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Dynamic and coordinating domain motions in the active subunits of the F_1-ATPase molecular motor

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Abstract

F_1-ATPase is a rotary molecular motor crucial for various cellular functions. In F_1-ATPase, the rotation of the $\gamma\delta\varepsilon$ subunits against the hexameric $\alpha_3\beta_3$ subunits is highly coordinative, driven by ATP hydrolysis and structural changes at three β subunits. However, the dynamical and coordinating structural transitions in the β subunits are not fully understood at the molecular level. Here we examine structural transitions and domain motions in the active subunits of F_1-ATPase via dynamical domain analysis of the $\alpha_3\beta_3\gamma\delta\varepsilon$ complex. The domain movement and hinge axes and bending residues have been identified and determined for various conformational changes of the β-subunits. P-loop and the ATP-binding pocket are for the first time found to play essential mechanical functions additional to the catalytic roles. The cooperative conformational changes pertaining to the rotary mechanism of F_1-ATPase appears to be more complex than Boyer's 'bi-site' activity. These findings provide unique molecular insights into dynamic and cooperative domain motions in F_1-ATPase.
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Keywords: F_1-ATPase; Molecular motor; Dynamic domain; Coarse-grained dynamics; Structural transition; Conformational cooperativity

1. Introduction

F_1F_o-ATPase is an enzyme critical for cellular energy conversion existing in bacteria, chloroplasts and mitochondria. It works as a multi-domain rotary molecular motor [1–4], in which the hydrolysis/synthesis or proton-motive energy is converted into mechanical rotation of functional subunits [1–4]. Isolated F_1-ATPase can also work independently as a rotating motor fueled by ATP hydrolysis and has been used as a biological nanomotor [5–9]. Understanding the operating mechanisms and dynamics of F_1F_o-ATPase is important for exploring its applications as a molecular motor.

Boyer's binding change scheme [1,2] is currently the most accepted mechanism for F_1F_o-ATPase. As shown in Fig. 1A, three active β-subunits are in different conformation states at any given time, namely ATP-binding tight state TB (β_{TP}), ADP-binding loose state LB (β_{DP}), and empty binding E (β_E) state. The catalytic sites at three ($\alpha\beta$) pairs work in a sequential collaboration in catalysing ATP. Consequently the collaborative conformational changes in $(\alpha\beta)_3$ induce a torque between the hexamer $(\alpha\beta)_3$ and the central stalk γ subunit, causing the F_1-ATPase motor to rotate [1,2]. Although structural investigations [10,11] and motor experiments of F_1F_o-ATPase [5] confirmed Boyer's mechanism, profound issues were raised concerning more complex binding-change schemes (Fig. 1B) and cooperativity of the multiple subunits in the F_1-ATPase molecular motor [4,12–18]. Considerable efforts have been made to reveal the intrinsic mechanochemics and dynamics of F_1-ATPase. These include functional microbiology and single-molecular biophysics experiments [9,19], mechanochemical modelling [20–24] and atomistic simulation using molecular dynamics [25–30]. Nevertheless, how the different subunits of F_1-ATPase coordinate the conformational changes and couple the hydrolysis actions has not been fully resolved.

The major dynamics in F_1-ATPase appear to be a series of transitions throughout different conformational and binding states of the active subunits. The conformational transitions are normally broken down by domain motions along hinge-bending residues. Dynamical domain analysis algorithms, such as domain movement determination [31] and dynamic domain determination [32], have been developed to identify internal

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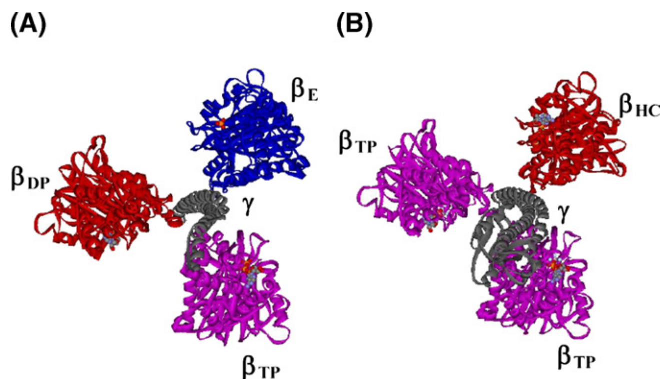

