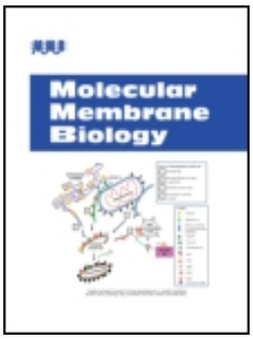

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Understanding ATP synthesis: structure and mechanism of the F1-ATPase (Review)

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Summary

To couple the energy present in the electrochemical proton gradient, established across the mitochondrial membrane by the respiratory chain, to the formation of ATP from ADP and Pi, ATP-synthase goes through a sequence of coordinated conformational changes of its major subunits (α, β). These changes are induced by the rotation of the γ subunit driven by the translocation of protons through the c subunit of the membrane portion of the enzyme. During this process, the F1-portion of the ATP-synthase adopts at least two major conformations depending on the occupancy of the β subunits: one with two nucleotides, the other with three. In the two-nucleotide structure, the empty β subunit adopts an open conformation that is highly different from the other conformations of β subunits: tight, loose and closed. The three-dimensional structures of the F1-ATPase in each of these two major conformations provide a framework for understanding the mechanism of energy coupling by the enzyme. The energetics associated with two different models of the reaction steps, analysed using molecular dynamics calculations, show that three-nucleotide intermediates do not occur in configurations with an open β subunit; instead, they are stabilized by completing a jaw-like motion that closes the β subunit around the nucleotide. Consequently, the energy driven, major conformational change takes place with the β subunits in the tight, loose and closed conformation.

Keywords: ATP synthesis; rotational catalysis; F1-ATPase; jaw-like motion.

Introduction

The ATP-synthase uses the proton gradient generated by the respiratory chain to synthesize ATP at the concentrations necessary to drive many of the cellular energy-requiring processes. The last few years have seen major advances in our understanding of the mechanism of this fundamental biological process. Important insights were contributed by many experiments, especially determination of the three-dimensional structures of several forms of the enzyme (Abrahams *et al.* 1994, 1996, Shirakihara *et al.* 1997, Orriss *et al.* 1998, Bianchet *et al.* 1998, Braig *et al.* 2000, Gibbons *et al.* 2000, Groth and Pohl 2001, Groth 2002) and direct visualization of large conformational changes that take place during the catalytic cycle (Noji *et al.* 1997, Kato-Yamada *et al.* 1998, Hisabori *et al.* 1999, Hara *et al.* 2000, Hirono-Hara *et al.* 2001, Yasuda *et al.* 2001).

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ATP-synthase is a multisubunit enzyme composed of two major parts: the F0 portion, an integral membrane complex involved in the translocation of protons, and the F1 portion, a large extramembrane complex that, when separated from the membrane, behaves as a soluble ATPase. The process of ATP synthesis involves major conformational changes in both portions of the enzyme. This paper deals with the changes that occur in the F1-ATPase.

F1-ATPase is composed of five different subunits with stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$. The molecular weights of the subunits, although they differ among enzymes from different species, are on average (kD): α, 55; β, 50; γ, 35; δ, 25; and ε, 12. Both α and β subunits bind nucleotides, but only the nucleotide binding sites of the β subunits participate in catalysis.

A large number of biochemical experiments have revealed, among many properties of the ATP-synthase, three features that have direct mechanistic relevance. First, the three catalytic sites in the three β subunits have different affinities for nucleotides. Second, ADP and Pi bound in the high-affinity site are in equilibrium with bound ATP, with a $K_{eq} \approx 1$. Third, during ATP synthesis, energy from the proton gradient is used to release ATP from the high-affinity site. These and other results were used to propose a 'binding change' mechanism in which the β subunits alternate through three different conformations during the catalytic cycle of the enzyme (Boyer 1989, 1993, 1997). It should be clear, although it is not always said explicitly, that the energy of the proton gradient is used not only to release ATP from the tight site, but also to change the loose site that contains ADP and Pi to a tight site that contains ATP about half of the time.

