}} . \tag{2}$$

The partition function for the problem is just [3]

$$Z = \int_0^\infty \mathrm{d}x \int_x^\infty \mathrm{d}l \exp\left[- E_0(l) - W(x, l) - \frac{1}{2} k(x - x_0)^2 \right], \tag{3}$$

in terms of which we can derive the various thermal averages of interest, *e.g.*, $f(x_0) = -k\langle(x - x_0)\rangle = -\partial_{x_0} \ln(Z)$. The fluctuations in l for given x_0 can also be calculated from (3) by putting l and l^2 inside the integral.

Force curves for the DNA/RNA parameter values are shown in fig. 3. A value of $k \sim 30/a^2$ has been chosen which maximized the oscillations in $(x - x_0) \equiv -f/k$, normalized by the thermal r.m.s. at fixed x_0 *vs.* $k^{-1/2}$. Even so, for the DNA parameters measurement of the 4th oscillation requires a position sensitivity of $5 \cdot 10^{-3}$ nm or 5% of $k^{-1/2}$. The RNA curve is much more encouraging because we have simply assumed ~ 10 bp stems break open as a unit described in its entirety by eq. (1). Of course E_0 will in no sense be a periodic functional l for the RNA. Figure 3*b*) should be read as the attenuation suffered by a force fluctuation of $\sim \varepsilon/a$ when filtered through a given stretch of ss filament.

Evaluation of $\langle(l - \langle l\rangle)^2\rangle^{1/2}(x_0)$ gave values $\lesssim a$ (together with considerable oscillation for the RNA parameters) so long as there were appreciable oscillations in fig. 3*a*), *b*). Therefore the number of broken bonds at fixed x_0 is quite well defined and we do not expect long relaxation times to arise from nucleation over barriers. These calculations can be generalized to allow for ds nucleic-acid linker segments, between the molecule and the cantilever, etc.

To assess the behavior of (3) in the «thermodynamic limit» (in practice limited to where there are many slowly decaying oscillations as for RNA) it suffices to expand W for fixed x to quadratic order in l and then do the l and x integrals.

Let $c_n = \int_{-\pi}^{\pi} \exp[-\varepsilon' \sin^2(x) - 2inx]\,\mathrm{d}x$, define β implicitly by $4\varepsilon A_{ss}/a = \beta^2(1 + 2/(1+\beta)^2)$ (the l.h.s. is known), then $e_0 = (\beta^2 + 2\beta + 4\beta/(1+\beta))/(4A)$, $\omega = (2\pi/a)(1 + \beta^{-1})$, $\lambda = A_{ss}(2\pi/a)^2(1 + \beta^{-1})^3/(2 + (1+\beta)^3)$, and

$$Z = \text{const} \exp[-kx_0^2/2][c_0 \exp[(kx_0 - e_0)^2/2k] + 2c_1 \operatorname{Re}(\exp[kx_0 - e_0 - \lambda - i\omega]^2/2k)] . \tag{4}$$

Thus ω is the oscillation frequency, and λ^{-1} the decay length in fig. 3*b*). Note that, while the nonlinearity in (1), ε', sets the magnitude of the force oscillations, their damping with increasing x_0 is governed by the average force $\sim \varepsilon/a$. When this force is greater than that necessary to extend the ss filament to $\sim l/2$, *i.e.* $\sim 1/A_{ss}$, β is large and

---

[3] It makes no difference if the ss filament occurs in several pieces provided in each we can use the thermodynamic limit for W (2), *i.e.* $l \gg A_{ss}$, and all are subject to the same tension. This situation is apt to occur when unfolding RNA.

$\lambda^{-1} \sim (2/\pi^2)\,\varepsilon^{3/2}(aA_{ss})^{1/2}$ Eventhough we are not yet in a high-force regime, the attenuation length improves by a factor of ~ 50 in physical units in passing from fig. 3*a*) to *b*).

A further limitation on any mechanical measurement of secondary structure is the intrinsic, zero-temperature elasticity for chain elongation studied in ref. [9] which makes the entire system softer as l increases. This effect could be incorporated into our calculation by setting $k^{-1} \to k^{-1} + (l/a)/k_{ss}$, though current estimates imply that thermal fluctuations are a more serious limitation.

To limit the flexing of the ss filament, one could attempt to increase the ds binding energy and thus the tension. For instance, examining a string of GC repeats would increase λ by $\sim 2^{3/2}$ in comparison with our assumed parameters. Attempts to rigidify the ss filament by rehybridizing it with complementary ss segments or single-strand binding protein [18], are apt to prove counterproductive if they lower the ΔG for the ss-ds transition, *e.g.*, as measured by the melting temperature. As an extreme example consider the strand exchange reaction implicated in homologous recombination [19]. In equilibrium, no force is required for one ss filament to displace another.

