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Zooming in on ATP Hydrolysis in F_1

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We summarize our current view of the reaction mechanism in F_1-ATPase as it has emerged from experiment, theory, and computational studies over the last several years. ATP catalysis in the catalytic binding pockets of F_1 takes place without the release of any significant free energy and is efficiently driven by the combined action of two water molecules utilizing a so-called protein-relay mechanism. The chemical reaction itself is controlled by the spatial position of a key arginine residue.

KEY WORDS: F1-ATPase; ATP; hydrolysis; QM/MM; molecular mechanics.

INTRODUCTION

In the decade that has passed since the first X-ray crystal structure of F_1 was determined in 1994 by Abrahams *et al*. (1994), our understanding of the microscopic processes underlying F_1 function has advanced steadily. In addition to the vast amount of information contained in the structures themselves, this advance can be attributed also to the fact that biochemical experiments may now be interpreted in a structural context. The atomic resolution data permit computer simulations to study F_1 function, e.g., stalk rotation (Böckmann and Grubmüller, 2002) or the ATP hydrolysis reaction in the catalytic sites (Dittrich *et al*., 2003, 2004; Strajbl *et al*., 2003).

Our present understanding of the chemomechanical coupling in F_1-ATPase, i.e., the coupling of ATP hydrolysis to stalk rotation during F_1's rotatory catalytic cycle, is still incomplete. However, recent experiments (Nishizaka *et al*., 2004; Shimabukuro *et al*., 2003) and computer simulations (Dittrich *et al*., 2003, 2004; Strajbl *et al*., 2003) have provided a much clearer picture of the underlying hydrolysis events, particularly their energetics and their coupling to the protein environment. It can be hoped, therefore, that further investigation will lead to a more complete understanding of how F_1 achieves its coupling of ATP synthesis/hydrolysis to stalk rotation and proton current through F_0.

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Here, we will summarize our understanding of the ATP hydrolysis reaction in the catalytic sites of F_1-ATPase and its coupling to protein conformational changes that follows from recent computer simulations as well as experimental findings and theoretical modeling. To assist our discussion, we will refer to "ATP hydrolysis" as the overall reaction process starting from an ATP molecule in solution and ending with ADP and P_i in solution. We will use the term "ATP catalysis" to describe the actual chemical bond breaking event in the catalytic sites of F_1-ATPase.

THE ROLE OF ATP CATALYSIS

The ATP hydrolysis reaction powering F_1 and many other molecular motors and cellular processes is exothermic with a ΔG_0 of approximately -7 kcal/mol (Berg *et al*., 2002). As single-molecule experiments (Nishizaka *et al*., 2004; Shimabukuro *et al*., 2003) have shown, the ATP hydrolysis reaction driving the rotatory catalytic cycle of F_1 can be divided into at least three distinct events: ATP binding, ATP catalysis, and unbinding of products ADP and P_i. In principle, each of these processes individually or any combination of them could be the force generating step, i.e., the one in which the free energy stored in ATP is being extracted by the protein and converted into mechanical torque acting on the F_1 stalk. Experimentally, both ATP catalysis (Weber *et al*., 2000) and the initial binding of ATP to the catalytic sites (Nishizaka *et al*., 2004; Shimabukuro *et al*., 2003) have been suggested as force generating steps.

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From an energetic point of view, however, it seems unlikely that the protein is able to extract, in an efficient manner, the free energy released during ATP catalysis, since the latter is an ultrafast process taking place on a femtosecond time scale. This was recently corroborated by computer simulations (Dittrich *et al.*, 2003, 2004) which revealed that no net free energy is released during ATP catalysis itself. In their paper (Dittrich *et al.*, 2003, 2004), the authors showed that the catalysis reaction energy profile changed from strongly endothermic in the β_{TP} catalytic site to approximately equienergetic in β_{DP}. This leaves either reactant binding (ATP), product unbinding (ADP, P_i), or both as possible candidates for force generation.

Considering the charged nature of the Mg-ATP complex and the multitude of possible hydrogen bond/salt bridge interactions of ATP's triphosphate moiety with the protein, an ATP binding-induced forced closure of the previously open β_E pocket as the force generating step seems very attractive. This type of mechanism has been proposed by Oster and coworkers (Oster and Wang, 2000) in their theoretical modeling studies and termed *ATP binding zipper*.