Structures of F1-ATPases

The three-dimensional structures of several forms of the F1-ATPase have been determined, some at atomic resolution (Abrahams *et al.* 1994, 1996, Shirakihara *et al.* 1997, Orriss *et al.* 1998, Bianchet *et al.* 1998, Braig *et al.* 2000, Gibbons *et al.* 2000, Groth and Pohl 2001, Groth 2002). In addition to being from diverse sources, the enzymes differ in the conditions used in their crystallization, in particular the concentration and nature of the nucleotides present in the crystallization media. All these crystals show similar structures for the F1-ATPase (Figure 1). The enzyme is a hexamer of alternating α and β subunits with a central hollow that contains helical portions of the γ subunit that form a coiled-coil running though the centre of the molecule.

The α and β subunits are structurally similar. Each encompasses three domains: a small N-terminal domain, a nucleotide binding domain and a helical C-terminal domain. They reportedly differ only in the conformation of one or more of the β subunits. The β subunits appear in at least four

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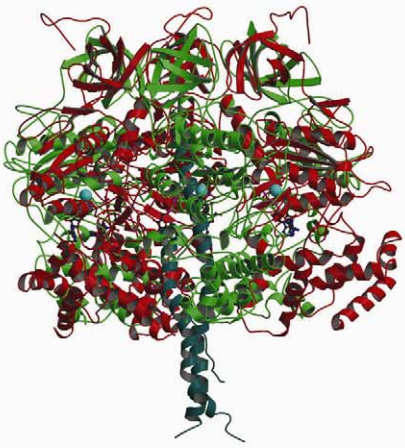