Fig. 1. The conformational states of active αβ subunits of F_1-ATPase exhibit different coordinating schemes of (A) 'bi-site' activities (e.g. PDB:1H8H) and (B) 'tri-site' activities (e.g. PDB:1H8E). In both cases, the α subunits are catalytically inactive and deliberately not drawn. The colours indicate different binding states of αβ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

domain movements in complex proteins. By comparing different conformational structures, these methods partition a protein into dynamic domains of relatively preserved geometry. The distinctive conformational structures may come from crystal structures or from trajectories of molecular dynamics simulation and normal mode dynamics (e.g. from the elastic network model) [33]. The application of dynamical domain analysis to F_1-ATPase and other motor proteins, as an effective coarse-grained way in describing the collectivity and flexibility in their conformational changes, is of fundamental and timely importance. For example, such work can provide insights into the intrinsic elasticity, collaborative and collective mechanisms of the motors' subunits [34,35] at a superior resolution than either experiments or atomistic simulations. In contrast to previous static structural [10,11,15] and manipulated molecular dynamics investigations [25–30], here we apply dynamical domain analysis extensively to all structural transitions of the active subunits and its complex of F_1-ATPase. In this way we obtain a quantitative and systematic description of dynamic domains and inter-domain motions for F_1-ATPase. We previously found a complex cooperativity in the F_1-ATPase motor [14,18]. We believe this complexity is the result of allosteric coordinating and interaction of different αβ subunits. Through dynamical domain analysis, an insightful cooperation and coupling dynamics in F_1-ATPase is also presented.

2. Methods

2.1. Structural models of F_1-ATPase

The functional F_1-ATPase is composed of subunits designated as α, β, γ, δ, and ε with a stoichiometry of 3:3:1:1:1 [10,11,15]. Three α and β subunits alternate to form a hexamer around a central cavity of helical γ, δ and ε subunits. As drawn schematically in Fig. 1, the crystal structures of three (αβ) pairs are homologous and have a strong symmetry, but are bound differently with nucleotides. The incorporation of the γδε subunits induces a structural and functional asymmetry between the three catalytic sites. Given that the α subunits are less directly involved in the hydrolysis and rotation of F_1-ATPase, the conformation of the active (αβ) pairs are classed by the binding state of β-subunit, namely the β_{TP}, β_{DP} and β_E state. The β_{TP} and β_{DP} subunits adopt similar closed conformations and β_E has an open conformation. In addition, a recent structure (Fig. 1B) provide evidence for the existence of a half-closed conformation [15], an intermediate state of β_{ADP+Pi} before reaching β_{TP} or β_{DP}.

Structures referring to different binding states of the active subunits of F_1-ATPase, namely the β_E, β_{TP} and β_{DP} states as well as the α and γ subunits, are based on bovine mitochondrial with Protein Data Bank codes of 1E79 [36], 1H8E [15], 1BMF [10], and 1H8H [37], in particular the half-closed β_{HC} is from 1H8E. Also, structures of F_1-ATPase from rat liver (PDB: 1MAB) and yeast (PDB:1E1Q) are also retrieved for extended analysis. These structures comprise the highest resolution structures and represent all possible conformations occurring in F_1-ATPase.

2.2. Dynamical domain analysis

Dynamical domain analysis was validated using the DynDom algorithm developed by Hayward et al. [32,38,39]. In the DynDom algorithm, conformational changes of protein, either from X-ray structures, normal mode analysis or molecular dynamics simulation are coarse-grained as quasi-rigid bodies on a mechanically large scale. The analysis of a conformational change in terms of domain movements is validated only when the inter-domain deformation is comparable to the intra-domain deformation. The structural basis for defining a dynamic 'domain' is based on the fact that any rigid body displacement can be decomposed by a screw motion about a certain screw axis [32,38].

The determination of dynamic domains, hinge axes, and bending residues in the active subunits of F_1-ATPase was done mainly with different conformations

