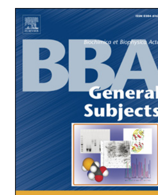
Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen



Effect of external pulling forces on the length distribution of peptides☆

Matthew Batchelor, James Gowdy, Emanuele Paci *

School of Molecular and Cellular Biology and the Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, UK

ARTICLE INFO

Article history:
Received 3 July 2014
Received in revised form 15 September 2014
Accepted 16 September 2014
Available online xxxx

Keywords:
Peptides
Length distribution
Force
Freely jointed chain
Alpha helix
Random coil

ABSTRACT

Background: The distribution of the length of a polypeptide, or that of the distance between any two of its atoms, is an important property as it can be analytically or numerically estimated for a number of polymer models. Importantly, it is directly measurable through a number of different experimental techniques. Length distributions can be straightforwardly assessed from molecular dynamics simulation; however, true convergence and full accurate coverage of the length range is difficult to achieve.
Methods: The application of external constant force combined with the weighted-histogram analysis method (WHAM) is used to enhance sampling of unlikely 'long' or 'short' conformations and obtain the potential of mean force, while also collecting dynamic properties of the chain under variable tension.
Results: We demonstrate the utility of constant force to enhance the sampling efficiency and obtain experimentally measurable quantities on a series of short peptides, including charge-rich sequences that are known to be highly helical but whose properties are distinct from those of helical peptides undergoing helix–coil transitions.
Conclusions: Force-enhanced sampling enhances the range and accuracy of the length-based potential of mean force of the peptide, in particular those sequences that contain increased numbers of charged residues.
General significance: This approach allows users to simultaneously probe the force-dependent behaviour of peptides directly, enhance the range and accuracy of the length-based PMF of the peptide and also test the convergence of simulations by comparing the overlap of PMF profiles from different constant forces. This article is part of a special issue entitled Recent developments of molecular dynamics.

© 2014 Published by Elsevier B.V.

1. Introduction

The average and probability distribution of the end-to-end distance are important quantities for describing the physical properties of polymeric chains [1]. The simplest model to describe polymers is the freely-jointed chain (FJC) [2]. It only assumes a polymer as a random walk and neglects any kind of interactions among monomers. If each monomer is assumed to be a rigid rod of length d and N monomers form the polymer, the maximal polymer length is $L_{\max} = Nd$. The distribution of the length (or end-to-end distance, L) is

$$P(L) = 4\pi L^2 \left(\frac{3}{2\pi N d^2}\right)^{3/2} e^{-\frac{3L^2}{2Nd^2}}.$$

In the case of polypeptides and proteins, 'monomers' consist of a variety of amino acid residues, the sequence is variable in length, and the interactions between monomer units vary both in strength and specificity. Depending on factors such as length, temperature and solvent condition, they deviate more or less strongly from an ideal polymer. For example, at temperatures below the folding temperature, $P(L)$ for a single alpha-helical peptide will be peaked at about $N \times 1.5$ Å, while that of a perfect beta hairpin will be a few Å, i.e., the distance between two residues sharing a main-chain hydrogen bond. In both cases, above the folding temperature, $P(L)$ will be better approximated by an ideal chain result and increasingly so with increasing temperature, i.e., when intra-chain interactions become negligible. Under some conditions, such as for unfolded proteins under the effect of mechanical force, ideal models, and specifically the worm-like chain model adequately reproduce experimentally observed properties [3].

The potential of mean force (PMF) along L is simply related to the length distribution by $W(L) = -k_B T \ln P(L)$ [4]. The estimation of $W(L)$ from molecular dynamics simulation—or for that matter estimating the PMF associated with any parameter that is a function of the coordinates—is straightforward when all the values assumed by the function are accurately sampled during the simulation. This is not the case in many instances in which the process being studied is an activated one, i.e., when a sizeable free energy barrier appears in the potential of mean force and the transition between different states, identified by different values of the function, or reaction coordinate, are rare events that may not occur spontaneously during the simulation. The most broadly used approach to enhance the sampling of regions of the conformation space that would not be accurately sampled otherwise, consists of adding a biasing potential that harmonically restrains the excursion of

☆ This article is part of a special issue entitled Recent developments of molecular dynamics.

