Journal of Molecular Graphics and Modelling 31 (2011) 1–4

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

Minimum energy reaction profiles for ATP hydrolysis in myosin

Bella L. Grigorenko[a], Ilya A. Kaliman[a], Alexander V. Nemukhin[a,b,*]

[a] Chemistry Department, M.V. Lomonosov Moscow State University, Leninskie Gory 1/3, Moscow 119991, Russian Federation
[b] N.M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Kosygina 4, Moscow 119334, Russian Federation

ARTICLE INFO

Article history:
Received 1 June 2011
Received in revised form 14 July 2011
Accepted 14 July 2011
Available online 23 July 2011

Keywords:
Quantum mechanics–molecular mechanics (QM/MM)
Adenosine triphosphate (ATP)
Myosin
Hydrolysis reaction

ABSTRACT

The minimum energy reaction profiles corresponding to two possible reaction mechanisms of adenosine triphosphate (ATP) hydrolysis in myosin are computed in this work within the framework of the quantum mechanics–molecular mechanics (QM/MM) method by using the same partitioning of the model system to the QM and MM parts and the same computational protocol. On the first reaction route, one water molecule performs nucleophilic attack at the phosphorus center P_γ from ATP while the second water molecule in the closed protein cleft serves as a catalytic base assisted by the Glu residue from the myosin salt bridge. According to the present QM/MM calculations consistent with the results of kinetic studies this reaction pathway is characterized by a low activation energy barrier about 10 kcal/mol. The computed activation energy barrier for the second mechanism, which assumes the penta-coordinated oxyphosphorane transition state upon involvement of single water molecule in the reaction, is considerably higher than that for the two-water mechanism.

1. Introduction

Enzymatic hydrolysis of the natural 'fuel', the adenosine triphosphate (ATP) molecule, by the motor protein myosin [1] is still far from being fully understood despite numerous experimental and theoretical efforts to examine individual stages of this process. Here we focus on the part of the entire mechano-chemical ATP hydrolysis by assuming that the reagents, ATP + H_2O, as well as the products, adenosine diphosphate and inorganic phosphate, ADP + Pi, are trapped inside the protein matrix. If we also disregard changes in protein conformations on the route from the enzyme-substrate (ES) complex to the enzyme-product (EP) complex, the molecular events of the chemical step include: (i) cleavage of the phosphorus–oxygen bond (P_γ–$O_{\beta\gamma}$) in ATP; (ii) formation of a new chemical bond between the oxygen atom from the lytic water molecule and the γ-phosphorus atom; (iii) re-distribution of protons between reacting species leading to Pi formation.

Available crystal structures that map atomic configurations at presumably different stages of the reaction provide valuable information which can be employed to analyze reaction routes. For the case of ATP hydrolysis in myosin, the stable systems of ATP analogues, in particular, ADP-BeF_x and ADP-VO_4^-, were used to obtain crystal structures of the myosin-ATP complex in different states [2–4]. These structures were employed in several theoretical papers [5–9] to rationalize the reaction mechanism. In majority of these studies [5–7,9], the minimum energy reaction pathways assuming the penta-coordinated oxyphosphorane transition state (often linked to the associative type mechanism) were calculated and analyzed. The most discouraging feature of all these simulations is that the computed activation barriers on the potential energy surface for the chemical step could not be obtained lower than 20 kcal/mol. The corresponding mechanism of the chemical step of ATP hydrolysis in myosin is not completely consistent with the results of the kinetic [10,11] and mutational [12,13] studies.

Previously, following consideration of available crystal structures in which the ATP binding site is tightly locked with the help of the Arg–Glu salt bridge, Onishi et al. [14,15] put forward a hypothesis that two water molecules actively participate in the intrinsic ATP hydrolysis reaction in myosin: one is the lytic water, and another is the proton acceptor aided by the Glu residue from the salt bridge. It should be noted that location and the role of water molecules in the closed cleft of myosin were discussed in other papers as well [16–18]. The 'two-water' hypothesis gained support and development when first quantum mechanics–molecular mechanics (QM/MM) calculations of the minimum energy profile for the chemical step were carried out [8].

