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Letter to the Editor

On the Precise Meaning of Extension in the Interpretation of Polymer-Chain Stretching Experiments

In their recent article, Keller et al. (2003) emphasize that the outcome for a single-polymer-molecule stretching experiment wherein the end-to-end distance of the polymer is fixed and the force fluctuates (as described by a fixed-displacement or Helmholtz ensemble) may differ from that wherein the force is fixed and the end-to-end distance fluctuates (as described by a Gibbs ensemble). Their specific findings are that the two force laws are identical 1), if the force-extension behavior is linear for the Helmholtz chain (regardless of its size); and 2), when the molecule becomes long, i.e., in the thermodynamic limit.

We argue that the overriding criterion for obtaining equivalent results in the differing experiments (an equivalence of ensembles) is that the polymer molecule be moderately stretched (i.e., having an end-to-end separation r greater than several times its random-coil size, r_p); the thermodynamic limit is, in itself, not a sufficient criterion to ensure an equivalence of ensembles in the zero-force limit, nor is linear-extension behavior in the Helmholtz case. Here $r = |\mathbf{r}|$, where $\mathbf{r}$ is the end-to-end displacement, and $\mathbf{f}$ is the stretching force. In the weak-stretching regime ($r_p < r < 4r_p$), the force laws differ considerably, regardless of the size of the chain, and are critically dependent on how the *extension* of the polymer chain is defined. Whereas Keller et al. (2003) discuss a nonspecific, generic, one-dimensional polymer model, we will use the *ideal* freely jointed (three-dimensional) chain in the Gaussian approximation, having a normalized field-free end-to-end distribution function $P(\mathbf{r})$ equal to $b^3\pi^{-3/2}\exp(-b^2r^2)$ for $r \ll Na$ (Hill, 1962). $b^2 = 3/(2Na^2)$, and N is the number of links, each of length a. We note that the random-coil size is roughly equal to the most-probable end-to-end separation r_p, where $r_p = 1/b$.

For a polymer chain, $\mathbf{r}$, $\mathbf{f}$, r, and f ($f = |\mathbf{f}|$) are state variables whose interrelationship is made explicit when deriving an equation of state. In the present context, three different ensembles are of particular interest in the weak-stretching regime.

1. In the constant-$\mathbf{r}$ (displacement) ensemble, the ends of the chain are fixed, and an average force in the direction of $\mathbf{r}$ is determined (Hill, 1962);

$$\beta\langle\mathbf{f}\rangle = -(d/d\mathbf{r})\ln[P(\mathbf{r})] = 2b^2\mathbf{r}, \quad [\beta\langle f\rangle = 2b^2 r]. \quad (1)$$

2. In the constant-$\mathbf{f}$ (force) ensemble, the chain ends are subject to a fixed-force couple, and the average value of the desired variable is calculated. The force is assumed to act in a direction parallel to the x-axis; $\mathbf{r} = x\mathbf{i} + y\mathbf{j} + z\mathbf{k}$. The average displacement is $\langle\mathbf{r}\rangle = Q^{-1}\int \mathbf{r}P(\mathbf{r})\exp(\beta\mathbf{f}\cdot\mathbf{r})d^3\mathbf{r}$, where $Q = \int P(\mathbf{r})\exp(\beta\mathbf{f}\cdot\mathbf{r})d^3\mathbf{r}$, resulting in

$$\beta\mathbf{f} = 2b^2\langle\mathbf{r}\rangle, \quad [\beta f = 2b^2\langle x\rangle]. \quad (2)$$

Historically, Eqs. 1 and 2 have been regarded as describing a Hooke's law dependence between force and displacement, with their formal similarity taken as implicit evidence for an equivalence of ensembles, as in the macroscopic limit (Hill, 1962).