* Corresponding author.
E-mail address: e.paci@leeds.ac.uk (E. Paci).

http://dx.doi.org/10.1016/j.bbagen.2014.09.019
0304-4165/© 2014 Published by Elsevier B.V.

Please cite this article as: M. Batchelor, et al., Effect of external pulling forces on the length distribution of peptides, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagen.2014.09.019

UNCORRECTED PROOF

ARTICLE IN PRESS

the reaction coordinate around chosen values along the whole range of values assumed by the reaction coordinate, thus enforcing an approximately uniform sampling [5]. The bias on the potential of mean force can be readily removed for the results, but general thermodynamic and kinetic properties of the system in the absence of the bias cannot be obtained. A variation of the umbrella sampling method has been recently proposed where the reaction coordinate is confined by reflecting boundaries [6]; a continuous PMF can be obtained by imposing that the forward and backward flux be equal at each boundary, with the advantage relative to umbrella sampling being that rates can also be obtained. A PMF can also be obtained by constraining the reaction coordinates at specific values [7–9].

In the specific case in which the reaction coordinate is the distance between two atoms, for example, the distance between the two ends of a polymer, a novel class of methods, based on the discoveries of Jarzynski [10] and Crooks [11], have shown that free energy differences can be obtained from non-equilibrium measurements. Such measurements have been made possible by single molecule manipulation techniques [12–15] and their simulation counterparts [16–18].

Experimental observation of the end-to-end distance under the application of a constant force is possible, thanks to techniques such as force-clamp atomic force spectroscopy and magnetic tweezers [19]. The potential of mean force along the extension may not be directly measurable, but it can be probed by single molecule force spectroscopy experiments that measure the force at which proteins ‘snap’—when they cross the free energy barrier that separates the native compact state from the denatured extended state when the two ends are pulled apart at constant velocity (or equivalently, the average time it takes the native protein to snap when a constant force is applied to its ends) [20]. If the process of mechanical fracture is thought of as diffusion over a free energy barrier on the potential of mean force defined by the extension of the protein (i.e., distance between the points where force is applied), then the unfolding rate depends exponentially on the applied force [21]. This relation, which goes under the name ‘Bell model’, seems to be generally obeyed although a number of exceptions have been reported [22,23].

The application of a constant force, parallel to the vector joining any two atoms of a polypeptide, modifies the probability of different lengths of the vector, a positive force favouring longer conformations and a negative force favouring shorter conformations. When modified by a force F, the length distribution is given by the relation $P_F(L) = P(L)e^{FL/k_BT}$. This relation is simply obtained by observing that the PMF ($W(L) = -k_BT \ln P(L)$) is modified by the application of an external force parallel to the end-to-end vector by $W_F(L) = W_0(L)–FL$ (in units of k_BT). For the FJC this is $W_F(L) = \frac{3L^2}{2Nd^2} - 2 \ln L - FL$.

In this paper, we exploit the relationship $W_F(L) = W_0(L) - FL$ to accurately determine the equilibrium potential of mean force associated with the distance between two atoms of a polypeptide chain. Unlike the methods mentioned above where an artificial biasing term is added to the Hamiltonian, the application of a constant force is also possible experimentally, and the equilibrium and kinetic properties of the real system are not perturbed.

We focus on relatively short peptides that have high intrinsic helical propensity. The α helix is a ubiquitous motif found throughout the proteome. Its structure is stabilised by hydrogen bonds between the backbone carbonyl oxygen of residue i and the backbone N–H group of residue $i + 4$. This pattern causes the backbone of the polypeptide chain to form a right-handed helix, with side chains pointing out from the core and slightly toward the N-terminus. Most α helices are found within globular proteins where interactions between neighbouring secondary structure elements stabilise the structure. When investigated in isolation, the short peptide sequences that exist as helices within globular proteins are often not helical. However, certain short sequences, notably the C-peptide from RNase [24–26] and several synthetic peptides [27,28], have since been shown to form stable helices in solution.