Fig. 1

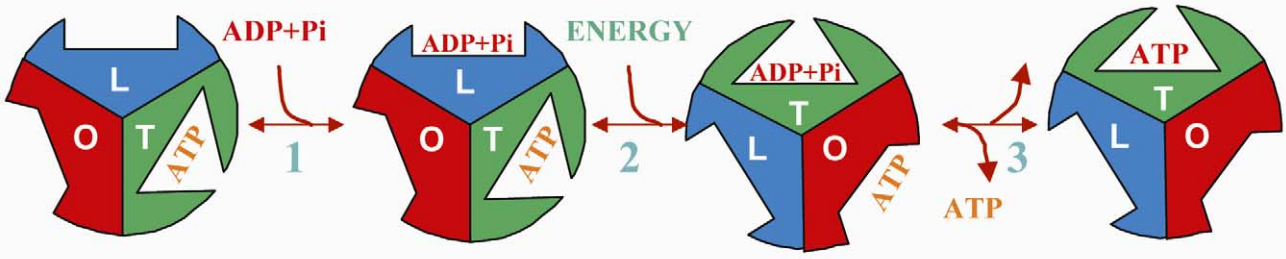

Fig. 2

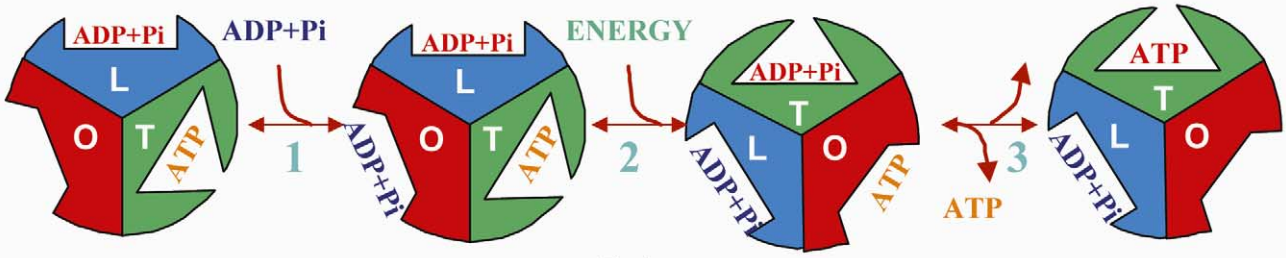

Fig. 3

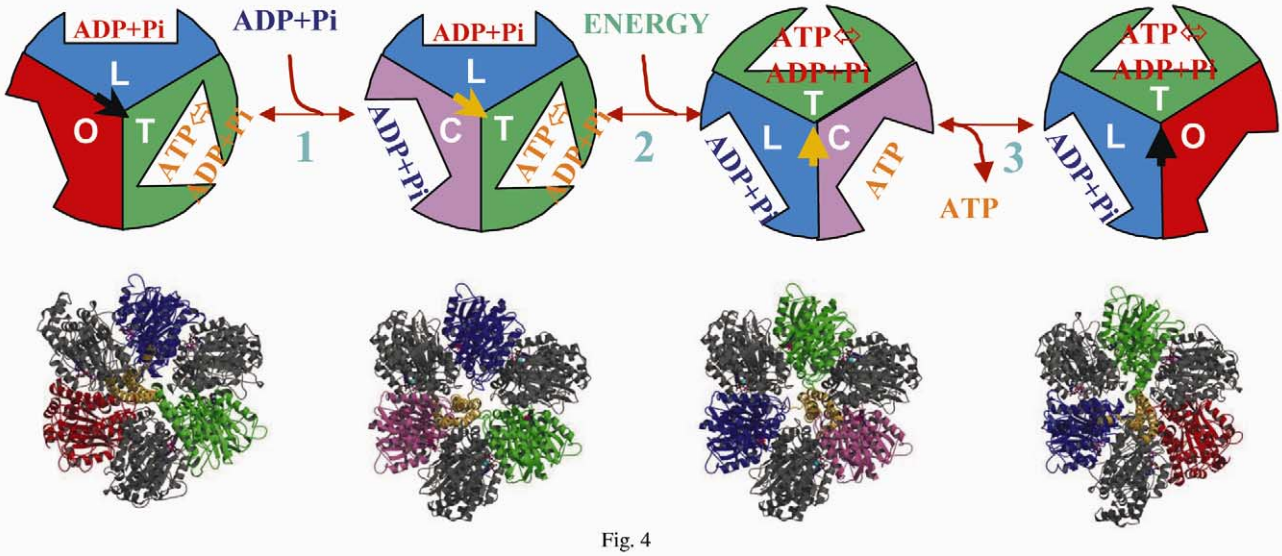

Fig. 4

different conformations: tight (T), loose (L), closed (C) and open (O). (A fifth, intermediate conformation is observed in one β subunit in the presence of a possible transition state analogue (Menz *et al.* 2001).) There is a perfect correlation between site occupancy and the conformation of the β subunit: the O conformation only occurs in β subunits whose binding site is not occupied by nucleotide. Two of the structures — one with two nucleotides bound (Abrahams *et al.* 1994), the other with three nucleotides bound (Bianchet *et al.* 1998) — are the most relevant for the coupling mechanism. The conformations of the T and L β subunits are highly similar in both structures and similar to each other (Bianchet *et al.* 1998). The major difference between the two structures is the conformation of the third β subunit, open in the two nucleotide structure, closed in the three nucleotide structure. The C conformation is highly similar to L and T, but the O conformation shows a major hinge motion ($> 20^{\circ}$) of its C-terminal domain that separates the halves of the nucleotide binding so that it no longer has in position the residues necessary for binding nucleotide.

Mechanism of ATP synthesis/hydrolysis

The structures of the F1-ATPase provided insight into the 'binding change' mechanism: the different conformations of the β subunits observed in the three-dimensional structures account for the conformations proposed to occur during the catalytic cycle. Initially, the mechanism shown in Figure 2 (2N; two-nucleotide) was proposed involving only the two-nucleotide structure (Abrahams *et al.* 1994). Many features of this proposal suggest that although it captures some of the expected characteristics of the mechanism, most notably the rotation of the γ subunit, it can not represent the actual steps that occur during catalytic cycle. Two problems are the most evident. First, for the enzyme working in the direction of ATP hydrolysis (right to left in Figure 2), the second step requires that ATP binds to the O-site without binding to the L-site, even though the affinities of the two sites differ in several orders of magnitude in the opposite direction.

Second, recent experiments have shown that the catalytic cycle of the enzyme requires simultaneous occupancy of all three catalytic sites (Lobau *et al.* 1997, 1998, Weber and Senior 2001). (Although Dong *et al.* (2002) suggest that the enzyme may function with two sites occupied, they do not show that it actually does.)

Several modifications of this mechanism, also involving only the two-nucleotide structure, have been proposed, including the one shown in Figure 3 (Duncan *et al.* 1995). One major problem with the mechanism in Figure 3 (3NO; three-nucleotide with one β subunit open) is that going in the direction of ATP synthesis, ADP and Pi must bind to the open site *and remain bound with the site open* until the major conformational change, the change that *requires the energy input*, takes places.

