Analysis of the conformational space of murine prion protein: an amyloidogenic protein involved in neurodegenerative disorders

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Introduction

Protein folding involves a stochastic search through the configurational energy landscape of the protein to find the native structure. Although most proteins have evolved to fold efficiently into a unique native structure, misfolding (the formation of non-native structures) occurs frequently *in vivo*. Biophysical studies of protein misfolding and early stage aggregation processes are very complex due to the presence of many conformations and different misfolding routes. Single-molecule approaches have proven to be good methods to evaluate the conformational heterogeneity of biological macromolecules, because they can discern amongst different subpopulations, rare or transient states and their energy barriers.

The cellular form of the prion protein (PrP^{C}) is a highly conserved membrane-bound protein that is able to misfold into an infectious conformation (PrP^{Sc}), which can form aggregates and fibrils with different biochemical and biological properties. Such conformational polymorphisms have been proposed to reflect the conformational heterogeneity of the monomer.

Using Atomic Force Microscope (AFM) force spectroscopy, we investigated the conformational equilibria of mouse (Mo) prion protein (PrP) using two different polymeric protein constructs.

Materials and Methods

Heteropolymeric protein constructs flanked by GB1 domains bearing one MoPrP molecule from residue 89 to 230 $(GB1)_4$-$(MoPrP_{89\text{-}230})$-$(GB1)_4$ and four MoPrP molecule copies *in tandem* $(GB1)_2$-$(MoPrP_{89\text{-}230})_4$-$(GB1)_2$ were cloned inside pET11a between BamHI and NdeI restriction sites. Proteins were expressed in BL21(DE3) *E. coli* cells, first purified by SEC and then by Ni^{2+} affinity chromatography. Proteins were concentrated to 1-10 µM and stored in Tris 20 mM pH7.4 NaN_3 0.05% at +4°C.

Constant velocity mechanical unfolding experiments were performed with a Veeco Picoforce AFM on a Multimode Nanoscope IIIa (Bruker) using gold-coated triangular silicon nitride cantilevers (NPG, Bruker) with nominal spring constants of 0.06 N/m. The effective spring constant was determined by characterizing the thermal noise spectrum. Ten microliters of protein specimen was deposited on a flame-cleaned glass coverslip, mounted on a fluid cell and equilibrated with buffer. Pulling velocity was 2180 nm/s. Refolding experiments were carried out by stretching the protein to a fixed length, refolding it at low force for 50 ms and eventually stretching it again; the second unfolding pulse was evaluated.

Analysis was performed by custom designed software [1]; curves were selected depending on the number of GB1 unfolding events and molecule length. Force peaks were fitted using the worm like chain (WLC) model.

Results and Conclusions

In order to establish the conformational heterogeneity of the monomeric MoPrP, we performed pulling experiments using the construct bearing one $MoPrP_{89\text{-}230}$ molecule flanked by four GB1 domains, denoted TR_{x1}. We analyzed the behavior of the construct under different buffer conditions, particularly at different pH and ionic strength, since these two factors are known to modulate the transition from the α-helical form to the β-enriched one [2,3]. Among all recorded single-molecule unfolding events, less than 21 % showed signals not interpretable as GB1 unfolding event (as they show event lengths >30 nm) (Table 1). This finding suggests that a fraction of the sampled PrP molecules might be in a β-enriched and stable structure, rather than in the native α-helical form. Experiments of mechanical unfolding following refolding showed a reduced proportion of such events, probably due to the longer folding time required by β structures.

To understand how more monomeric PrP molecules associate with each other, we pulled the construct bearing four tandem copies of MoPrP molecules in a head-to-tail orientation, denoted TR_{x4}. Interestingly, more than 70 % of the molecules exhibited a strong mechanical behavior (Table 1), which had different lengths and forces (Figure 1). Many of these unfolding events were longer than the unfolded monomeric protein, hence suggesting that strong associations among PrP molecules inside the same polypeptide chain occur. Refolding of such constructs confirmed the presence of strong associations among protein monomers, some of which encompassed the whole length of the four-tandem repeats of MoPrP, indicating that long-range contacts can be established as well. Finally the presence of mild ionic strength (150 mM NaCl) inhibited the formation of such structures only during refolding, suggesting that salt bridges play a major role in the early stages of the association process.

All the data we collected indicate that PrP can undergo conformational changes in the monomeric form, but this is a rare event. The presence of more PrP molecules along the same polypeptide chain can induce them to associate and the buffer can modulate these interactions.

Protein	Experiment	Buffer			
		Tris 20 mM pH 7.4	NaOAc 20 mM pH 5.5	NaOAc 20 mM pH 4.0	PBS pH 7.4
TR_{x1}	Unfolding	20.3 %	13.1 %	16.9 %	-
	Refolding	17.9 %	13.3 %	4.5 %	-
TR_{x4}	Unfolding	71.6 %	78.9 %	66.7 %	70.3 %
	Refolding	71.4 %	100 %	-	43.2 %

Table 1. Percentage of unfolding events from prion protein on the overall curves

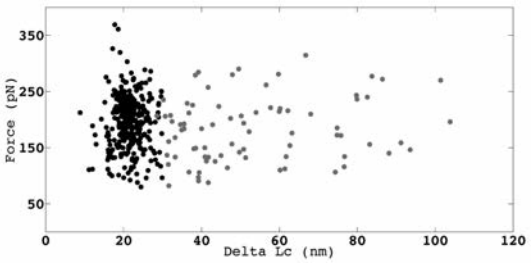

Figure 1. Scatterplot from TRx4 unfolding experiments: GB1 distribution (black dots) and PrP events (gray dots).

References

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