ACTIN MONOMER-MONOMER INTERACTION
– A MOLECULAR MECHANICS STUDY

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Abstract

Knowledge of the interaction properties of the actin monomers is critical for understanding the molecular mechanism of the filament polymerization. The aim of the present paper was to provide information about the interaction properties of actin monomers using molecular mechanics approach. In order to be characterized, the atomic structure of the protein was taken from the Protein Data Bank. We used the atomic coordinates set in 1ATN.pdb which is a 3D-model of the actin monomer from rabbit. The commercial software package used to perform the molecular simulations was Hyperchem 6.01. Using the molecular mechanics approach, the interaction energies between actin monomers were evaluated for different intermolecular distances, after a preliminary minimization. Starting from these values, the binding force and binding stiffness were calculated as the first and the second order derivative of the interaction energy with respect to the intermolecular distance. According to the results of our simulations, the complex between the actin monomers is characterized by a minimum interaction energy of -740.6 kJ/mol and a maximum binding force of about 3.2 nN. Our results match a number of experimental data, thus supporting the idea that molecular mechanics may be a powerful tool to find a way to characterize biological macromolecules.

**Key words***: actin monomer, interaction properties, molecular mechanics*

1. Introduction

Actin is a major component of the microfilament system in eukaryotic cells (Otterben *et.al*, 2001) and plays an important role in muscle contraction (Geeves *et.al*, 1999).

Monomeric actin (G-actin) is a highly conserved eukaryotic protein which in physiological salt conditions polymerizes and forms actin filaments (F-actin) (Holmes *et.al*, 1990). F-actins are helical polymers made up of G-actin monomers arranged in six left-handed turns repeatingevery 36 nm. Along each of the morphological helices the actin monomers are spaced by 5.5 nm (Geeves *et.al*, 1999).

Atomic structure of G-actin was resolved by crystallizing it in complex with other proteins, such as deoxyribonuclease I (Kabsch *et.al*, 1990), gelsolin (McLaughlin *et.al*, 1993), Robinson *et.al*, 1999) and profilin (Schutt *et.al*, 1993), which prevent actin polymerization. The crystallographic studies show that actin monomers consist of two similar domains each containing a 5-stranded β-sheet and associated α-helices. Each domain of actin molecule can be further subdivided into two subdomains, termed 1, 2, 3, and 4, which are stabilized by bonds to an adenine nucleotide and a divalent cation (figure1). In the filament model, subdomains 3 and 4 form the core of the filament, while subdomains 1 and 2 project toward the periphery. Most of the amino acids that are involved in the interaction with myosin are located in subdomain 1.
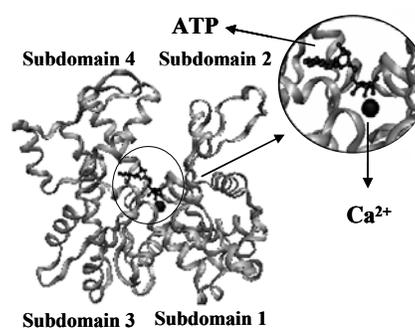
Figure 1. Ribbon representation of the structure of uncomplexed actin monomer in ATP state. ATP is bound to the center of molecule, where the four actin

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subdomains meet; the catalytic Ca^{2+} ion is bound in close association with the nucleotide (insert)

There are different experimental studies performed on actin which report details about its interaction with myosin (Nishizaka *et.al*, 1995), about its elastic properties (Liu and Pollack, 2002, Kojima *et.al*, 1994, Dupuis *et.al*, 1997), and polymerization - depolymerization mechanism of the filaments (Goddette and Frieden, 1986).

Knowledge of the mechanism of association and dissociation of actin monomers within filaments is important for many biological structures and processes. During the past several years two types of techniques were used to study the mechanics of protein interaction (Weisel *et.al*, 2003). The first one refers to atomic force microscopy, optical trap, magnetic tweezers, and hydrodynamic methods. The second technique uses molecular modelling approach to investigate the disruption of specific pairs of molecules in atomic details (Aprodu *et.al*, 2006, Vesentini *et.al*, 2005).