Clearly this problem is solved if the three nucleotide structure is used as part of the mechanism (Figure 4) (Bianchet *et al.* 1998, 2000) (3NC mechanism; three-nucleotide with β subunit closing upon binding).

In this scheme, binding of ADP and Pi to the β subunit in the O conformation drives a local conformational change that changes the β subunit from open to closed. In the direction of ATP hydrolysis, a similar conformational change would occur when ATP binds to the open site. The low affinity of the C-site for nucleotide reflects not only an 'intrinsic' low affinity, but also the cost of closing the β subunit. Conformational changes of this kind, in which two domains of a protein undergo a jaw-like motion that closes the binding site upon binding substrate, have been observed before in many enzymes, including hexokinase (Bennett and Steitz 1978, McDonald *et al.* 1979, Bennett and Steitz 1980a,b, Steitz *et al.* 1981) and phosphoglycerate kinase (Pickover *et al.* 1979). Once the β subunits are in the T, L and C conformations, the energy requiring conformational change driven by the rotation of the γ subunit requires only minor changes in the β subunits, because the T, L and C conformations are much more similar to each other than they are to the O conformation. Significantly, the mechanism shown in Figure 4 requires that the major conformational change energized by the rotation of the γ subunit can only take place when the equilibrium in the T-site is in the direction of ATP. In the ATPase direction, the enzyme can only go back with ADP and Pi in the T-site, reversing the rotation of the γ subunit and translocating protons against their electrochemical gradient.

The mechanism in Figure 4 has an additional important characteristic: phosphate bond formation/breakage, even though it is tightly coupled to energy coupling, does not take place simultaneously with the major conformational change. That is, the two steps of the reaction that are kinetically least favourable — energy coupling and bond formation/breakage — do not occur at the same time. The transition state of bond formation/breakage occurs only while

Figure 1. F1-ATPase in the three nucleotide conformation. The α subunits are in green, the β subunits in red and the γ subunit in dark green. The nucleotides in all six subunits as well as the Mg^{2+} ions (cyan) in the α subunits are shown.

Figure 2. Two nucleotide mechanism of ATP synthesis (Abrahams *et al.* 1994, Boyer 1997) (2N mechanism). Each α/β pair is shown as a 120° sector, green for the T-configuration (tight), blue for the L (loose) and red for the O (open). The rotation of the γ subunit (black arrow) drives the conformational change of step 2. Reproduced in modified form, with permission, from Bianchet *et al.* (2000).

Figure 3. Three-nucleotide mechanism using only the two-nucleotide structure. In this modification of the mechanism in Figure 2, the site occupancy is incremented by always occupying the L-site and occupying the O-site before the conformational change (Duncan *et al.* 1995) (3NO mechanism). Colours are the same as in Figure 2. Reproduced in modified form, with permission, from Bianchet *et al.* (2000).

Figure 4. Mechanism using the two and three nucleotide structures. The schematic representation is on the top and the actual structures on the bottom. In both representations, the colours of the β subunit (or α/β pair) in the T-, L- and O-configurations are as before, but the β subunit (or α/β pair) in the C-configuration is pink. In the bottom diagrams, the α subunits are grey. As seen in the actual structures (bottom), when the β subunits closes, there is a small change in the position of γ. In the schematic drawing (top), these two positions are shown as black and ochre arrows. Note that the change from the O- to the C-configuration occurs before the energy input using binding free-energy to close the subunit (Bianchet *et al.* 1998, 2000) (3NC mechanism). Reproduced in modified form, with permission, from Bianchet *et al.* (2000).