Alanine-rich peptides are often used as the archetype of ‘normal’ alpha helices, and there exists a great deal of literature describing their properties [29]. The substitution of alanine for charged residues, glutamic acid (E), lysine (K) and arginine (R), originally to improve the solubility of these peptides, has been shown to have a significant effect on peptide helicity [27,28,30–32]. A dramatic example is the recent experimental comparison of short E-R/K-rich peptides using CD spectroscopy [33]. We use this study as an experimental touchstone for comparison with our simulations.

The peptides studied here are in general polyalanine based, 10 residues in length but have different numbers and patterns of charged residues (glutamic acid, arginine and lysine). We also use a glycine-rich sequence as a random coil model. Each peptide has an N-terminal YS ‘tag’, used in experiments as a means of accurately calculating peptide concentration, which is retained in simulations to allow for direct comparison, and for consistency, all other peptide sequences start in this manner. As expected, the glycine-rich sequence is completely disordered, alanine-rich sequences show a helical propensity, and that propensity increases as a pattern of charged residues sequentially replace alanine. The PMF properties of the charge-rich sequences are distinct from those of alanine-rich sequences. We also highlight some advantages of using mechanical force to enhance sampling relative to other computational methods, in part related to the fact that constant force can also be applied experimentally to individual proteins.

2. Methods

The equilibrium dynamic behaviour of the peptides was simulated using a united-atom force field (CHARMM19) and implicit solvent model (FACTS) [34]. The force field and the solvation model were chosen after testing several alternatives. Simulations performed using the CHARMM22/CMAP force field [35] with FACTS showed that helical conformations for charged sequences were excessively stable and reversible helix formation was never observed. This same behaviour was observed using CHARMM22/CMAP and explicit solvent (with TIP3P water). A similarly initiated 1.25 μs simulation using a fully solvated recently revised all-atom model of YSE_4R_4 (CHARMM36 [36], run using NAMD [37]) did not show any significant helicity (4%, most of which being 3_{10} helix). Simulations performed using CHARMM19 with the SASA implicit solvent model [38] showed, in clear disagreement with experiment, poor differentiation between charged and non-charged sequences, likely due to the neutralization of charged side chains imposed with the SASA implicit solvation model. The combination of CHARMM19 and FACTS was recently shown to give excellent agreement with experiment for a highly charged system [39]. The ‘standard’ CHARMM19 FACTS parameters for structured peptides were used: dielectric constant = 2.0, nonpolar surface tension coefficient = 0.015 kcal mol^{-1} $Å^{-2}$.

Unless otherwise stated, all simulations were performed at 300 K, with Langevin dynamics using the leapfrog integrator, a time step of 2 fs and a friction coefficient of 3 ps^{-1}, and run using CHARMM [38]. Trajectory frames (and associated analysis parameters) were recorded every 500 steps. A constant force of between −50 and 50 pN was applied between the N atom of the first residue and the carbonyl carbon atom of the last (tenth) residue. The N and C termini were capped with acetyl (ACE) and methylamine (CBX) groups, respectively [40]. Simulations lasted between 1 and 4 μs. Starting structures for simulations were prepared by performing a steepest descent minimisation (1000 steps) from an all-*trans* backbone conformer followed by a short (20 ps) dynamics run. The first 100 ns of each of the simulations was removed prior to analysis in an effort to remove starting structure bias.

Wordom (version 21) [41] was used to analyse the simulation trajectories. The secondary structure of the peptide was assigned for each timeframe using the DSSPcont [42] criteria. This was then used to calculate the helicity (or helical fraction) of the peptide overall, with helicity defined as the fraction of 3_{10}, α, and π residues (i.e., G + H + I) [43]. It

UNCORRECTED PROOF

Please cite this article as: M. Batchelor, et al., Effect of external pulling forces on the length distribution of peptides, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagen.2014.09.019

Download English Version:

https://daneshyari.com/en/article/10800044

Download Persian Version:

https://daneshyari.com/article/10800044

Daneshyari.com