Structural Conformations of ATP/Mg:ATP and Mg^{2+} Coordinating Dynamics in Motor Proteins

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ABSTRACT

The conformational diversity of ATP/Mg:ATP in motor proteins is investigated using molecular dynamics and data mining. ATP conformations were found to be constrained mostly by inter cavity motifs in the motor proteins. It is demonstrated that ATP favors extended conformations in the tight pockets of motor proteins such as F_1-ATPase and actin whereas compact structures are favored in motor proteins such as RNA polymerase and DNA helicase. The incorporation of Mg^{2+} leads to increased flexibility of ATP molecules. The differences in the conformational dynamics of ATP/Mg:ATP in various motor proteins was quantified by calculating the radius of gyration. The data mining analysis of motor proteins supports the conformational diversity of the phosphate group of ATP obtained computationally.

1 INTRODUCTION

Adenosine triphosphate (ATP) is known as the universal currency of energy in biological systems [1]. The structural conformation of ATP tightly coupled with its efficient hydrolysis is essential for both the stability and functionality of motor proteins. Motor proteins are natural proteins that exert forces and motions at cellular levels. They produce the mechanics while fulfilling various biochemical functions.[2][3][4] In addition to efficiently catalysing ATP, ATPase systems have other important roles such as importing metabolites from the outer cell environment and releasing cellular wastes that can perturb the cell function.[1] Knowledge of the structural states of ATP and its coordination [5] [6] [7] by Mg^{2+} is important for our understanding of the functions of motor proteins. A detailed analysis of the structural role of ATP in specific motor proteins is also desirable for understanding the therapeutic use of inhibitors efficient drug design, the development of hybrid bio-nano-devices, and the stability and efficiency of nano-engines.

The structural snapshots of ATP/Mg:ATP from the crystal structures of motor proteins in the Protein Data Bank (PDB) [8] provide a rich dataset of structural conformations. Molecular simulation [2] is a powerful method to study conformational dynamics of proteins at the molecular level. In this work, conformational changes of ATP/Mg:ATP in motor proteins are assessed by data mining crystal structures and performing molecular simulations. The conformational dynamics of ATP/Mg:ATP is simulated in different motor proteins such as F_1-ATPase, RNA polymerase, DNA helicase. The structural states obtained from molecular dynamics are analysed in comparison with those from crystal structures of motor proteins derived from PDB. Data mining was employed with the goal of identifying structural diversities of the ATP/Mg:ATP phosphate group in crystal structures of motor proteins.

2 METHODS

In this work, data mining is used to explore the large number of crystal structures of motor proteins available in the PDB. Structural coordinates of ATP/Mg:ATP/Mg:ANP in motor proteins, including F_1-ATPase, actin, dynein, myosin, DNA helicase, DNA polymerase and RNA polymerase, were data-mined and retrieved from PDB.

Visual molecular dynamics (VMD) was used to: (a) build the simulated systems; (b) check for steric clashes; (c) analyse the simulation trajectory; and (d) perform structural analyses. The main representation and coloring method used for presenting atomic structure of Mg:ATP was Corey, Pauling and Koltun (CPK) space filling molecular model. [10]

Molecular dynamics (MD) [2] was used in this work to characterize the dynamics of ATP/Mg:ATP in motor proteins summarized in Table I. All simulations were performed using NAMD [11] with the CHARMM [12] The TIP3P model was used as the interactions potential for water molecules because it is specifically optimized for biomolecular simulations. [13]

3 RESULTS AND DISCUSSION

3.1 Alignment of ATP/Mg:ATP molecules

The structural changes triggered by the presence of Mg^{2+} have a critical impact on the hydrolysis of ATP. Figures 1(a) and (b) indicate that ATP adopts conformations characterized by high fluctuations of the phosphate group and smaller structural orientations of the adenine. These correspond to ATP structures obtained from simulations in αβ subunits of F_1-ATPase and of RNA polymerase. When Mg:ATP is simulated in αβ subunits of F_1-ATPase and in DNA helicase it can be observed that adenine adopts two distinct orientations (Figures 1(c) and (d)). The conformational changes in the presence of Mg^{2+} coordination are more evident if the structures are rotated by 90 degrees. Analysing these figures it can be noted that different orientation of ribose ring correspond to different orientations of adenine and phosphate group.

The structural orientation of ATP is essential in the catalytic process. The results show that besides its critical role in cleaving the phosphate bond of the γ -phosphate, Mg^{2+} plays a role also in the structural orientation of ATP in motor proteins.