There are several reasons to re-consider the mechanism of the intrinsic chemical reaction of ATP hydrolysis in myosin and to calculate the corresponding minimum energy pathway. First of all, we note the important recent results of kinetic studies by Song et al. [11] on myosin catalyzed hydrolysis of ATP. From the Arrhenius plot between 20 and 50 °C the authors obtained the apparent activation

* Corresponding author at: Chemistry Department, M.V. Lomonosov Moscow State University, Leninskie Gory 1/3, Moscow 119991, Russian Federation.
Tel.: +7 495 9391096; fax: +7 495 9392035.
E-mail address: anemukhin@yahoo.com (A.V. Nemukhin).

1093-3263/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmgm.2011.07.005

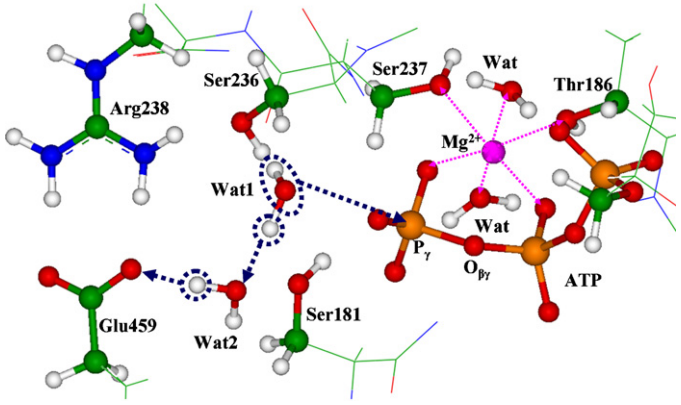

Fig. 1. QM subsystem in the ball and stick representation; link hydrogen atoms are not shown. Here the carbon atoms are distinguished by green, oxygen atoms by red, nitrogen atoms by blue, phosphorus atoms by brown, magnesium by magenta. Selection by dashed lines and arrows illustrates the two-water mechanism.

energy 10.3 kcal/mol. These data augment the widely cited experimental result for the rate constant for the chemical step of ATP hydrolysis in myosin forward reaction estimated as 160 s^{-1} [10]. The latter value may be converted to the free energy activation barrier 14.6 kcal/mol at room temperature by relying on the simplest transition state theory formula. From the theoretical side, we should note that the calculations described in paper [8] were performed with the restricted Hartree–Fock method and limited basis sets in the quantum subsystem which might be criticized because of potential low accuracy. Secondly, it is important to analyze an alternative pathway for this reaction, which assumes the penta-coordinated oxyphosphorane transition state [5–7], along with the two-water mechanism [8,14,15] using a common computational protocol.

The minimum energy reaction profiles corresponding to these two reaction mechanisms were computed in this work within the framework of the QM/MM method. The same partitioning of the model system to the QM and MM parts and the same computational protocol were employed in all simulations. On the first reaction route, corresponding to the two-water mechanism [8,14,15], one water molecule performs nucleophilic attack at the phosphorus center P_γ from ATP while the second water molecule in the closed cleft serves as a catalytic base assisted by the Glu residue from the myosin salt bridge. The second mechanism assumes the penta-coordinated oxyphosphorane transition state upon involvement of single water molecule in the reaction [5–7].

2. Methods

The X-ray structure 1VOM [3] from the Protein Data Bank of the Mg^{2+}–ADP–vanadate (VO_4^-) complex of *Dictyostelium discoideum* myosin II motor domain solved at a resolution of 1.9 Å, which represents the so-called closed form of the protein, served as a source of initial coordinates of heavy atoms when constructing the model system. In the initial structure 1VOM, the missing heavy and hydrogen residue atoms were restored; the VO_4 moiety was replaced by a PO_3 phosphate group in the γ position. The complex with the 705 crystallography resolved water molecules was energy-minimized relative to the AMBER force field [19]. Complete solvation was then obtained by adding a 5-Å layer of water, followed by an energy minimization [8]. It is important to note that the size of the VO_4 group in the initial crystal structure is larger than that of the PO_3 group, and replacement of VO_4 by PO_3 allows enough space to accommodate the second water molecule near the reactive site of ATP.

The QM part as illustrated in Fig. 1 included all three phosphate groups from ATP terminated by the methyl species, two water molecules indicated as Wat1 and Wat2, the functional groups of the salt bridge residues Glu459 and Arg238, the magnesium ion surrounded by two water molecules and the functional groups of Ser237 and Thr186, which together with the oxygen atoms of the γ- and β-phosphates formed the six-fold coordination shell of Mg^{2+}, and the functional groups of Ser236 and Ser181. In total, 74 atoms constituted the quantum subsystem, and 1823 atoms were assigned to the MM part.