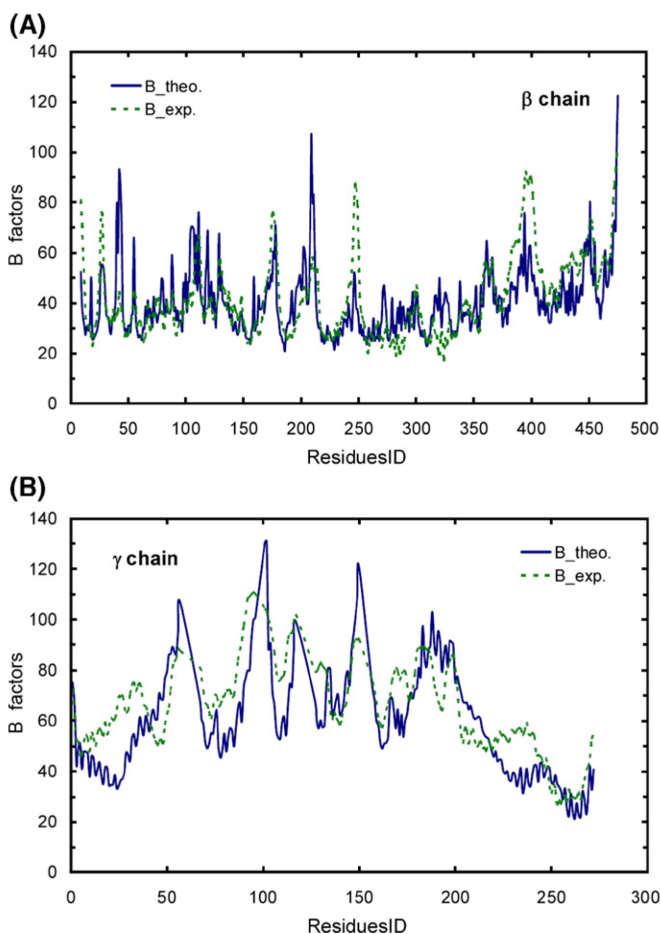

Fig. 2. The temperature factors (B-factors) calculated by the elastic network model of backbone C_α of (A) the active β_{DP} subunit and, (B) the γ subunit. A cutoff value is set at r_c=7 Å. Good agreement is found with experimental measurements (PDB:1H8H).

from crystal structures of the α, β and γ subunits and its complex, as well as from the lowest mode of elastic network computation. The structures of each α, β and γ subunit or their complex were first relaxed by molecular mechanics (MM) minimization before the DynDom calculations. The MM minimization was done in about 1000 steps by NAMD [40] with threshold energy less than 10^{-3} kcal mol^{-1} $Å^{-1}$. To confirm the reliability of coarse-grained dynamics of F_1-ATPase, we checked the dynamical fluctuation with the improved Gaussian elastic network model [41]. Fig. 2 shows that the theoretical temperature factors of the active β-subunit and the γ-subunit agree very well with the measurements. This ensures that the coarse-grained dynamics analysis of F_1-ATPase is accurate and reliable in terms of elasticity and the collective motions.

In the DynDom analysis, the window length was set initially at 5 residues until successful domain decomposition (≤15 residues). RMSD of the moving domains' best-fit was in the range of 0.9–1.5 Å, and was 0.4–0.9 Å for the fixed domains. The sub-domain matches and mismatches (e.g. hinge) were determined with pair-wise sequence identities above 80%. The domain similarity cutoff was set as the RMSD of $C_\alpha \leq 2.0$ Å, and dissimilarity cutoff should be RMSD of $C_\alpha \geq 3.0$ Å (while at least 7 residues are provided).

The dynamics minimization, the normal modes and DynDom analysis were carried out on a cluster of HP7500 workstations. Typically it takes a few CPU minutes (~5 min) to accomplish a DynDom calculation for β-subunit (of about 474 residues). The residue stability constants of the βγ subunits were calculated within 24 CPU hours on the web server [42,43] for 10,000 Monte-Carlo ensembles with window-size of 9 residues. The dynamic domains are structurally aligned by SuperPose [44] and all visualization graphics are processed by the VMD package [45].

3. Results and discussion

3.1. Dynamic domains and their motions

In the crystal structures of F_1-ATPase, the active α, β subunits have similar tertiary constructs and conformations [10,11,15]. However, superimposition of $\beta_{DP/TP}$ against β_E provides strong evidence of internal domains within the active β subunit upon binding the nucleotides. Physically, it can be expected that these inter domains are a reflection of coordinating and dynamical conformational changes in the β subunits when F_1-ATPase is in operation. The dynamic domains and their structural transition in the β and γ subunits of F_1-ATPase are identified as listed in Table 1. However, as with the static superimposition, no dynamic domains were found within the active α subunits of F_1-ATPase. In other words, the structural changes in the α subunits are not dynamically collective enough to be interpreted as any rotational or translational internal domain movement. For the sake of mechanical functions, the α subunits are likely to play the roles of structural framework and stabilisers for the βγ subunits.

In the active β subunits, dynamical domain analyses find essentially one 'fixed' domain and one 'moving' domain. The fixed domain consists of about 280 residues including Thr9–Glu131 and Asn172–Asp330, mainly the N-terminal portion and most of the ATP-binding region of β-subunit [10,15]. The fixed domains of $(\alpha\beta)_3$ show little motion and structurally form a stable crown to the whole complex. The moving domain has about 182 residues of mainly the C-terminal-leading α-helical portion of β-subunit. A complete view of dynamic domains and its structural transition between β_E and other conformational states are illustrated in Fig. 3. In Fig. 3, (A) β_E: The rotation occurs in the direction perpendicular to the plane of the paper. Rotational angle and conformational translation of (B) β_{DP} versus β_E, (C) β_{TP} versus β_E, (D) β_{HC} versus β_E are about 28.4°, 27.8°, 14.5°, respectively. The domain motions show high degree of closure, e.g. 65.3% from β_{TP} to β_E. β_{TP} conformation is slightly more open than β_{DP} conformation. There is virtually no dynamical change in between $\beta_{TP} \leftrightarrow \beta_{DP}$ except a subtle move of β-strand of Gly373–Asp394. This is consistent with the various investigations, where for the active β subunits, a significant conformational change occurs only after ATP hydrolysis or before ADP synthesis [46], namely the $\beta_{TP} \leftrightarrow \beta_E$ or $\beta_{DP} \leftrightarrow \beta_E$ transitions. These are completed via the state represented by the 'half-closed' β_{HC} structure.