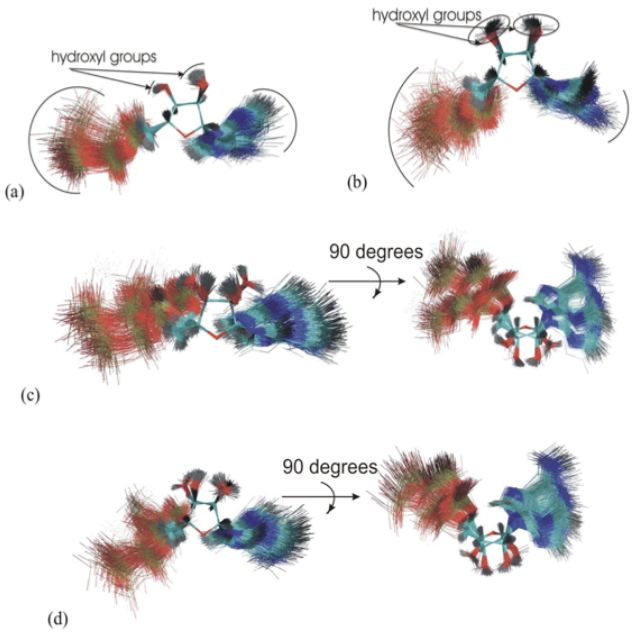

Figure 1. Alignment of 400 ATP/Mg:ATP molecules on their ribose ring. The structures were extracted from 2ns simulations. One structure was extracted at every 5000 time step. The structural changes of the ribose ring determine larger conformational changes of ATP. (a) Alignment of ATP structures simulated in αβ (BF – segments) subunits of F_1-ATPase. (b) Alignment of ATP structures simulated in RNA polymerase. (c) Alignment of Mg:ATP structures simulated in αβ subunits (BF – segments) of F_1-ATPase. The structural changes of ribose ring are shown by a side view. (d) Alignment of Mg:ATP structures simulated in DNA helicase. The structural changes of ribose ring are shown by a side view.

3.2 Distance constraint in ATP/Mg:ATP

The conformational flexibility of the phosphate group of ATP/Mg:ATP was explored from results obtained by simulating ATP/Mg:ATP in RNA polymerase, DNA helicase and F_1-ATPase motor proteins. The inter-atomic distance between P^{γ} and C4' is presented in Figure 2. Measurements of this distance revealed that the conformational changes in ATP/Mg:ATP/Mg:ANP are conserved. However ATP in the absence of Mg^{2+} adopts more extended structures than in the presence of Mg^{2+}. As shown in Figure 2, in the absence of Mg^{2+} this inter-atomic distance peaks at about 7.75 Å whereas in the presence of Mg^{2+} the same inter atomic distance peaks at a value of about 7.5 Å.

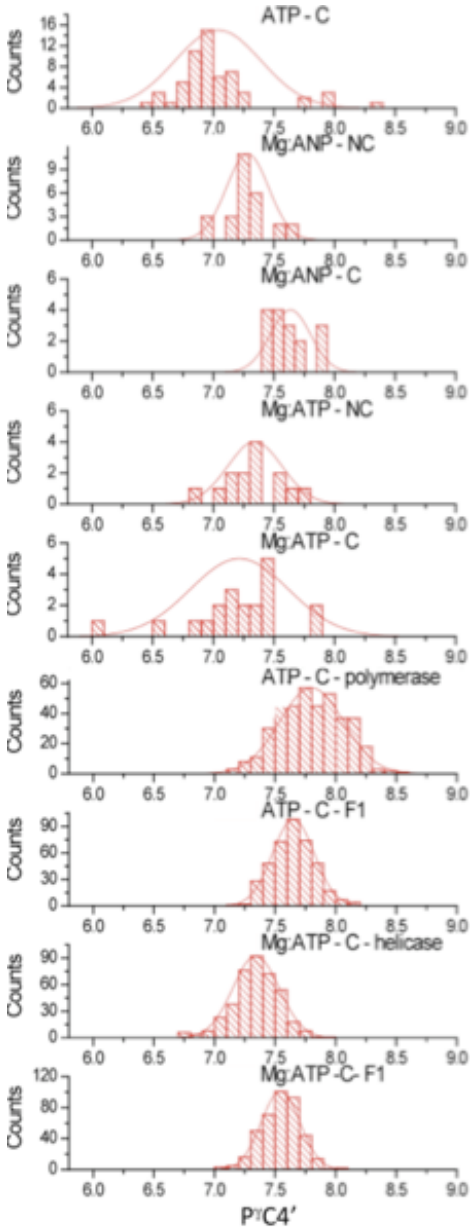

Figure 2. The probability of distribution of the distance between P$^{\gamma}$and C4' atoms of ATP/ANP. The inter-atomic distance is presented for ATP/ANP from crystal structure of motor proteins and from simulation. In the plot, C – denotes the fact that ATP/ANP is situated in a catalytic pocket while NC – indicates a non-catalytic pocket. Mg:ATP/Mg:ANP indicates that the nucleotides are coordinated by Mg^{2+}, while ATP indicates that there was no Mg^{2+} coordination in crystal or simulation. In the first five plots of this image the P$^{\gamma}$C4' inter atomic distance is from crystal structures. In the last four the same distance was determined from the simulation results. F1- indicates the fact that ATP in the absence respectively presence of Mg^{2+} was simulated in the αβ (BF segments) subunits of F_1-ATPase. Helicase – indicates that ATP was simulated in DNA helicases and polymerase – corresponds to simulation of ATP in RNA polymerase.