Assuming that the tight binding of ATP into the binding pockets is the actual force generating event during rotatory catalysis and taking into account the fact that no free energy is released during ATP catalysis, it follows that the unbinding of products ADP and P_i from β_{DP} will actually require the input of free energy. This energy could, e.g., be supplied by ATP binding to the neighboring empty β_E site. Oster *et al.* have suggested an elastic recoil driven by energy stored during the initial ATP binding event as free energy source for P_i unbinding (Oster and Wang, 2000). However, it is not clear how proteins could achieve such a localized and efficient storage of elastic energy without much dissipation.

What, then, is the role of the actual chemical step if not force generation? Computational mutation studies (Dittrich *et al.*, 2004) have shown that the spatial position of important residues in the catalytic binding sites, in particular the arginine finger residue αR373, can switch on and off catalysis by means of adjusting the reaction energy barrier and profile. More importantly, this switch mechanism can enforce cooperativity between the catalytic sites. For example, F_1 might inhibit ATP hydrolysis in β_{TP} via retracting the guanidinium group of αR373 from the bound nucleotide until solution ATP binds to the empty β_E site. This binding event causes movement of αR373 back into the β_{TP} catalytic site, thereby, reenabling ATP hydrolysis and the progression of the rotatory catalytic cycle, effectively establishing a line of communication between the β_E and β_{TP} catalytic sites.

EFFICIENT ATP HYDROLYSIS IN F_1 INVOLVES TWO WATER MOLECULES

ATP hydrolysis in an aqueous environment is initiated by nucleophilic attack of a single water molecule (or hydroxide anion) on the γ-phosphate group of the nucleotide. A similar mechanism was also assumed to hold inside the catalytic binding sites of F_1-ATPase and other ATP hydrolyzing enzymes. Support for this view came from the presence of electron density for a single water molecule in the first X-ray crystal structure of F_1 (Abrahams *et al.*, 1994), that was located between the γ-phosphate group and the carboxyl group of βE188. The latter was assumed to polarize the nucleophilic water molecule or to act as a general base, thereby, facilitating nucleophilic attack (Abrahams *et al.*, 1994).

In contrast, recent computational studies employing combined quantum mechanical/molecular mechanical simulations (Dittrich *et al.*, 2003, 2004) revealed that ATP catalysis and nucleophilic attack in the catalytic binding sites of F_1 involves two water molecules working together in a concerted fashion. This is illustrated in Fig. 1, which shows the reactant, transition, and product state conformations during the ATP hydrolysis reaction in the β_{DP}-binding site of F_1. Figure 2 shows a schematic view of this so-called *multicenter protein-relay mechanism*, in which a second water molecule, WAT2, abstracts a proton from the nucleophilic water, WAT1, thereby, acting as a base. This leads to an excess electron density on WAT1-O, facilitating nucleophilic attack on ATP-P_γ. The abstracted proton on WAT2 contributes to the formation of an hydronium ion-like construct in the transition state, and is electrostatically stabilized by the protein environment, predominantly the negatively charged carboxyl group of βE188. The electron density transferred from the nucleophilic WAT1 to the terminal phosphate group upon bond formation in turn facilitates protonation of ATP-O_γ by the hydronium ion. Interestingly, this protonation event does not involve the proton originally abstracted from WAT1, but rather one that stems from WAT2.

Dittrich *et al.* (2004) have shown that such a protein-relay mechanism is energetically favorable compared to nucleophilic attack of a single water molecule. This is mainly due to the fact that the involvement of a second water molecule offers a very efficient mechanism for nucleophile polarization and proton transfer from the nucleophile to ATP. As shown in Dittrich *et al.* (2004), the proton relay mechanism allows the nucleophilic WAT1 to perform an in-line attack on P_γ giving rise to a WAT1-O–ATP-P_γ separation in the transition state that is only slightly longer (0.2 Å) than the final bonding distance without any distortion of the planar penta-covalent