The dynamic domains in the γδε subunit are less observable due to the limited number of conformations. From the current analysis, there are about one fixed domain and one moving domain in γ-subunit. The fixed domain is made of residues Ala1–Phe250. The moving domain is formed by residue Phe250–Leu272. The moving domain is the top of the long helices portion that is believed to play essential mechanical roles of contacting the ATP binding pockets and the DELSEED region (~residues 394–400) of the β subunit [4,15]. This suggests that the rotation of γ-subunit (against the αβ hexamer) is initiated and propelled by the top end of the coiled-coil helices. The possible contacts between $\beta_E/\beta_{TP}/\beta_{DP}/\beta_{HC}$ and γ are in β's fixed domain (the coiled-coil turns of ASP315↔Pro320 and ILE 275↔GLY280). This gives a picture that the fixed domain of β is tightly mounted with the top of γδε, whereas the moving domain of β opens/closes to push γ twisting its orientation.

The pathway of conformational transitions in β-subunit is believed to be $\beta_E \leftrightarrow \beta_{HC} \leftrightarrow \beta_{TP}/\beta_{DP} \leftrightarrow \beta_{HC} \leftrightarrow \beta_E$ [3,4]. Our analysis showed (Fig. 3) that both $\beta_{HC} \leftrightarrow \beta_E$ and $\beta_{HC} \leftrightarrow \beta_{TP/DP}$ transitions are bending off the centre of the $(\alpha\beta)_3$ hexamer and take trajectories away from the path of $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$. Dynamically the course of $\beta_{HC} \leftrightarrow \beta_{TP/DP}$ has more rotation and closure than the $\beta_{HC} \leftrightarrow \beta_E$ transition with $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}/\beta_{HC}$ all out of the $\beta_E \leftrightarrow \gamma$ plane. These introduce an eccentric 'ratchet' force toward γ by every β from a biased direction (about 30°). As a result, the γ-subunit alters from the convex or neutral orientation to the concave position (with respect to β) and rotates all the way in one direction. Similarly the quantitative elastic energy profiles and molecular dynamics simulation [23,25,29] also found that the asymmetric structural transitions at β-subunits drive the γ subunit like a propeller from an off-center direction.

The compelling unidirectional rotation of γδε-subunit of F_1-ATPase requires sophisticated cooperativity between three active β-subunits, provided mountain evidences [9,13–15] suggest that steady F_1-ATPase is a 'tri-site' motor. Although γδε-subunit of F_1F_0-ATPase during ATP synthesis is believed to be in the opposite direction from the case of hydrolysis, our finding above indicates another possibility for F_1-ATPase. If the structural 'ratchet' is the reason for unidirectionality, we would argue that, for either hydrolysis or synthesis, the γδε-subunit of F_1-ATPase might rotate in the same direction. In fact, β-subunit is in a fast equilibrium between β_{TP} and β_{DP} for synthesis and hydrolysis [4]. Both counter-clockwise and clockwise rotation