Whereas $|\langle\mathbf{r}\rangle|$ is the magnitude of the average value of the projection of $\mathbf{r}$ in the direction of $\mathbf{f}$, $\langle x\rangle$, it is illuminating to calculate $\langle r\rangle$ (Neumann, 1985) in the force ensemble and compare it with Eqs. 1 and 2. $\langle r^2\rangle$ is presented here for subsequent use.

$$\langle r\rangle = (\langle r\rangle_0/2)\left[\exp(-\nu^2/4) + (\nu + 2/\nu)\int_0^{\nu/2}\exp(-t^2)dt\right] = \langle r\rangle_o[1 + \nu^2/12 + O(\nu^4)]; \quad (3)$$

$$\langle r^2\rangle = (3/2 + \nu^2/4)/b^2, \quad (4)$$

where $\nu = \beta f/b$ and $\langle r\rangle_0 = 2/(b\pi^{1/2})$. Eqs. 3 and 4 remain valid only within the Gaussian approximation, $r \ll Na$. Under the influence of a small force, $\nu < 1$, the chain maintains a nearly constant average end-to-end separation, $\langle r\rangle_0$ (the random-coil size), while "rotating" analogous to an electric dipole; hence $\langle\mathbf{r}\rangle$ increases linearly, beginning at zero, with the force (Neumann, 1985). It can be readily shown that Eq. 3 reduces to the Hooke's law expression, Eq. 1, in the limit of moderate stretching.

3. In the constant-r (length) ensemble, the scalar separation between the chain ends is maintained fixed while the orientation of $\mathbf{r}$ is permitted to vary. An "imaginary rod" of length r is inserted between the ends of the chain to fix the length. The equation of state is determined by calculating the average tension in the rod as a function of r (Neumann, 1986).

$$\beta\langle f\rangle = 2(b^2 r - 1/r). \quad (5)$$

The force vanishes when $r = 1/b = r_p$, rather than when $r = 0$ as in Eq. 1. Here, the ideal chain behaves as a spring of *finite* unstretched length, which may be compressed as well as extended; a negative value for $\langle f\rangle$ corresponds to compression.

Fig. 1 shows the extension-versus-force behavior predicted by the force laws derived from the three different ensembles, Eqs. 1, 3, and 5. As expected, in the moderate-stretching region ($4r_p < r \ll Na$) the curves described by

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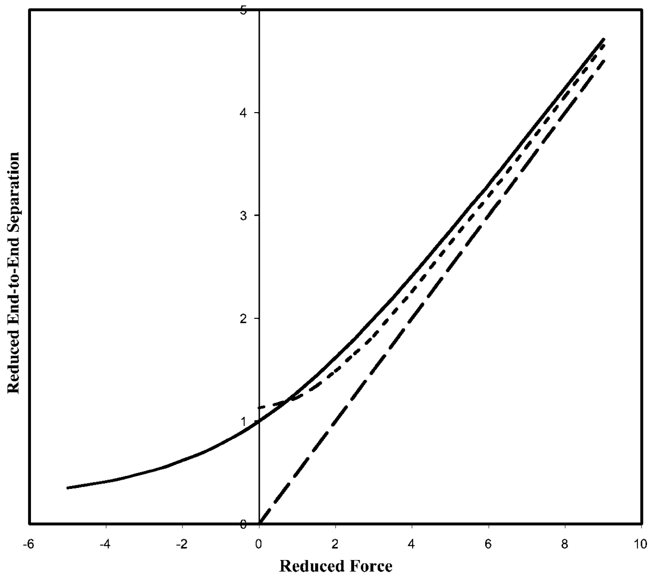

FIGURE 1 The reduced end-to-end separation is shown as a function of the reduced force for the three ensembles. The long-dash line depicts r/r_p versus $\beta r_p\langle f\rangle$ from Eq. 1 of the Helmholtz ensemble; the short-dash line depicts $\langle r/r_p\rangle$ versus $\beta r_p f$ from Eq. 3 of the Gibbs ensemble; and the solid line depicts r/r_p versus $\beta r_p\langle f\rangle$ from Eq. 5 of the constant-length ensemble.