The present study focuses on the molecular mechanics characterization of the interaction between actin monomers at the single molecule level.

Molecular mechanics is a computer based technique which uses classical mechanics equations to predict the energy of the molecule as a function of its conformation. The potential energy of the systems is calculated using a force field, considering (i) each atom as a single particle to which is assigned a radius, polarizability, and a constant net charge on the basis of quantum calculations and/or experiments, and (ii) bonded interactions as springs with an equilibrium distance equal to the experimental or calculated bond length. Molecular mechanics can be used to describe physical properties of molecules based on nuclear positions, and to confirm experimental work on biomolecules or to simulate different situations, which cannot be realized experimentally.

2. Materials and Methods

The atomic structure of the actin monomer was taken from RCSB Protein Data Bank. We used the atomic coordinates set by Kabsch *et al.* (1990) in 1ATN.pdb file, which is an atomic model of the complex between rabbit skeletal muscle actin and bovine pancreatic deoxyribonuclease I, determined through X-ray analysis with a resolution of 2.8 Å. In order to be suitable for characterization, all water

molecules, deoxyribonuclease I and chemical compounds, such as N-acetil-D-glucosamine, αD-mannose, acetyl and methyl groups, which are not physiologically present in the molecule, were removed from the initial 1ATN.pdb file. The atomic model of the monomer-monomer complex (figure 2) was constructed using the 3D S1-decorated F-actin (1O1B.pdb) (Chen *et.al*, 2002) as a template. In particular Least Squares Fitting (LSQ-fitting) algorithm was used to set the relative positions of the proteins.
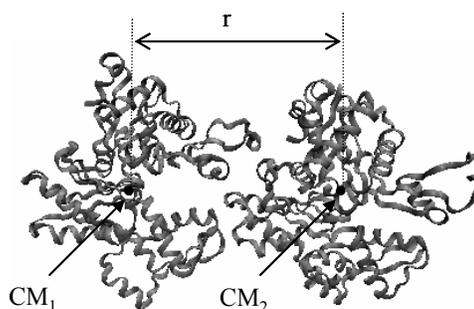
Figure 2. 3-D structure of the actin monomers complex. *CM$_1$* and *CM$_2$* represent the centers of mass of the two chains, while *r* is the intermolecular distance, calculated as the distance between the CMs

Molecular mechanics optimization setup

Potential energy and interaction properties of the actin monomers system were described with the equations of classical mechanics by carrying out the molecular mechanic simulations with Hyperchem 6.01 (Hyperchem®, Hypercube, Canada) commercial software package. All simulations were carried out on a personal computer Pentium-m, 2GHz. The energy calculations were performed with AMBER 3 (Assisted Model Building and Energy Refinement) force field, which was specially developed for protein and nucleic acid computations. During the optimizations, a distance dependent dielectric constant equal to 78 was used to define the screening effect of solvent molecules on electrostatic interactions, in the absence of explicit water molecules. The use of a distance-dependent dielectric constant mimics the polarization effect in attractive interactions, and compensates for the lack of explicit salvation by implicitly damping long range charge interactions more than short range ones.

Due to the large number of atoms involved in the analyzed molecular systems, a cutoff distance was

introduced in order to reduce the computational costs by ignoring long-range interactions. To minimize the edge effects of the cutoff, a switching function was used to allow the interactions to go smoothly to zero. A 10 Å inner radius and a 14 Å outer radius were set to define the switching function.

Each structure was energetically minimized using a sequence of two algorithms in series. For the first steps of optimization the Steepest Descent algorithm was used in order to obtain a local minimum of the system. Afterwards, the second order derivative algorithm, Polack Ribiere was used. Each optimization was halted when the potential energy gradient became lower than 10^{-3} kcal/Åmol.