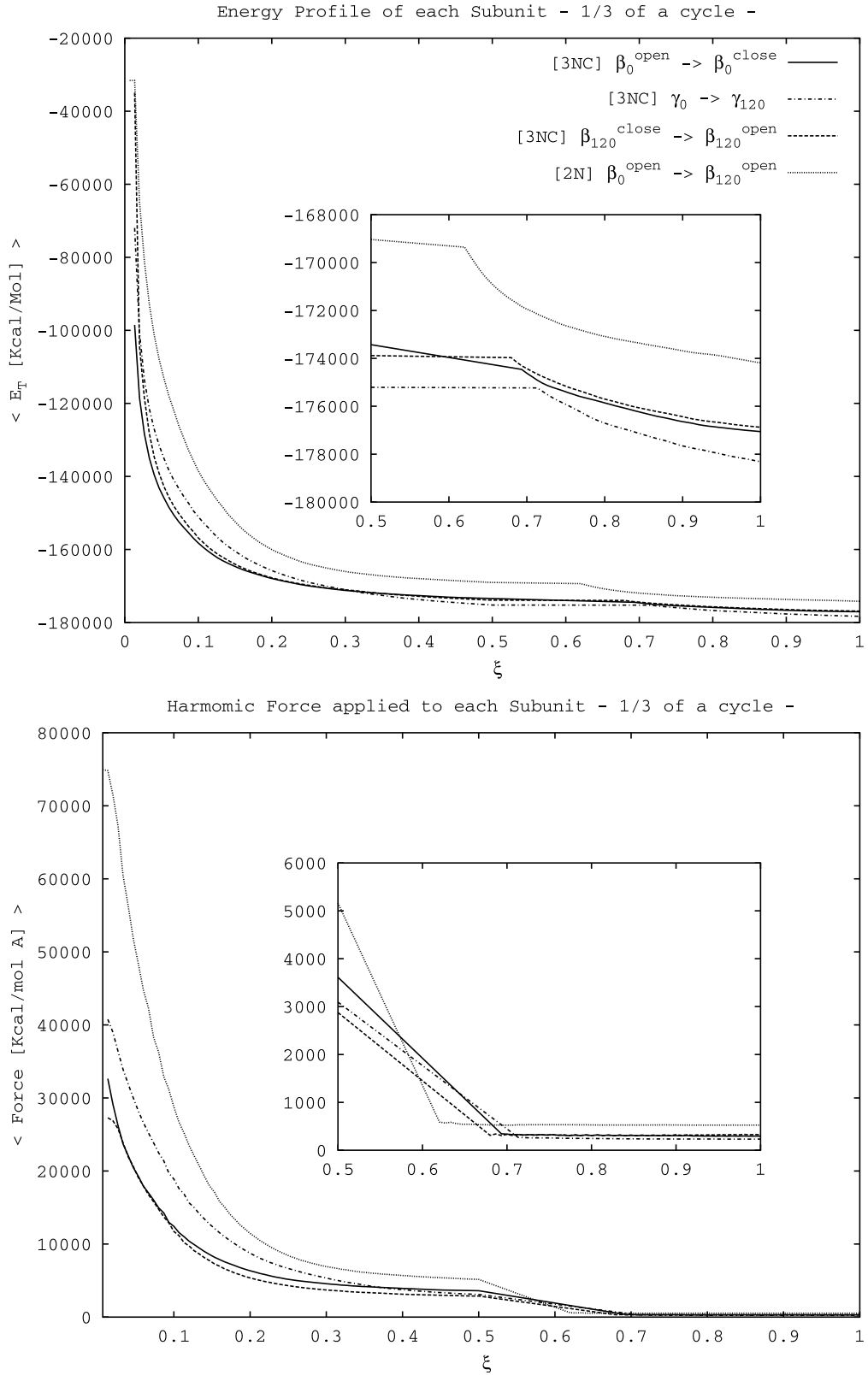

Figure 5. Identification of intermediate structures between open and closed conformations. An arbitrary reaction coordinate ξ is used to indicate the distance between the open and the closed states ($\xi = 0$ is open, $\xi = 1$ is closed). All intermediate structures are obtained by energy minimization of the initial structure using a harmonic constraint tethering the atomic positions to those of the final state. (a) Total energy, including the energy of the harmonic constraint is plotted; (b) force being exercised by the harmonic constraint during minimization.