However, schemes to chemically alter the ss-ds equilibrium merit consideration, since the potential payoff is large. The enthalpic gain and entropic loss are both 3-5 times larger than the net ΔG for the ss $\to$ ds transition [10, 12]. It is natural to attribute the former to the ds regions and the latter to ss filaments since the chain entropies should crudely scale with the inverse stiffness constant and $A_{ds}/A_{ss} \sim 10^2$. Thus one in principle could increase both ε and A_{ss} with the same agent. In this context, it should be noted that polyamines such as spermine increase the melting temperature of ds DNA [19]. Whether they also rigidify single-stranded nucleic acids is unclear.

We do not believe nonequilibrium kinetic effects can be used to enhance the measurement of base unpairing. It is not feasible to limit the phase space accessible to the ss-filament since its equilibration times are crudely of order the Zimm time for $a \sim 1$ nm sphere or $< 10^{-7}$ s. So long as there is a reasonable oscillation in $f(x_0)$, the variance in the length of free chain is $< a$, so we expect bases to unstack abruptly; whatever nucleation is required is internal to a base pair and presumably fast. There may be measurable hysteresis when one tries to refold an RNA molecule by relaxing the force; which would be quite interesting.

RNA remains a more appealing target than DNA for the experiments envisaged in this paper eventhough the average unbinding forces are comparable because there is interesting structure on the scale of many bases, and the decay length λ in (4) scales with the *square* of the block size at fixed ε/a.

* * *

The authors thank M. FRANK-KAMENETSKII and W. OLSON for supplying important references, and them together with C. BUSTAMANTE, J. MARKO, and D. THALER for discussions. Our research was supported by the NSF under grant number DMR-9012974.

REFERENCES

[1] SVOBODA K., SCHMIDT C. F., SCHNAPP B. J. and BLOCK S. M., *Nature*, **365** (1993) 721; FINER J. T., SIMMONS R. M. and SPUDICH J. A., *Nature*, **368** (1994) 113.
[2] SMITH S. B., FINZI L. and BUSTAMANTE C., *Science*, **258** (1992) 1122.
[3] BUSTAMANTE C. *et al.*, *Science*, **265** (1994) 1599.
[4] FLORIN E.-L., MOY V. T. and GAUB H. E., *Science*, **264** (1994) 415; FINZI L. and GELLES J., *Science*, **267** (1995) 378.

[5] LEE G. U., CHRISEY L. A. and COLTON R. J., *Science*, **266** (1994) 771.
[6] There are many references, for a critical review see HUNT A. J., GITTES F. and HOWARD J., *Biophys. J.*, **67** (1994) 766. Among recent references are: MAGNASCO M., *Phys. Rev. Lett.*, **71** (1993) 1477; ASTUMIAN R. D. and BIER M., *Phys. Rev. Lett.*, **72** (1994) 1766; PROST J., CHAUWIN J.-F., PELITI L. and AJDARI A., *Phys. Rev. Lett.*, **72** (1994) 2652.
[7] MARKO J. F. and SIGGIA E. D., *Science*, **265** (1994) 506.
[8] BEECHEM J. M., *Biophys. J.*, **67** (1994) 2133.
[9] VIOVY J. L. *et al.*, *C. R. Acad. Sci. Paris*, **317** (1994) 795.
[10] BRESLAUER K. J., FRANK R., BLOCKER H. and MARKY L. A., *Proc. Natl. Acad. Sci.*, **83** (1986) 3746.
[11] GESTELAND R. F. and ATKINS J. F. (Editors), *The RNA World* (Cold Spring Harbor Laboratory Press) 1993.
[12] FREIER S. M. *et al.*, *Proc. Natl. Acad. Sci.*, **83** (1986) 9373.
[13] SMITH S. B. *et al.*, *Biophys. J.*, **68** (1995) A250.
[14] HAGERMAN P. J., *Annu. Rev. Biophys. Biophys. Chem.*, **17** (1988) 265.
[15] TURNER D. H., SUGIMOTO N. and FREIER S. M., *Annu. Rev. Biophys. Biophys. Chem.*, **17** (1988) 167.
[16] HINGERTY B. E., BROYDE S. B. and OLSON W. K., *Biopolymers*, **21** (1982) 1167; ERIE D. A., BRESLAUER K. J. and OLSON W. K., *Biopolymers*, **33** (1993) 75.
[17] MAYER P., SLATER G. W. and DROUIN G., *Appl. Theor. Electrophoresis*, **3** (1993) 147.
[18] ALBERTS B. *et al.*, *Molecular Biology of the Cell*, 3rd edition (Garland Publishing, New York, N.Y.) 1994.
[19] FEUERSTEIN B. G., PATTABIRAMAN N. and MARTON L. J., *Nucleic Acids Res.*, **18** (1990) 1271.