Determination of the interaction forces

The interaction properties of the actin monomers were estimated using molecular mechanics approach, by evaluating the potential energy which characterizes the system for different distances within the monomers, according to Vesentini's technique (2005).

The two monomers were moved apart along the line of their interaction to obtain different intermolecular distances (r). The intermolecular distance (r) was calculated as the distance between the centers of mass (CM_1 and CM_2) of the two actin monomers:

$$r = \sqrt{\left(x_{CM_1} - x_{CM_2}\right)^2 + \left(y_{CM_1} - y_{CM_2}\right)^2 + \left(z_{CM_1} - z_{CM_2}\right)^2} \quad (1)$$

The CMs of both actin monomers were estimated using the Cartesian coordinates (x_i, y_i, z_i) and the atomic weight (m_i) of each atom i:

$$x_{CM} = \frac{\sum_i m_i \cdot x_i}{\sum_i m_i}$$

$$y_{CM} = \frac{\sum_i m_i \cdot y_i}{\sum_i m_i}$$

$$z_{CM} = \frac{\sum_i m_i \cdot z_i}{\sum_i m_i} \quad (2)$$

Potential energy was measured for each intermolecular distance after performing molecular mechanics simulations during which the entire system was left free to move. The molecular mechanics set-up was similar to the one used for the initial optimization. Due to the large dimension of the molecular system (5813 atoms) the CPU time, necessary to reach the gradient of 10^{-3} kcal/Åmol for

each intermolecular distance, varied between 40-60 hours.

The interaction energy (E) was obtained by subtracting the energy of each actin monomer (E_1 and E_2) from the total potential energy (E_T):

$$E = E_T - E_1 - E_2 \quad (3)$$

The interaction energy-intermolecular distance data were interpolated with a third order polynomial function. The binding force and the binding stiffness, which characterize the monomer-monomer interaction, were estimated as the first and the second order derivative of the interaction energy (E) with respect to the intermolecular distance (r).

3. Results and Discussion
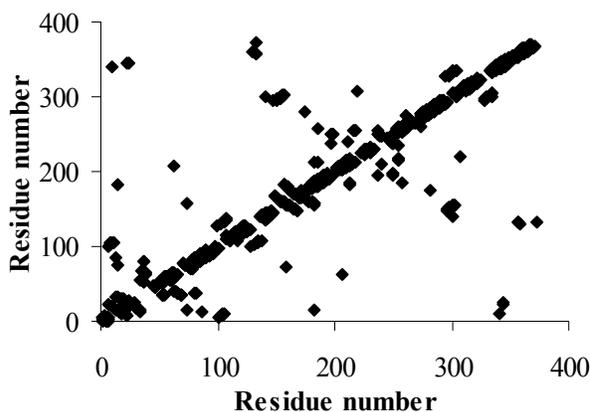
Analysis of the actin monomer structure

The analysis of the secondary structure of the actin monomer (figure 1.) shows that the protein is made up of complex motifs such as: strands (20.7%), alpha helixes (34.9 %) and 3-10 helixes (5.1%). The Ramachandran plot analysis of the atomic structure used for the present analysis (1ATN.pdb) shows that 263 residues (70.89%) are placed in fully allowed region, 88 residues (23.72%) in additionally allowed region, while 10 residues (2.70%) are placed in the generously allowed regions, thus indicating a good general conformation of the actin monomer.

A detailed analysis of the protein secondary structure motifs was computed using v.3.0 of Gail Hutchinson's PROMOTIF program, and it was possible to individuate the following structural motifs in protein structure: 6 mixed and antiparallel beta shets, 2 bete-alpha-beta motifs (strand 1: Thr 103 − Glu 107 and Asn 297 − Ser 300; strand 2: Ala 131-Ile 136 and Ile 329 to Ile 330), 6 beta hairpins considered to play a critical role in ATP hydrolysis (Kabsch and Holmes, 1995, Hurley, 1996), 1 antiparallel classic beta bulge (between Ala 19, Arg 28, Ala 29), 20 strands, 22 helices with 26 interactions between them, 26 beta turns and 3 gamma turns.