The QM/MM calculations were performed with the NWChem program suite [20]. The AMBER force field parameters [19] were used to compute energies and forces in the MM subsystem. For modeling the QM part we applied the density functional theory (DFT) approaches considering two versions: the conventional B3LYP approximation and the recently suggested BB1K variant [21] potent for kinetic studies. Equilibrium geometry configurations of the stationary points corresponding to the enzyme-substrate (ES), enzyme-product (EP) and transition state (TS) complexes were computed with the 6-311++G(d,p) basis sets, and the energies at the located points were re-computed with larger sets 6-311++G(2d,2p). We found only slight differences in equilibrium geometry configurations of all stationary points obtained with the B3LYP and BB1K approaches, and the computed relative energies on the potential energy surfaces were fairly close (within 2 kcal/mol) as well.

Initial atomic coordinates corresponding to the TS structure for the two-water mechanism were taken from the results of previous QM/MM calculations [8] performed at the different computational level in the quantum part. Since direct calculations of the Hessian matrices were not technically feasible for such large molecular systems within the QM/MM approach and straightforward procedures to locate saddle points on potential energy surfaces could not be applied. Instead we computed dense grids of points along the O(Wat1)-P_γ coordinate (Fig. 1) each time optimizing all internal coordinates in the QM + MM model system. We searched for the points where change of sign of energy gradient occurred and unconstrained minimization from which led directly to the reagent (ES) and product (EP) configurations. The geometry parameters of the penta-coordinated oxyphosphorane TS are listed in the paper of Schwarzl et al. [7]. The latter were used in this work as initial coordinates to refine the search of a corresponding saddle point for our model system and our computational scheme.

3. Results

Fig. 1 shows the computed structure of the ES complex of the two-water mechanism. The reaction flow is consistent with the route schematically illustrated with dashed lines and arrows: the position of the lytic water molecule Wat1 coordinated by hydrogen bonds with the Ser236 side chain and Wat2 allows a direct attack of the hydroxyl ion on P_γ from ATP while the protons are transferred through the second water molecule Wat2. In the EP complex, still in the closed cleft of myosin, the inorganic phosphate, HPO_4^-, is formed and the side chain of Glu459 is protonated. Fig. 2 illustrates schematically transformations with the main participants in the enzymatic active site. More detailed structures corresponding to ES, TS and EP complexes are presented in Figs. S1–S3 in the Supplementary Materials.

The computed activation energy considered as the energy difference between TS and ES is either 8.1 kcal/mol (B3LYP result) or 10.4 kcal/mol (BB1K result). These values are perfectly consistent with the recent experimental studies by Song et al. [11] who reported the results of kinetic studies on myosin catalyzed hydrolysis of ATP: from the Arrhenius plot between 20 and 50 °C the authors obtained the apparent activation energy 10.3 kcal/mol. In accord with the previous calculations of minimum energy profiles for hydrolysis of different triphosphates in different environments

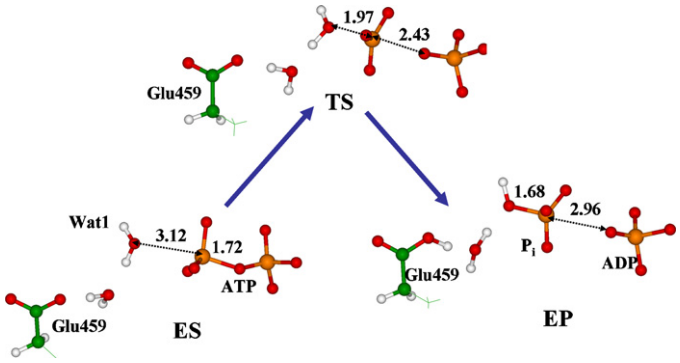

Fig. 2. Transformations with the reagents on the route from ES to EP according to the two-water mechanism. The energy of TS relative to the energy of the ES complex is 8.1 kcal/mol for the B3LYP functional and 10.4 kcal/mol for the BB1K functional. The corresponding energies of EP relative to ES are 3.2 and 2.8 kcal/mol. Detailed features of these structures are given in Supplementary materials (Figs. S1–S3).

[8,22–24], the TS structure is characterized by the almost perfect planar arrangement of the $P_\gamma O_3$ moiety with the broken P_γ–$O_{\beta\gamma}$ chemical bond. It is important to stress that the present conclusions are qualitatively consistent with the results for ATP hydrolysis in myosin [8], performed with a different version of the QM/MM theory, different program suites and different quantum chemical approaches for the QM subsystem.