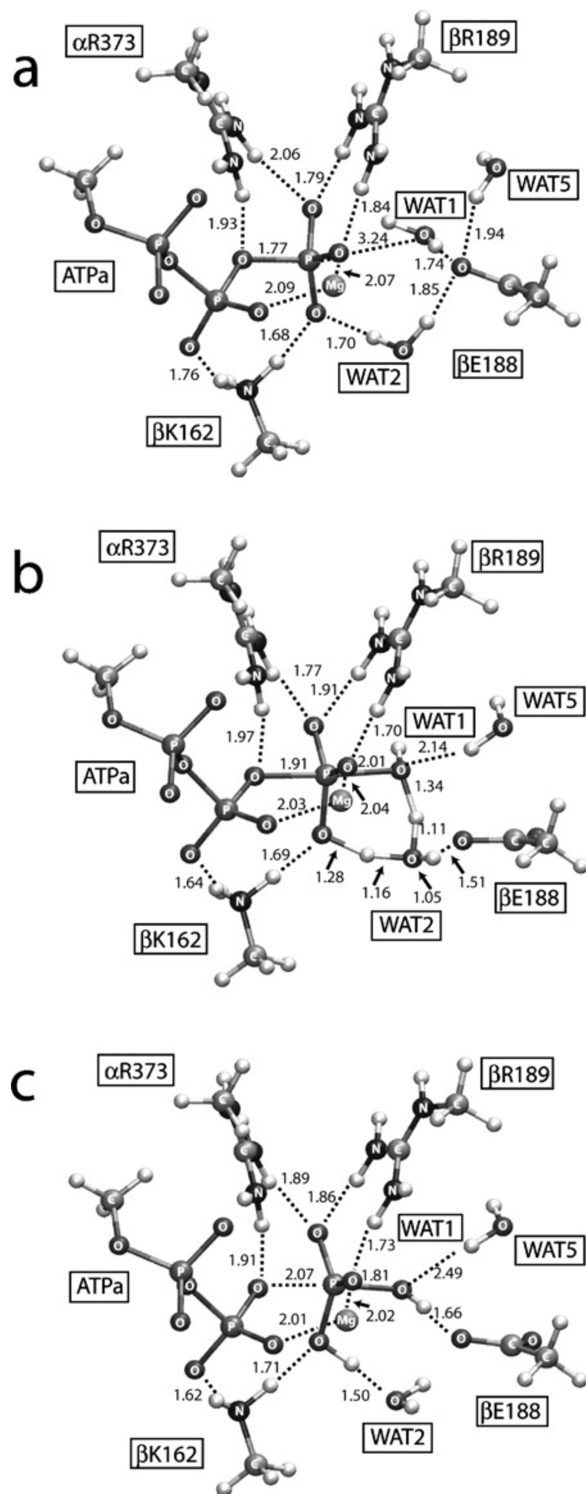

Fig. 1. ATP hydrolysis reaction in the β_{DP} catalytic site. Shown are the reactant (a), transition (b), and final (c) state conformations. In (b) the involvement of two water molecules is clearly discernible in the form of an intermediate H_3O^+-like construct. (Figure adapted with permission from Dittrich *et al*., 2004. Copyright 2004 Biophysical Society.)

arrangement of the terminal phosphate group. This facilitates the efficient transfer of electron density from the nucleophile to the site of protonation on one of the γ-phosphate oxygens and, consequently, assists proton transfer. In contrast, the nucleophilic attack of a single water molecule and the associated direct proton transfer to the γ-phosphate group requires the system to assume a non-inline and distorted penta-covalent transition state conformation. This leads to a significantly longer WAT1-O–ATP-P_γ separation compared to the final bonding distance (~0.5 Å), making proton transfer via the latter mechanism less efficient and energetically unfavorable.

In this context it is important to realize that such a proton-relay mechanism is made possible exclusively by the protein environment provided by the binding pocket and does not exist in solution, making this a genuine enzymatic pathway.

Several other studies have found evidence for the involvement of such proton relays for efficient nucleotide hydrolysis. Scheidig *et al.* (1999) proposed the participation of two water molecules during GTP hydrolysis in $p21^{ras}$ on the basis of X-ray crystal structure data. A recent theoretical study by Li and Cui (2004) investigating ATP hydrolysis in myosin also identified a proton-relay mechanism involving the hydroxy group of a serine residue as the energetically most favorable one. Finally, an involvement of two water molecules during ATP catalysis in F_1 is also consistent with recent experimental mutation studies (Ahmad and Senior, 2004), which found that residue βN243 in F_1 is involved in the orientation of the attacking and a second, associated water molecule, which could correspond to WAT1 and WAT2 in the computational studies of Dittrich *et al.* (2004).