Since the hydrogen bond within the proteins is one of the most important interatomic interaction in protein folding, dynamics and function, the intramolecular hydrogen bond network of the actin monomer was analyzed. The plot (HB) hydrogen bonds was obtained by plotting the aminoacid residues involved in hydrogen bonding horizontally and vertically.

Analyzing the HB plot of the actin monomer (figure 3) the following elements of the secondary structure

were recognised: helices (which includes α-, 3₁₀- and π-helices) as adjacent strips to the diagonal, antiparallel β sheets as cross-diagonals, parallel β sheets as parallel to the diagonal, and loops which appear as breaks in the diagonal between the cross-diagonal beta-sheet motifs (Bikadi *et.al*, 2007).

Figure 3. HB plot of the equilibrated structure of the actin monomer

The statistic indicates that there are 362 H bonds which stabilize the structure of the protein, with the length ranging mainly from 2.5 to 3.2 Å. The long hydrogen bonds, greater than 3.2 Å, represent 5.83% of the total. These H bonds act within distant residues of aminoacids and are considered to be responsible for 3D structure stabilization and flexibility (McDonald and Thomton, 1994)

**Analysis of the interaction between actin monomers**

The atomic coordinates of the actin monomer from 1ATN.pdb file were used to construct the monomer-monomer complex. The relative positions of the two proteins (chains 1 and 2) within the complex were set on the basis of the 1O1B.pdb. The aminoacids found to be involved in the interaction are: Thr 148, Glu 167, Ser 323, Pro 322, Thr 324, Asp 286, Asp 288, Ile 287 and Arg 290 in case of Chain 1, and Val 45, Met 44, Val 43, Asp 244, Pro 243, Leu 242, Thr 203, Thr 202 and Ala 204 in case of chain 2 (figure 4). The interface areas of the chain 1 and chain 2 are 536 Å and 549 Å, respectively.

In order to be mechanically characterized, the complex of actin monomers was first minimized. The Ramachandran plot analysis (figure 5) of the minimized complex indicates that 88.5% of the residues are in the most favoured regions, while 11.5% are in additionally allowed regions.

The interaction properties were studied through molecular mechanics calculations on the minimized complex by evaluating the potential energy at 12 different intermolecular distances.
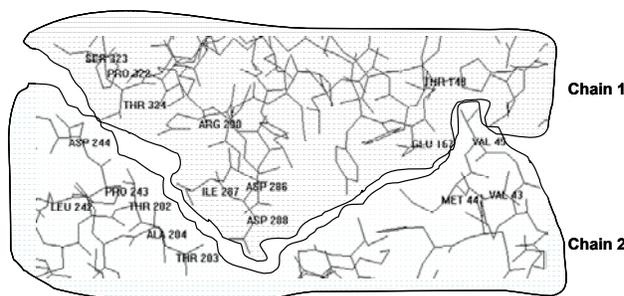
Figure 4. The atom-atom interactions across protein-protein interface
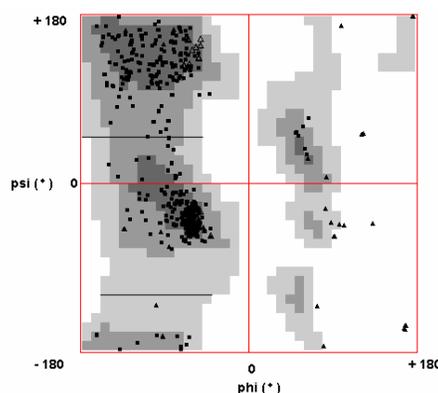
Figure 5. The Ramachandran plot generated for the complex of actin monomers (▨ fully allowed region, ▨ additionally allowed region, ▨ generously allowed region, ▲ glycine residue, Δ proline residue, ■ other residue

The potential energy is calculated as the sum of the bonded and non bonded energy terms. The bonded energy term is made up of bonding energy (E_{bond}), dihedral energy ($E_{dihedral}$) and angle-bending energy (E_{angle}), while the non-bonded energy term refers to the van der Waals dispersion forces (E_{vdW}), to the hydrogen bond contribution (E_{Hbond}) and to electrostatic potential ($E_{Coulomb}$). For each intermolecular distance tested, the contribution of each component to the total potential energy was investigated and the results are presented in Table 1.