Geometry parameters and relative energies of the ES, TS and EP complexes for the alternative mechanism assuming the penta-coordinated oxyphosphorane TS are presented in Supplementary materials (Figs. S4–S6). Fig. 3 illustrates the corresponding transformations schematically. On this pathway, the lytic water molecule Wat1 donates both the hydroxyl ion and the proton to the γ-phosphate group. We found that in our scheme the energy difference between TS and ES was about 34 kcal/mol, thus strongly overestimating the experimental data [10,11].

4. Discussion

Modeling chemical reactions in proteins and in solutions suitable for a direct comparison to the experimental conditions requires an evaluation of the free energy profiles. The latter can be principally computed in molecular dynamics (MD) simulations taking into account an extensive configurational averaging. Using rigorous QM based methods within the QM/MM scheme in extensive conformational sampling is prohibitively expensive nowadays [25]. However, the results obtained for the minimum energy reaction profiles are of value in many applications including that considered in this work. As formulated in Section 1 we focus on the transformations referring to the chemical step of ATP hydrolysis inside the closed cleft of myosin. Substantial conformational changes are not assumed for this stage. Consideration of related stages of ATP hydrolysis in myosin, e.g., the stage of product release, of course requires large conformational sampling and estimates of free energy profiles [26].

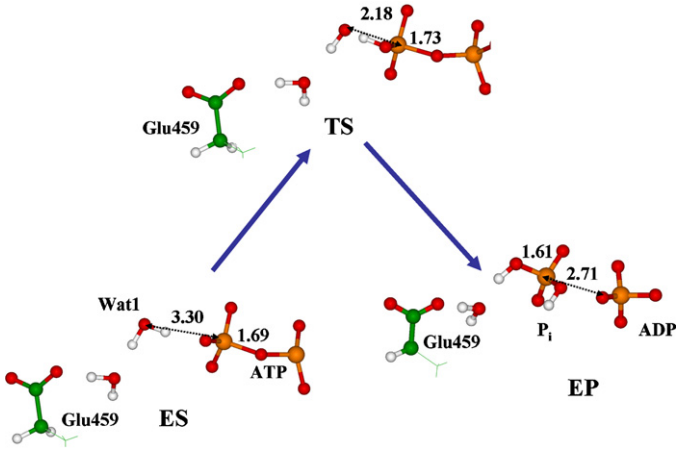

Fig. 3. Transformations with the reagents on the route from ES to EP according to the mechanism with the penta-coordinated oxyphosphorane TS. The energy of TS relative to the energy of the ES complex is 34.4 kcal/mol for the B3LYP functional and 33.7 kcal/mol for the BB1K functional. The corresponding energies of EP relative to ES are 3.7 and 3.1 kcal/mol. Detailed features of these structures are given in Supplementary materials (Figs. S4–S6).

The qualitatively correct computed minimum energy profile provides an estimate for the reaction activation barrier which is unlikely to be strongly modified when entropic terms are taken into account. In this respect the paper of Cisneros and Yang [27] provides an important contribution showing that the computed differences in the activation barriers along the potential and free energy profiles for enzymatic reactions are typically within 2–5 kcal/mol. Even if we assume such corrections for the activation barriers, the main conclusion of our work is not affected: the two-water mechanism is better consistent with the experimental results than the mechanism assuming the penta-coordinated oxyphosphorane transition state.

5. Conclusion

According to the QM/MM calculations performed at the reasonable level the chemical step of the ATP hydrolysis in the closed form of myosin is consistent with the two-water mechanism: one water molecule performs nucleophilic attack at the phosphorus center P_γ while the second water molecule serves as a catalytic base assisted by the Glu residue from the salt bridge. The computed activation energy not exceeding 10 kcal/mol agrees well with the kinetic measurements.

Acknowledgements

This work is partly supported by the grant from the Russian Foundation for Basic Research (project no. 10-03-00139). We thank the Research Computing Center of M.V. Lomonosov Moscow State University and the Joint Supercomputer Center of Russian Academy of Sciences for providing computational resources.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmgm.2011.07.005.