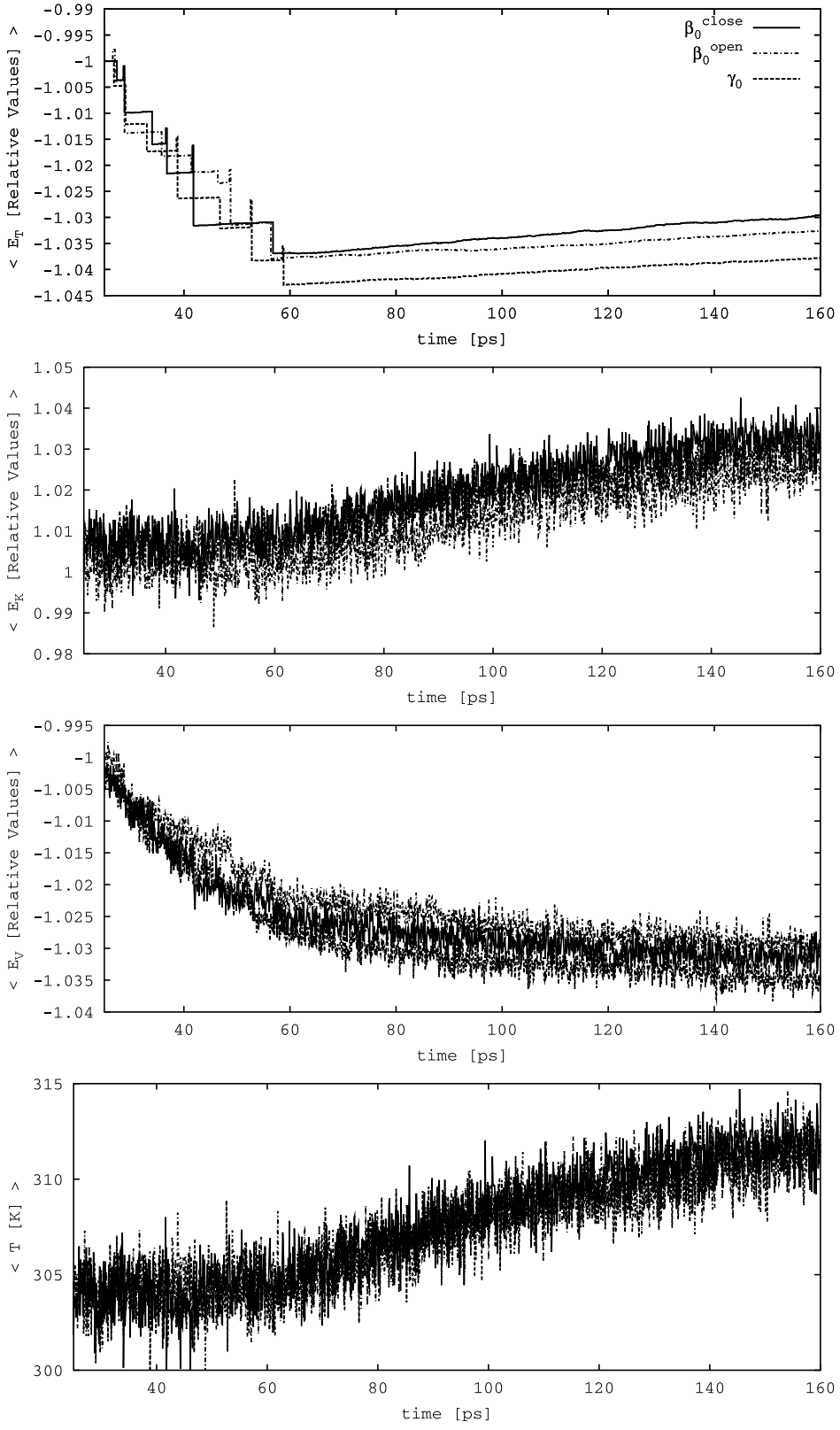

Figure 6. Examples of molecular dynamics (MD) runs. The graphs show the time evolution of the relative values of the total energy E_t, the kinetic energy E_k, the potential energy E_v and the temperature T during an MD simulation. Calculations for several of the intermediates presented in Figure 7 are shown. No harmonic constraints were used in this portion of the calculation.

the enzyme is pausing, waiting for the major, energy-requiring/releasing conformational change.

Energetics of the conformational changes

Information about the type of detailed movements that take place during the catalytic cycle of the enzyme, as well as about the energy of the intermediates that occur in the different mechanisms, was obtained using molecular mechanics/dynamics (MM/MD) calculations. We carried out calculations for the transition proposed for the mechanisms showed in Figures 2 and 4 (J. A. Leyva, M. Bianchet and L. M. Amzel, unpublished data). Coordinates for the major conformations were obtained by combining the models of the two- and three-nucleotide structures.