Analyzing the numerical values presented in table 1, it possible to observe that in general the main terms of the total potential energy are the van der Waals and H bond terms. In case of performing molecular mechanics energy minimization of the complex in

which the actin monomers are placed to an inferior distance with respect to the physiological one in the filament, the values of all energy terms are higher.

This is due to the fact that, in case of bringing the two proteins closer the tertiary and secondary structure of each chain is affected.

Table 1. The contributions of individual energy components to total potential energy

r, nm	E_T, kJ/mol	E_{bond}, kJ/mol	$E_{dihedral}$, kJ/mol	E_{angle}, kJ/mol	E_{vdW}, kJ/mol	E_{Hbond}, kJ/mol	$E_{Coulomb}$, kJ/mol
4.66	-6.3	8100.4	3115.5	5198.0	-14901.4	-1099.2	-419.7
4.89	-12179.2	441.4	3066.7	4208.3	-18356.9	-1114.7	-423.9
4.99	-12137.2	441.3	3023.4	4202.0	-18250.7	-1126.2	-426.9
5.03	-12072.0	442.8	3025.5	4199.9	-18189.5	-1123.9	-426.8
5.11	-12004.1	443.5	3031.5	4219.7	-18156.7	-1114.4	-427.7
5.22	-12124.2	435.0	2965.0	4151.4	-18139.8	-1105.1	-430.7
5.31	-12108.7	438.0	3013.4	4139.0	-18173.8	-1095.4	-430.0
5.41	-12061.0	434.3	2974.6	4160.4	-18084.2	-1114.4	-431.6
5.46	-12105.9	433.6	2949.7	4144.9	-18093	-1108.9	-432.3
5.47	-12111.4	438.1	3020.4	4150.0	-18161.9	-1124.7	-433.3
5.63	-12122.8	435.5	2972.9	4153.0	-18137.1	-1114.6	-432.0
5.68	-12114.1	437.9	2989.2	4149.4	-18140.1	-1118.5	-429.9

The values of the angles, dihedrals and bond length between atoms change with respect to the equilibrium causing an increase of the total potential energy of the system. After surpassing the intermolecular distance which physiologically characterize the actin monomers within filaments, the values of the bond, angle and van der Waals energy terms present a constant behavior, the electrostatic energy terms decreases, while the dihedral and H bonds term present a random behavior (Table 1).

In physiological conditions the interaction between proteins is controlled by a complex array of intermolecular forces. In the case of the interaction between actin monomers, it was possible to individuate 127 non-bonded contacts, 27.8% of the residues involved in the interaction being neutral, 27.8 charged % (positively and negatively) and 30% aliphatic (very hydrophobic). Only one hydrogen bond 2.54 Å long was observed between ASP 288 of the chain 1 and THR 203 of the chain 2. The strongest non-bonded interactions between residues of the two chains were noted between: Ile 287 of the chain1 and residues Thr 202 and Thr 203 of the chain 2, which involves 40 non-bonded contacts; residues Pro 322 and Thr 324 of the chain 1 and residues Asp 244 and Pro 243 of the chain 2 with 38 non-bonded contacts; Glu 167 of the chain 1 and Met 44 of the chain 2 with 11 non-bonded contacts.