Table 1
Dynamical domain analysis of F_1-ATPase

Conformational states	Dynamic domains	Size (residues)	Backbone RMSD (Å)	Residues*	Domain pairs dynamics	Bending residues (green)
β_{TP} versus β_E	1 Fixed (blue)	280	1.46	11–131	Rotation angle (deg) 27.8±0.3	131–132
				173–330	Translation (Å) 1.3	171–173
					Closure (%) 65.3	330–331
	2 Moving (red)	182	1.12	132–171		
				331–472		
β_{DP} versus β_E	1 Fixed (blue)	280	1.51	11–131	Rotation angle (deg) 28.4±0.6	131–132
				173–330	Translation (Å) 1.2	171–173
	2 Moving (red)	182	0.95	132–171	Closure (%) 53.7	330–331
				331–472		
β_{HC} versus β_E	1 Fixed (blue)	286	0.68	11–132	Rotation angle (deg) 14.5	130–133
				151–153	Translation (Å) 0.8	150–154
				173–333	Closure (%) 47.9	172–181
				354–355		332–334
	2 Moving (red)	164	0.95	133–150		353–356
				154–172		
				334–353		
				356–462		
β_{TP} versus β_{DP}	1 Fixed (blue)	193	0.54	122–184	Rotation angle (deg) 14.9	372–373
				231–233	Translation (Å) −0.2	394–407
				235–236	Closure (%) 40.2	
				254–255		
				308–324		
				334–353		
				358–362		
				364–372		
				395–467		
	2 Moving (red)	22	0.89	373–394		
$\beta_{TP,\ DP}$ versus β_{HC}	1 Fixed (blue)	271±1	1.36±0.03	11–130	Rotation angle (deg) 20.5	130–131
				151–151	Translation (Å) 1.0	150–152
				172–185	Closure (%) 89.3	171–180
				187–187		185–190
				189–218		218–222
				222–255		255–257
				258–329		330–331
	2 Moving (red)	179±1	0.99±0.10	131–150		
				152–171		
				186–188		
				219–221		
				256–257		
				330–462		
$\gamma_{tri\text{-}site}$ versus $\gamma_{bi\text{-}site}$	1 Fixed	22	0.39	3–24	Rotation angle (deg) 11.5	250–251
		216	0.67	25–250	Translation (Å) −0.1	
	2 Moving	20	0.24	251–270	Closure (%) 20.5	

The color scheme of the fixed domain (blue), moving domain (red) and bending residues (green) refers to the colors in Figs. 3 and 4. 'Domain pairs dynamics' indicates the relative domain motions between the moving domains.
* *Note*. residues 1–10 in the 'fixed' domain are either missing or non-determinable.

were found for the same F_1 motor [9,47]. The direction of synthesis or hydrolysis could also be regulated by the orientation of γδε-subunit [48].

3.2. Bending residues and hinge axis

Bending residues are normally at the inter-domain boundaries, plus the neighbouring residues whose rotations are outside the main distribution. Depending on flexibility, it is also possible for the inter-domain screw axis to be located far away from the inter-domain connections. Only in the case that the inter-domain screw axis is located near to those residues involved in the inter-domain bending, the axis is regarded as a hinge axis [32,38]. In F_1-ATPase, the bending residues for $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$ are Glu131–Ile132, Asn171–Asn172 and Asp330–Ala331, respectively. Fig. 4 gives a magnified view of the bending residues and ATP pocket versus the whole β subunit. The bending residues for various structural transitions are listed in details in Table 1. Interestingly for the $\beta_E \leftrightarrow \beta_{HC}$ transition, several residues (i.e. Lys151–Gly153 and Thr354–Ser355) around the binding pocket have dynamically become more stable. This results in more complicated bending moves. For structural transition in the γ subunit, the bending residues are residues Phe250–Asn251. This bending area is exactly the joint that makes γ take either a concave or convex orientation about the β subunit [10].

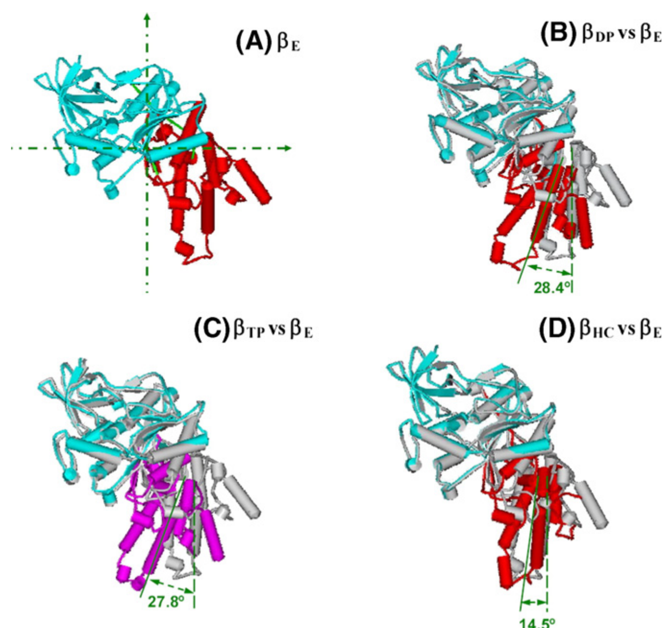

Fig. 3. Dynamic domains identified in the active β subunits of F_1-ATPase. (A) In β_E: portion in cyan colour is the fixed domain and red indicates the moving domains. Rotational and conformational transition between (B) β_{DP} and β_E, (C) β_{TP} and β_E, and (D) β_{HC} and β_E are shown in angles where β_E is referenced in grey color. In β_{DP}, β_{TP}, and β_{HC}, residues forming the fixed domain, moving domain and bending residues are depicted in colours of cyan, red (pink) and green, respectively, with reference lines (dotted green) crossing at the centre of rotation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

As for the hinge axis in F_1-ATPase, Fig. 3 shows that the rotational/twist axis in the β subunit is almost passing residues Asp330–Ala331 (the bending residues) and very close to the bending residues Asn171–Asn172 (still quite a distance from the bending residues Glu131–Ile132, more clearly seen in Fig. 4). Thus the twist axis in the β subunit, which is crossing the centre of rotation and is perpendicular to the dotted reference lines in Fig. 3, could be regarded as the hinge axis in F_1-ATPase. Nonetheless, there is no hinge axis identified in the γ subunit.