Eqs. 3 and 5 merge with the Hooke's law description, Eq. 1, and the variation among the different calculated forces is <5%. On the other hand, in the weak-stretching regime ($r_p < r < 4r_p$), the appropriate equation of state depends on the particular physical environment of the chain, i.e., which ensemble is used to describe the chain. This is true for any finite N because it is always possible to remain in the weak-stretching regime by adjusting the force acting on the chain. Eqs. 1 and 3, which correspond to the Helmholtz and Gibbs cases, respectively, are not consistent with the first finding of Keller et al. (2003), requiring "identical" force laws when the stretching behavior for the Helmholtz chain is linear. This is particularly apparent at zero stretching force where the Helmholtz chain is collapsed, but the Gibbs chain is in its normal random-coil configuration.

Whereas the magnitude of the force in the weak-stretching regime is of the order of 4 pN/r_p (where r_p is in units of nm), which is typically <0.1 pN and well below the magnitude of the force involved in biological elastic function (typically 10–100 pN), the differing force laws in this regime are, nevertheless, apparent in some DNA-stretching experiments (Perkins et al., 1995; Smith and Bendich, 1990). In these experiments, a force is applied to a strand of DNA by means of hydrodynamic flow or an electric field, and the scalar end-to-end separation is measured as a function of the applied force. When using extension-versus-force plots to interpret their results, the authors include data points in the weak-stretching regime, along with a theoretical curve that is equivalent to Eq. 1 in this regime. The lack of agreement between their data and a Hookean analysis is particularly evident at *zero* external force, where the measured extension is of the order of $\langle r\rangle_0$, whereas the theoretical value is *zero*. We have reanalyzed the published results of a DNA-stretching experiment wherein the molecule was stretched by means of an elongational flow, taking note of the differing force laws (Neumann, 1999).

The second finding of Keller et al. (2003) concerning the equivalence of ensembles in the thermodynamic limit is based on their Eq. 18, which examines the difference (ε) between the average value and most-probable value of the *relative extension* as calculated from the Gibbs ensemble and shows this difference to vanish as the chain length becomes infinite, regardless of the magnitude of the stretching force. Whereas we are in agreement with Keller et al. (2003) concerning the equivalence of ensembles in the moderate-stretching regime and beyond, the weak-stretching regime requires further attention. The authors define relative extension as the polymer's end-to-end separation divided by its fully extended length, which in the present context is r/Na. Thus, at zero force, one would find that $\varepsilon = [\langle r\rangle_0/Na] - [r_p/Na] = 0.1N^{-1/2}$, which does indeed vanish in the thermodynamic limit. This behavior stems from the use of relative extension rather than extension, which causes each bracketed term to approach zero in the limit $N \to \infty$, rather than each approach the same *finite* limit as occurs for $f > 0$. If the relative extension is regarded as the thermodynamic length, with ε the measure of dispersion, then the standard statistical-mechanical criterion for the absence of fluctuations (or equivalence of ensembles) requires a comparison of this quantity with the length itself, i.e., an examination of the relative fluctuation, $\varepsilon/(\langle r/Na\rangle_0)$, which does not vanish in the thermodynamic limit if $f \to 0$. This is an essential yet subtle point because in macroscopic statistical mechanics, the relative volume fluctuation for an ideal gas (whose dependence on the number of molecules n is given by $\sigma_V/\langle V\rangle \approx n^{-1/2}$) can be made arbitrarily small by a sufficient increase in n (Hill, 1962). For the polymer chain, however, using Eqs. 3 and 4 in the weak-stretching region ($0 \leq \nu \leq 1$, for instance), one has $\sigma_r/\langle r\rangle = (\langle r^2\rangle - \langle r\rangle^2)^{1/2}/\langle r\rangle \approx 0.4$. Because $\nu \propto fN^{1/2}$, for any finite N, *no matter how large*, the relative fluctuation in extension can be maintained at a finite value by reducing the force sufficiently.

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