References

[1] H.L. Sweeney, A. Houdusse, Annu. Rev. Biophys. 39 (2010) 539–557.
[2] A.J. Fisher, C.A. Smith, J.B. Thoden, R. Smith, K. Sutoh, H.M. Holden, I. Rayment, Biochemistry 34 (1995) 8960–8972.
[3] C.A. Smith, I. Rayment, Biochemistry 35 (1996) 5404–5417.
[4] C.B. Bauer, H.W. Holden, H.B. Thoden, R. Smith, I. Rayment, J. Biol. Chem. 275 (2000) 38494–38499.
[5] N. Okimoto, K. Yamanaka, J. Ueno, M. Hata, T. Hoshino, M. Tsuda, Biophys. J. 81 (2001) 2786–2794.
[6] G. Li, Q. Cui, J. Phys. Chem. B 108 (2004) 3342–3357.
[7] S. Schwarzl, J.C. Smith, S. Fisher, Biochemistry 45 (2006) 5830–5847.
[8] B.L. Grigorenko, A.V. Rogov, I.A. Topol, S.K. Burt, H.M. Martinez, A.V. Nemukhin, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 7057–7061.
[9] Y. Yang, H. Yu, Q. Cui, J. Mol. Biol. 381 (2008) 1407–1420.
[10] D.R. Trentham, J.F. Eccleston, C.R. Bagshaw, Q. Rev. Biophys. 9 (1976) 217–281.
[11] Z. Song, K.J. Parker, I. Enoh, H. Hua Zhao, O. Olubajo, Anal. Bioanal. Chem. 395 (2009) 1453–1459.
[12] X. Li, T.E. Rhodes, R. Ikebe, T. Kambara, H.D. White, M. Ikebe, J. Biol. Chem. 273 (1998) 27404–27411.

[13] J.J. Frye, V.A. Klenchin, C.R. Bagshaw, I. Rayment, Biochemistry 49 (2010) 4897–4907.
[14] H. Onishi, T. Ohki, N. Mochizuki, M.F. Morales, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 15339–15344.
[15] H. Onishi, N. Mochizuki, M.F. Morales, Biochemistry 43 (2004) 3757–3763.
[16] T.D. Minehardt, N. Marzari, R. Cooke, E. Pate, P.A. Kollman, R. Car, Biophys. J. 82 (2002) 660–675.
[17] T.F. Reubold, S. Eschenburg, A. Becker, F.J. Kull, D.J. Manstein, Nat. Struct. Biol. 10 (2003) 826–830.
[18] J.D. Lawson, E. Pate, I. Rayment, R.G. Yount, Biophys. J. 86 (2004) 3794–3803.
[19] W.D. Cornell, C.I. Bayly, I.R. Gould, K.M. Merz, D.M. Ferguson, D.C. Spellmeyer, T. Fox, J.W. Caldwell, P.A. Kollman, J. Am. Chem. Soc. 117 (1995) 5179–5197.
[20] M. Valiev, E.J. Bylaska, N. Govind, K. Kowalski, T.P. Straatsma, H.J.J. van Dam, D. Wang, J. Nieplocha, E. Apra, T.L. Windus, W.A. de Jong, Comput. Phys. Commun. 181 (2010) 1477–1489.
[21] Y. Zhao, B.J. Lynch, D.G. Truhlar, J. Phys. Chem. A 108 (2004) 2715–2719.
[22] B.L. Grigorenko, A.V. Rogov, A.V. Nemukhin, J. Phys. Chem. B 110 (2006) 4407–4412.
[23] B.L. Grigorenko, A.V. Nemukhin, M.S. Shadrina, I.A. Topol, S.K. Burt, Proteins 66 (2007) 456–466.
[24] B.L. Grigorenko, M.S. Shadrina, I.A. Topol, J. Collins, A.V. Nemukhin, Biochim. Biophys. Acta, Proteins Proteomics 1784 (2008) 1908–1917.
[25] S. Braun-Sand, P.K. Sharma, Z.T. Chu, A.V. Pisliakov, A. Warshel, Biochim. Biophys. Acta 1777 (2008) 441–452.
[26] I. Kaliman, B. Grigorenko, M. Shadrina, A. Nemukhin, Phys. Chem. Chem. Phys. 11 (2009) 4804–4807.
[27] G.A. Cisneros, W. Yang, in: D.M. York, T.-S. Lee (Eds.), Comparison of Reaction Barriers in Energy and Free Energy for Enzyme Catalysis. Multi-Scale Quantum Models for Biocatalysis, Springer-Verlag, London, 2009, pp. 57–78.