3.3. Stability of the active subunits

Based on the distinct conformational transitions and dynamic domains of the active subunits, the residue stability of F_1-ATPase can be determined from the dynamical ensembles. To do this, a COREX/BEST algorithm [42] was employed where the fluctuations for different conformational states, e.g. of β_E, β_{TP}, β_{DP} and its complexes with γ, are sampled by statistical thermodynamics rather than structural states [43]. The dynamical nature of stability is that the residues with high stability constants will be folded in the majority of the highly probable states, and residues with low stability constants will be less folded in many of the highly probable states.

Fig. 5A shows the residue stability of the βγ subunits upon binding ATP. There is no difference in stability between the β_{DP} conformation and the β_{TP} conformation, neither are for three α subunits. Examining stability residue by residue, the major stability changes of the β subunit upon binding ATP is the 'flip-flops' of stability constants from residue Gly150 to Tyr180, and from residue Ile275 to Thr325, respectively. As a result, a mechanical 'clutch' mainly formed by P-loop (from Gly159 and so forth) and a β-strand-α-helix motif supporting the P-loop (from Gly190 and so forth) occurs around the ATP-binding pocket in F_1-ATPase (Fig. 5B, details reported in the following section). In γ-subunit, stability constants change behaviour in-between residue Glu175 and residue Gln225. This is just the part of the helix connected to the bending residues of γ. This change likely incorporates the twist motion of γ when the αβ subunits undergo structural transitions from $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$, or *vice versa*. However, whether this is triggered by or is the original drive of the $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$ transitions remains unknown.

3.4. Mechanical function of P-loop and the ATP-binding pocket

In F_1-ATPase, two G-rich motifs of P-loops [49] (Ala158–GVGKT–Val164 and Arg189–GGAGVGKT–Val198, Fig. 5B) are regarded as the major structural constraint for ATP binding. In addition to chemically binding/unbinding nucleotides, P-loops may function as a possible bending axis or mechanical amplifier to transduce the structural changes to larger scale. There is considerable evidence for its chemical function, e.g. in ATP/ADP binding and catalytic residues (e.g., of βGlu188, βArg189 and αArg373) [15,37,46] and Pi binding [50]. For the mechanical function, no convincing evidence is available, even though the internal rotation of β was believed to be driven by the sliding of P-loop over the ATP phosphates [23].

The dynamical domain analysis does not show that the P-loop plays a bending or hinge role in β. As in Fig. 4, the actual bending residues are quite distanced from the ATP pocket. While the Ala158–GVGKT–Val164 loop is in the moving domain, the Arg189–GGAGVGKT–Val198 loop is in the fixed domain. From the residue stability properties (Fig. 5A), the Ala158–GVGKT–Val164 loop becomes more stable upon binding but the Arg189–GGAGVGKT–Val198 loop does not. Thus for the first time we can see that the P-loop and the pocket (Fig. 5B) work together like a mechanical 'clutch' for nucleotides. The

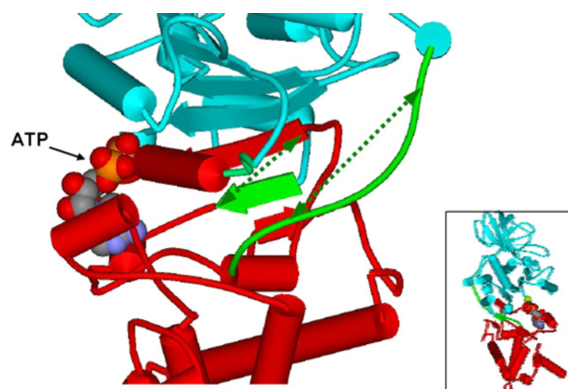

Fig. 4. Close-up view of bending residues (colored in green), hinge axis and the ATP-binding pocket. The dashed green lines indicate the direction of closure and sliding for the moving domain. Inset shows a perspective of the bending residues and ATP pocket versus the whole β subunit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