Instead of attempting to reproduce a complete time course (Böckmann and Grubmüller 2002) — far too long for an actual MD calculation — we used a scheme similar to what it is called 'adiabatic approximation'. The initial coordinates for intermediates between the major conformations were obtained by energy minimization of one of the observed conformations, applying a soft harmonic potential ($E = \frac{1}{2}\,\mathrm{S}k(x_\mathrm{i} - x_\mathrm{f})^2$, where $k = 1.0$ kcal Å^{-2}, and x_i and x_f are the coordinates of the initial and final conformations. The evolution of the force and the energy during 350 cycles of minimization for some of the transitions are shown in Figure 5. The equilibration and MD runs for several of these conformations are shown in Figure 6.

The conformations shown in Figure 7 correspond to intermediates in the mechanisms showed in Figure 2 (2N mechanism) and Figure 4 (3NC).

Each of the conformations shown in Figure 7 was equilibrated at 300 K by running a 60-ps MD calculation followed by 100 ps of actual dynamics. The relative energies presented in Figure 7 (normalized with respect to the ground state) represent the overall tendencies of the energy of the individual conformations and not actual values of the energy.

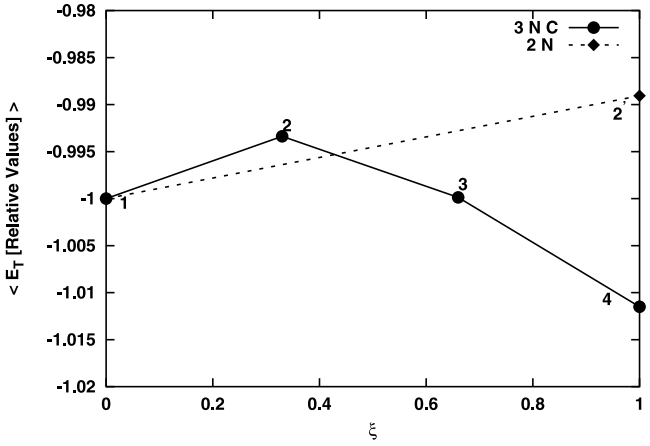

Figure 7. Equilibrium energy for selected intermediate states. The energies are relative averages (normalized to the first state) calculated over the last 50 ps of a 160-ps molecular dynamics (MD) simulation (Figure 6) run without harmonic constraints. Two models are considered: a 2N mechanism (Figure 2) and a 3NC mechanism (Figure 4). The points represent different conformations of one of the β subunits. In the 3NC mechanism, the four points are: open empty, open with ADP, closed with ADP and after the rotation of γ. In the 2N mechanism, the two points are open empty and after the rotation of γ.

The average equilibrium energy as a function of an arbitrary reaction coordinate ranging from 0 to 1, and spanning the first portion of the reaction cycles shown in Figures 2 and 4, is shown in Figure 7. Four states are represented for the mechanism in Figure 4 (3NC mechanism) and two for the mechanism in Figure 2 (2N). The results show that in the 3NC mechanism, adding nucleotide (state 2) to the open β subunit (state 1) increases the energy, but closing that subunit (state 3) lowers the energy significantly, even below the value of the open empty conformation. That is, in the presence of nucleotide and *before* the rotation of γ, the occupied closed conformation is the most stable species. The overall conclusion of these calculations is that the energies of the conformations in the 3NC mechanism are lower or equal to those of the 2N proposal. The lower energy of this pathway reflects the stabilizing energy provided by closing the open β subunit when it becomes occupied by nucleotide.

Conclusions

The results provide further evidence that during ATP synthesis/hydrolysis, binding of nucleotide to the open empty β subunit causes the subunit to close. This conformational change uses only binding free energy and does not require dissipation of the proton gradient. The major conformational change driven by the rotation of the γ subunit energized by the translocation of protons occurs after this β subunit closes such that the three β subunits in the T-, L- and C-conformations change to C-, T- and L-conformations. The mechanism shown in Figure 4, based on the two- and three-nucleotide structures, not only captures all of the known properties of ATP synthesis, but also, as shown in this work, uses a low-energy path stabilized by the closing of the open β subunit driven by binding nucleotide.

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