Short communication

Folding-unfolding of Immunoglobulin Domains in Titin: A Simple Two-state Model

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Abstract. The folding-unfolding reaction rate process in the giant protein titin is studied within a simple two-state model. The molecule is assumed to be stretched by an external force which modulates the potential barrier associated with the folded state. A two-state model for this process is assumed (*i.e.*, the immunoglobulin domains are considered to be either folded or unfolded, with no intermediate states at all). Simple calculations yield a relation between the force and the pulling speed that agrees fairly well with data from experiments and Monte Carlo simulations performed recently. Moreover, in a regime involving ultrafast pulling, the results show that the detailed form of the potential barrier is irrelevant, a conclusion that agrees with the current theoretical work on molecular dynamics.

Key words: Skeletal muscle — Two-state model — Folding-unfolding in titin — Passive forces

Introduction

The passive force and resting tension of striated muscle are derived from connecting filaments, each of which is composed of three to six molecules of the giant protein titin. This protein contains 140-160 immunoglobulin C2 domains and 132 fibronectin III domains (Yoshioka et al. 1986; Horowits and Podolsky 1987; Higuchi 1992; Wang et al. 1993: Granzier and Irving 1995; Granzier et al. 1996). Both of these domains consist of seven β-strands stabilized by hydrogen bonds and by side-chain interaction between the two faces of the sheets (Keller 1997). The section of the titin molecule at the I-band region has a segment that is called PEVK because it is rich in proline (P), glutamic acid (E), valine (V), and lysine (K) (Labeit and Kolmerer 1995). Recent studies on the mechanical properties of the isolated titin

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molecule show that under stretching, the PEVK segment unfolds to more than ten times its original size, then refolds as the stretch is released (Linke et al. 1996; Tskhovrebova et al. 1997). When the titin molecule is extremely stretched the immunoglobulin C2 domains are unfolded. Refolding of these domains is slower than the refolding of PEVK. The external force necessary to unfold the immunoglobulin domains depends on the extension speed. Small changes in amino acid sequence or the isomerization of proline could change the unfold-refold rate of the immunoglobulin domain (Erickson, 1994; Kellermayer et al. 1997; Rief et al. 1997). The aim of this present work was to devise a model to elucidate the characteristics of the energy barrier associated with the immunoglobulin unfolding process.

The Model

As briefly described in the Introduction, the extensibility of the titin filament resides mainly in the immunoglobulin domains (β-sheet-like domains) and the PEVK portion. For the purposes of this work, we shall restrict ourselves to analyzing the folding-unfolding properties of the immunoglobulin domains in the presence of an external force; *i.e.*, we shall for the present ignore the participation of the PEVK segment. This omission is justified by the fact that the PEVK segment is fully extended at a moderate stretch (Tskhovrebova et al. 1997) whose value is below those in the stretching regime considered in this work.

Let us assume that a β-sheet-like domain can be folded or unfolded without any intermediate state. If $\nu(\mu)$ is the unfolding (refolding) rate constant, f the stretching force, E_a the potential barrier that must be overcome to unfold a domain, and Δx the deformation produced in this barrier by the external force (Evans and Ritchie 1997), then

$$\nu = \mu \exp[-(E_a - f\Delta x)/kT] \tag{1}$$

where k is Boltzmann's constant and T the absolute temperature.

The pulling speed to unfold (refold) the domains, is given by

$$v = (\nu - \mu)\Delta x \tag{2}$$

Combining Eqs. (1) and (2), the force can be expressed as:

$$f = f_0 + f_1 \ln(av + 1) \tag{3}$$

where $f_0 = E_a/\Delta x$, $f_1 = kT/\Delta x$, and $a = 1/(\mu\Delta x)$.

Discussion and Further Remarks

In this section we shall test the main predictions of the proposed model by making a comparison between the present results and the available experimental and theoretical data.

As a starting point, we will assume that E_a is the equilibrium free energy for unfolding found experimentally (Soteriou et al. 1993; Politou et al. 1995); this is between 12 and 30 kJ/mol and so we used an average value of 21 kJ/mol. The most accepted values for the width of the potential barrier Δx, are in the range 0.3–0.4 nm (Tskhovrebova et al. 1997; Rief et al. 1997), and so the average value of 0.35 nm was used to estimate f_0 and f_1. To calculate a, we used a refolding time of 1s (Fong et al. 1996; Tskhovrebova et al. 1997). Since, at room temperature, kT = 0.025 eV = 4 pN-nm and that 4.186 kJ/mol = 0.0433 eV = 6.94 pN-nm, then f_0 = 99.14 pN, f_1 = 11.43 pN, and a = 2857 s/μm.

Figure 1 shows a comparison between the unfolding forces predicted by Eq. 3 and both the experimental data of Rief et al. (1997) and the Monte Carlo simulation made by the same authors. As can be readily seen from this Figure, the agreement between the predictions of the model presented in this work and the results obtained by these previous authors is fairly good, in spite of the crudeness and simplicity of the assumptions made. However, the Monte Carlo simulation of Rief et al. (1997) and the prediction of Eq. 3, seem to disagree for the extremes of pulling speed. This disagreement could be due to the fact that either approach neglects mechanisms that may play an important role when the pulling speed it too low or too high, but are unimportant at intermediate values of pulling speed. We must also remember that experiments in these extreme regimes could be less precise because of the difficulties experienced in controlling the pulling speed.

The discrepancy described above should motivate deeper research on this subject. This might involve study, for instance, of the optimization of the structure of

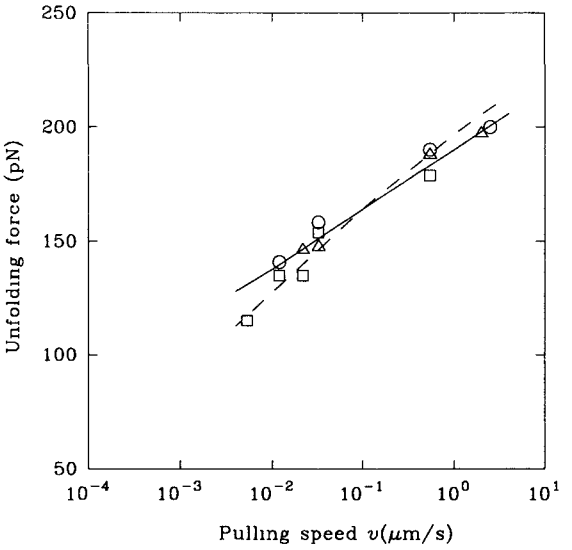

Figure 1. Force-Pulling speed relationship: comparison between the results of this work (continuous line) and the experimental results (open circles, triangles, and squares) and Monte Carlo simulation (dashed line) (Rief et al. 1997).

the immunoglobulin domain of titin and its energy surfaces to attempt to elucidate the characteristics of the energy barriers involved as well as the unfolding dynamics of the immunoglobulin domain at the molecular level, in much the same way as in a recent study of polysaccharide elasticity (Marszalek et al 1998).

Finally, Rief et al. (1998) have recently carried out experiments and Monte Carlo simulations to study the force-extensibility behavior of biopolymers (polysaccharide dextran and titin) They assumed an elastically coupled two-level system to make the simulations, and the results of their simulations are in good agreement with their experimental results.

Work is currently in progress to study the force-extensibility behavior of titin in the frame of the two-state model proposed in the present work, the results will be published elsewhere

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