CONCLUSIONS

Over the last years, the combination of experiment, theory, and computer simulation have provided new insights into the microscopic events during the chemomechanical coupling in F_1-ATPase. It has emerged that the actual ATP catalysis reaction does not release any significant free energy to drive stalk rotation, but rather furnishes a control mechanism governed by the spatial position of the arginine finger residue αR373 enabling catalytic site cooperativity. Efficient ATP hydrolysis is catalyzed by the cooperative action of two water molecules giving rise to rapid nucleophilic attack and proton transfer from the nucleophilic water to ATP. As studies of different ATP hydrolyzing enzymes have shown, this two-water mechanism can be expected to be of fundamental importance for protein catalyzed nucleotide hydrolysis in systems other than F_1.

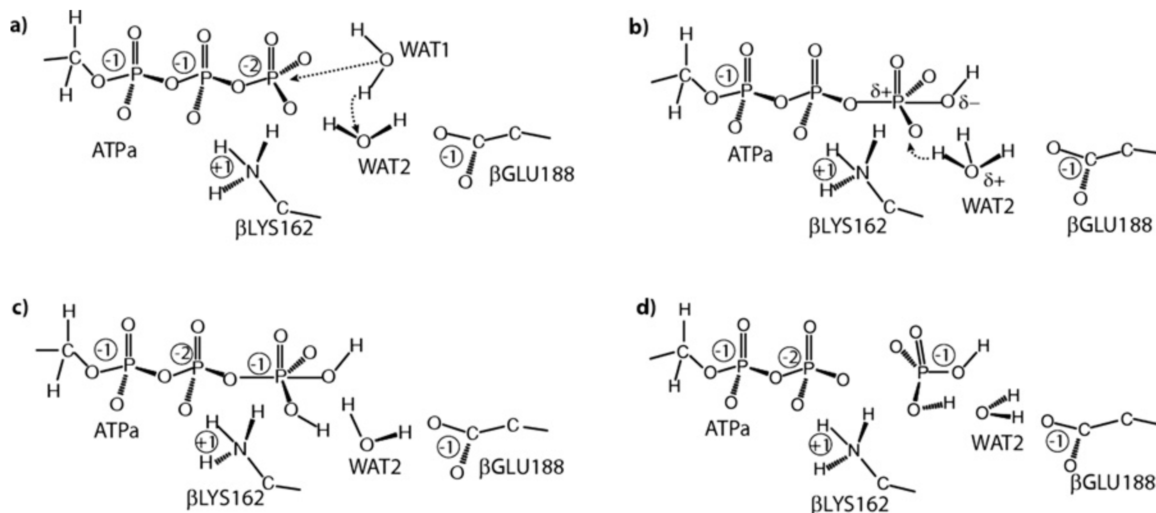

Fig. 2. Schematic representation of the multicenter reaction for ATP hydrolysis. Shown are the (a) reactant, (b) transition, (c) intermediate, and (d) product state. (Figure reprinted with permission from Dittrich *et al.* (2003). Copyright 2003 Biophysical Society.)

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REFERENCES

Abrahams, J., Leslie, A., Lutter, R., and Walker, J. (1994). *Nature* **370**, 621–628.

Ahmad, Z., and Senior, A. E. (2004). *J. Biol. Chem.* **44**, 46057–46064.

Berg, J. M., Tymoczko, J. L., and Stryer, L. (2002). *Biochemistry*, 5th edn., W. H. Freeman, New York.

Böckmann, R. A., and Grubmüller, H. (2002). *Nature Struct. Biol.* **9**, 198–202.

Dittrich, M., Hayashi, S., and Schulten, K. (2003). *Biophys. J.* **85**, 2253–2266.

Dittrich, M., Hayashi, S., and Schulten, K. (2004). *Biophys. J.* **87**, 2954–2967.

Li, G., and Cui, Q. (2004). *J. Phys. Chem. B* **108**, 3342–3357.

Nishizaka, T., Oiwa, K., Noji, H., Kimura, S., Muneyuki, E., Yoshida, M., and Kinosita, K., Jr. (2004). *Nat. Struct. Mol. Biol.* **11**, 142–148.

Oster, G., and Wang, H. (2000). *Biochim. Biophys. Acta* **1458**, 482–510.

Scheidig, A. J., Burmester, C., and Goody, R. S. (1999). *Structure* **7**, 1311–1324.

Shimabukuro, K., Yasuda, R., Muneyuki, E., Hara, K. Y., Kinosita, K., Jr., and Yoshida, M. (2003). *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14731–14736.

Strajbl, M., Shurki, A., and Warshel, A. (2003). *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14834–14839.

Weber, J., Nadanaciva, S., and Senior, A. E. (2000). *FEBS Lett.* **483**, 1–5.