Starting from the potential energy of the complex (E_T) and of each actin monomer (E_1, E_2), the interaction energy (E) was calculated with equation 3 and the results are presented in figure 6. The interaction energy-intermolecular distance data points were interpolated with a third order polynomial function (figure 6) and the first derivative of the energy fitting equation with respect to the intermolecular distance was used to estimate the interaction force which characterizes the complex (figure 7). As indicated by the energy curve, the complex of actin monomers is characterized by minimum interaction energy of 740.63 kJ/mol which corresponds to an equilibrium distance of 4.98 nm. The binding stiffness (k) was calculated for the equilibrium position as the second order derivative of the energy fitting and was found to be 38.86 N/m.
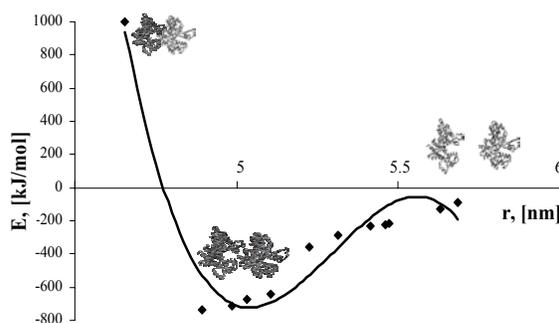
Figure 6. Interaction energy (E) data (squares) and energy fitting curve (line) as a function of intermolecular distance (r)

Concerning the interaction force which characterizes the complex (3.2 nN), our result are one order of higher magnitude with respect to the experimental

force used to unbind the actin monomers. The breaking force of the actin-actin bond, measured by Tsuda (1996) while twisting the filament through various angles using microneedles, was found to vary between 320 and 600 pN. The difference between the results in terms of interaction force can be explained by the fact that in the case of molecular mechanics all simulations were performed at equilibrium while experimentally they are twisting the protein thus submitting it to deformations.
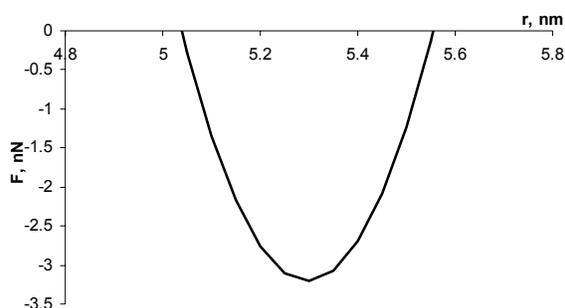
Figure 7. Interaction force (F) between the actin monomers as a function of intermolecular distance (r)

Different experimental studies give indications about actin filament stiffness. Liu *et al.* (2002) measured longitudinal elasticity of the 1-μm-long single actin filaments with microfabricated cantilevers and the resultant stiffness was 34.5±3.5 pN/nm. Kojima *et al.* (1994) estimated the axial stiffness of single actin filaments with and without tropomyosin through the use of an ultracompliant glass microneedle. The bonds between the two ends of actin filaments and the needles were not completely rigid since the attachment region was greater than 2 μm through myosins bound to the surface. In these experimental conditions the stiffness of 1-μm-long actin filaments with and without tropomyosin was found to be 65.3±6.3 pN/nm and 43.7±4.6 pN/nm, respectively. It can be observed that the values of the interaction stiffness obtained by performing molecular mechanics calculations for the complex actin monomers are higher than ones characterizing the actin filament. This is likely due to the hierarchy of the structure determined by the monomer assembly into filaments through both nonpolar dispersive and hydrophobic-exclusion interaction, as well as polar and ionic forces (Avraham and Tirion, 1995).

In order to reduce computational costs some approximations were applied. The water-like environment was simplified and the continuum implicit method was used. Other limitations occur from the use of molecular mechanics method, which assume the molecular system at 0 K and do not consider the temporal evolution of the molecular systems.

4. Conclusions

The present work consists in a computational study of the interaction properties of the actin monomers at single molecule level. This study was motivated by importance of the knowledge of details concerning binding and unbinding of the actin monomers within the filament. Our results show that the complex of actin monomers is characterized by a maximum attraction force of 3.2 nN, which is one order of magnitude higher than the experimental twisting force used to unbind the actin monomers, and a binding stiffness of 38.86 N/m.

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