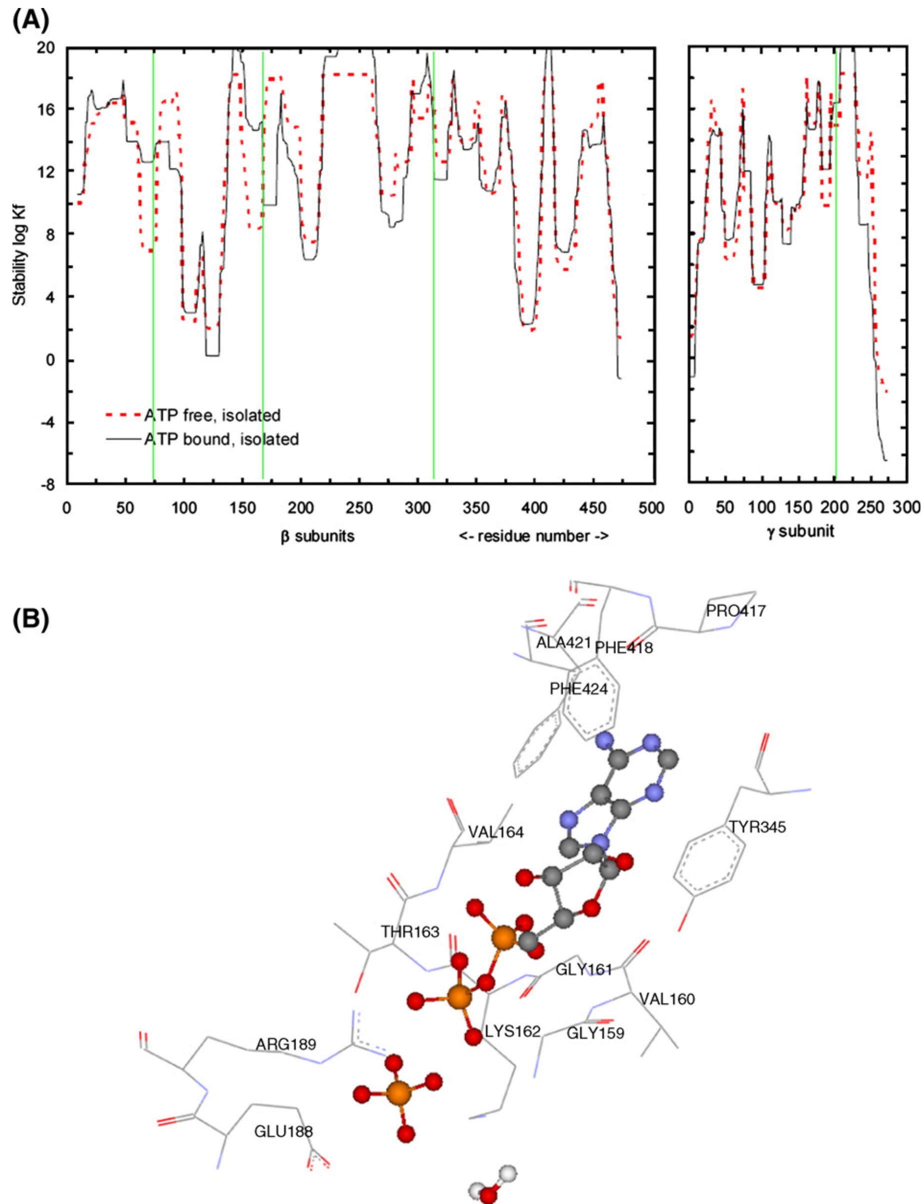

Fig. 5. (A) Residue stability of the active β and γ subunits when F_1-ATPase is bound (the solid lines) or bound free (the dash lines). The larger the value of $\log K_f$ indicates more stable dynamics and slow response to external mechanics. (B) The major changes in stability occur around the ATP-binding pocket, where the residues show a kind of 'flip-flop' in stability constants. These are marked out by the green lines in (A). Note that a dramatic stability change of β-strand around Val75 is due to its connection of the N-terminal barrier and the ATP-binding barrier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

binding/unbinding of nucleotides makes this 'clutch' go on and off, while the ATP-binding pocket takes the open or closed conformations. The conformational changes from this 'clutch' move should be further communicated and amplified into the structural transition of β-subunit. During this course, the P-loop of Ala158–GVGKT–Val164 serves as a mechanical device as believed [23] to drive inter domain motion, yet the real dynamical pathway is complicated and needs to be resolved.

In the ATP-binding pocket (Fig. 5B), whether or not the ATP reactions are the direct and original drive to the structural transitions of β-subunit has been a question. From our analysis, ATP reactions (making the $\beta_{TP} \leftrightarrow \beta_{DP}$ transition and producing a movement of the cleaved γ-phosphate and βGlu188 and βArg189 [15]) do not directly lead to the mechanical 'clutch' of P-loop and the ATP-binding pocket. The conformational changes of this 'clutch' can be only triggered by ATP binding/unbinding. This supports the arguments that only the binding/unbinding of nucleotides drive the inter-domain motions of the active subunits [4,23,51].