The possible values of this inter atomic distance as can be seen from the crystal structure investigations, which span from 6 Å up to 8.4 Å. The results show that this inter atomic distance difference is not only due to the presence or absence of Mg^{2+} but also depends on the state of the ATP binding pocket (open, loose or closed), the surrounding amino acids and the number of water molecules present in the pocket.

3.3 Radius of gyration for ATP/Mg

The flexibility and particularly the degree of extensibility of ATP/Mg:ATP can be assessed by determining the radius of gyration. Investigations of radius of gyration of ATP in crystal structures were performed separately for the catalytic and non-catalytic pocket and in the presence and absence of Mg^{2+} coordination. Alignment results showed that Mg^{2+} enhances the structural flexibility of ATP. The extensibility of ATP molecules is a reflection of the conformational structure of the pocket. The extent to which the pocket is open or closed has an important role on the conformation of ATP. Moreover the amino acids that surround the ATP in the pocket have a localized effect on ATP and determine its structural conformation. From crystal investigations it can be seen that, in the absence of Mg^{2+}, ATP has the most extended structures in actin followed by RNA polymerase, dynein and DNA helicase (Figure 3 (a)).

The degree of extensibility of ATP can be only assessed in actin for which a large number of crystal structures with ATP bound are available. For the other motors, the number of structures available does not allow an accurate statistical interpretation of extensibility of ATP. Comparing Figure 3(a) with Figure 3(b) it can be observed that the highest values for the radius of gyration are obtained for ATP from crystal structures in the presence of Mg^{2+}. The average value is ~5.1 Å for ATP in the catalytic pocket of actin motor without Mg^{2+} and ~5.2 Å with Mg^{2+}. The investigations of the radius of gyration in crystals with Mg^{2+} revealed that extended structures were mainly present in actin motors, medium extended structures in F_1-ATPase, DNA helicase and dynein motors, while less extended

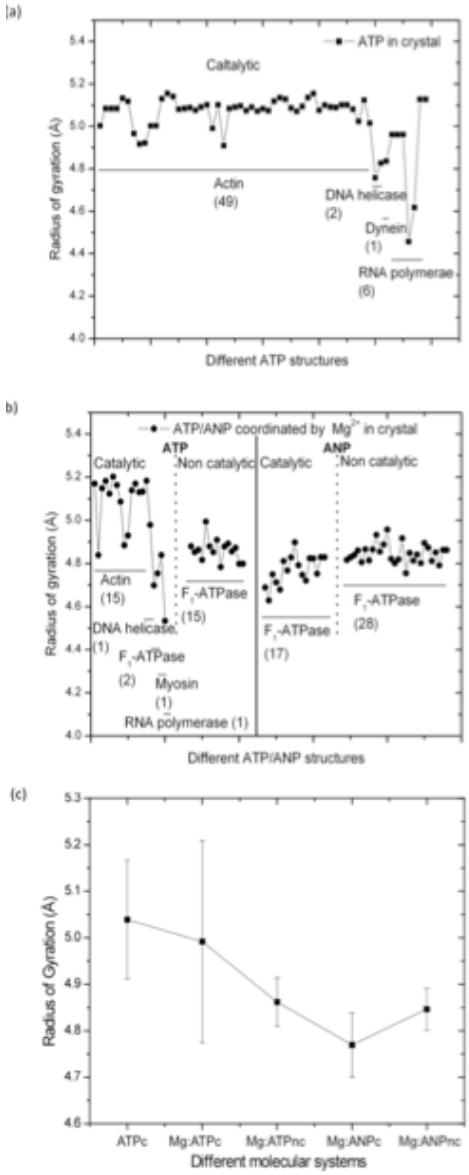

Figure 3. Radii of gyration for ATP and ATP/ANP coordinated by Mg^{2+} in catalytic and non-catalytic pockets. The structures of ATP/ANP were retrieved from crystal structures of motor proteins available in the PDB. The numbers in round brackets represent the number of ATP/ANP crystal structures used from all the available structures of the motor proteins indicated by their name. (a) Radius of gyration of ATP structures extracted from the catalytic pocket of motor proteins. (b) Radii of gyration of ATP/ANP structures extracted from the catalytic non-catalytic pocket of motor proteins. The average values and standard deviations for the data presented in (a) and (b) is shown in (c).

structures were present in myosin and RNA polymerase motors (Figure 3(b)).

In Figures 3(a) and (b) there is little difference between the degrees of extensibility of ATP in catalytic and non-catalytic pocket. This is further illustrated in Figure 3(c), which compares the mean and standard deviation of the radius of gyration of ATP and ANP from crystal structures of motor proteins. The main differences are for the mean values obtained from crystal structures of ATP in the catalytic pockets in the presence and absence of Mg^{2+} coordination.

4 CONCLUSIONS

The conformational diversity of ATP/Mg:ATP in motor proteins was studied via data mining of the PDB and molecular simulation. This was achieved by presenting structural results obtained from motor proteins in the PDB in tandem with the results obtained from simulations. The combination of molecular simulation results and data mining investigations provide a detailed picture of conformational dynamics of ATP in molecular motors.

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