3.5. *The torque generated by a structural $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$ transition*

From the dynamical domain analysis, the primary structural transition in F_1-ATPase is $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$. This transition is presumably powered by the energy released from reactions of

$ATP^{4-}+H_2O \rightarrow ADP^{3-}+H_2PO_4^-$. The tight coupling between the $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$ transitions of three β subunits is believed to provide the mechanical torque driving γ-subunit rotation [2,9,22]. If we assume a mechanical equilibrium exists between the β and γ subunits for F_1-ATPase under steady conditions, then the work from the $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$ transitions should equate to the net torque that γ -subunit applies on the loading objects. In terms of biomechanics, this torque should be determinable once we have identified the inter-domain rotation and translation in $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$ (i.e. a rotation of ~28° and a translation of 1.2 Å of the moving domain), which is not measurable by any present technologies. For β-subunit undergoing an inter-domain rotation, the magnitude of torque τ for the $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP}$ transition can be estimated by,

$$\tau = \left(\sum_i m_i |\mathbf{r}_i - \mathbf{r}_{\mathrm{cm}}|^2 + \sum_i m_i d^2 \right) d \left(\frac{\Delta \theta}{\Delta t} \right)^2 \tag{1}$$

where the sum applies to every atom, *i*, of the moving domain, $\mathbf{r}_i$ is the position of atom *i* and $\mathbf{r}_{cm}$ is the centre of mass. *d* is the distance from the centre of mass to the centre of rotation (i.e. the dotted intersection point in Fig. 3A). For the moving domain, the first-order approximation is that each atom rotates the same angle $\Delta\theta$ in the same time of Δt. Given that the structural conformational changes of the β subunit follows the cycle of $\beta_E \leftrightarrow \beta_{TP}/\beta_{DP} \leftrightarrow \beta_E$, Δt should be about half of the rotational period of γ -subunit.

In experiments of F_1 driving actin filaments or micro beads [6,8,47,51], the rotation speed of the γ subunit is about 10 r.p.s. at the steady and saturated [ATP] condition. This was translated into a period of about 0.1 s and a mean torque of about 80 pN nm by a 120° step of the γ subunit. Using the dynamic domain data of F_1-ATPase (Table 1) and Eq. (1), we determine that the torque in the $\beta_E \leftrightarrow \beta_{DP}$ transition is about 55 pN nm, and 52 pN nm for $\beta_E \leftrightarrow \beta_{TP}$ respectively. The work done by this torque is close to theoretical elastic energy when the β subunit fully bends [23] and the experimental torque of F_1F_O-ATPase obtained by the curvature and the flexural rigidity of filaments [52]. For the solo running F_1-ATPase motor, there appears a difference of approximately 40% between the theoretical values (i.e., ~50 pN nm) and experimental observation (~80 pN nm). This implies that, either the F_1 motor works at a mechanochemical coefficient much less than 100% that was reported [6,8,47,51] (e.g. some energy might be dissipated in inter-domain friction), or the torques from three αβ subunits are applied to the γ subunit in the plane of not perpendicular to the $\alpha_3\beta_3$ hexamer. Whatever the reason, it indicates that three active subunits have complex cooperative interactions and different operating scheme from Boyer's 'bi-site' activities.

4. Conclusion

Using dynamical domain analysis, dynamic inter-domains, hinge axes and bending residues in the primary structural transitions of the active subunits of F_1-ATPase were identified and determined. P-loop and the ATP-binding pocket of β-subunits were found to play unique mechanical roles in driving inter-domain conformational changes. Our dynamical domain analysis also helped to determine the residue stability and rotary torque of F_1-ATPase. The cooperative mechanism and conformational changes causing the unidirectional rotation of F_1-ATPase appeared to be more complex than Boyer's 'bi-site' activity. In conjunction with structural biology and molecular dynamics simulation, these findings provide fresh insights into the cooperative dynamics and coupling domain motions in F_1-ATPase at the molecular level.

In addition, dynamical domain analysis provides a coarse-grained computational algorithm to speed up molecular dynamics simulation of motor proteins. Given the large size, molecular dynamics of F_1-ATPase were only attempted by manually spinning the γ subunit [25] or forcing the β subunits via a targeted trajectory [26,30]. These treatments appear to be inadequate, for it is likely that the coordinated structural transitions in $(\alpha\beta)_3$ induce the torque and make γδε rotate, not vice versa. An improved strategy would be to mimic the cooperative inter-domain motions in $(\alpha\beta)_3$ (e.g. using the dynamic domain quantities from this study) and to check dynamics and conformational changes in γδε (e.g. whether or not it undergoes rotation coupled by the structural transitions in $(\alpha\beta)_3$).

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