MODAL ANALYSIS OF THE C-TERMINAL HELIX OF THE F_1-ATP SYNTHASE GAMMA SUBUNIT

ANÁLISIS MODAL DE LA HÉLICE TERMINAL C DE LA SUBUNIDAD GAMMA EN LA ENZIMA ATP

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ABSTRACT

In the analysis of protein dynamics an important goal is the description of slow large-amplitude motions. These motions describe configuration rearrangements which are essential for the function of the protein. The rearrangements can change the exposed surface of the protein and, therefore, influence the interactions with its environment. In this article, we study low-frequency modes of the C-terminal helix of the bovine ATP synthase Gamma subunit. Three methods are compared: Brownian modes, calpha modes and simplified potential modes. Results suggest that the F_1 end of the helix is more rigid than other parts. This is coherent with recent studies where is shown how the F_1 end of the helix works as a crankshaft converting the bending and unbending of the Beta subunit into rotational motion. Moreover, along the helix, flexible lengths are followed by rigid lengths. This feature may serve to smooth out the torque produced at the F_1 end, delivering a nearly constant torque necessary to achieve the highest efficiency possible.

Key words.- ATP synthase, Modal analysis, Brownian modes, Calpha modes.

RESUMEN

En el análisis dinámico de proteínas, un objetivo importante es la descripción de los movimientos lentos de grandes amplitudes. Estos movimientos describen configuraciones de reordenamiento que son importantes para la función de la proteína. Estos reordenamientos pueden cambiar la superficie de exposición de la proteína, y por lo tanto, influenciar las interacciones con su entorno. En este articulo, estudiamos los modos de baja frecuencia de la hélice Terminal C de la ATP Sintasa de la subunidad Gamma. Tres métodos son comparados: Modos Browinanos, Modos Calpha y Modos de Potencial Simplificado. Los resultados sugieren que el extremo F_1 de la hélice es más rígido que otras partes. Esto es coherente con los recientes estudios donde se muestra como el extremo F_1 de la hélice trabaja como un eje cigüeñal convirtiendo el movimiento de doblado y desdoblado de la subunidad Beta en movimiento rotacional. Además, a lo largo de la hélice, longitudes flexibles son seguidas de longitudes rígidas. Esta propiedad puede servir para suavizar el torque producido en el extremo de F_1, entregando un torque casi constante que es necesario para alcanzar la más alta eficiencia posible.

Palabras clave.- ATP sintasa, Análisis modal, Modos brownianos, Modos calpha.

INTRODUCTION

ATP synthase is the smallest rotary motor in nature. This biomolecular motor, whose high efficiency approaches 100%, converts torque into ATP, which is the universal fuel currency of life. ATP provides the chemical energy that fuels muscle contraction, transmission of nerve

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messages and many other biological functions. ATP synthase is the power plant of metabolism. In an active day an adult human can produce and consume more than its body weight in ATP.

Almost all of this quantity is produced by this enzyme.

Normal mode analysis has become one of the standard techniques in the study of the dynamics of biological macromolecules. It is primarily used for identifying and characterizing the slowest frequency motions in a macromolecule system, which are almost inaccessible by other methods. These motions typically describe rearrangements of domains which are essential for the function of the protein. Only such global motions can change the exposed surface of the protein significantly and hence influence interactions with its environment.

On the other hand higher frequencies represent more localized motions in the interior or on the surface of the protein involving few atoms. These localized motions play an important role in signal transmission mechanisms.

In this article we analyze the lowest frequency modes of the C-terminal helix of the Gamma subunit on the bovine ATP synthase (1E79). First we explain briefly how the ATP synthase works. Next, in the section Normal modes section, three methods are presented: Brownian modes, Calpha modes and simplified potential modes. After, we show the results comparing the three methods exposed.

Finally in Conclusion section we discuss the functional relevance of the normal mode results and summarize our findings.

A BRIEF DESCRIPTION OF THE ATP SYNTHASE MOLECULAR MOTOR

ATP (Adenosine TriPhosphate) is used to provide energy for different biochemical reactions such as muscle contraction, transport of nutrients and neural activity to name just a few. ATP synthase is the enzyme that synthesizes (or hydrolyzes) ATP.

ATP synthase efficiently converts a cell's transmembrane proton gradient into chemical energy stored as ATP. The protein is made of two molecular motors, F_0 and F_1, which are coupled by a central stalk, known as the Gamma subunit. This feature makes ATP synthase the smallest rotary machine ever known.

The membrane embedded F_0 unit converts the proton-motive force into mechanical rotation of the central stalk inside the solvent-exposed F_1 unit.

The rotation causes cyclic conformational changes in F_1, which drives the ATP synthesis. The ATP enzyme can also rotate in the reverse direction hydrolyzing ATP and consuming the released energy to pump protons across the membrane [1, 2, 3, 4,5,6], see Fig. 1.

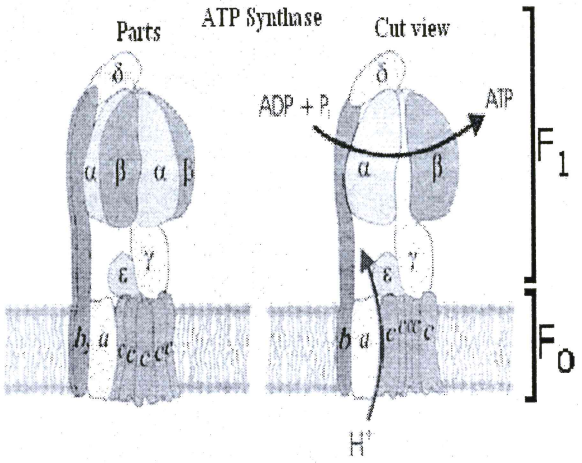

Fig. 1 *Structure and functioning of the ATP synthase.*

The overall equation of the ATP hydrolysis-synthesis is:

$$ADP^{3-} + HPO_4^{2-} + H^+ + nH^+_{out} <=> ATP^{4-} + H_2O + nH^+_{in} \quad (1)$$

Where subscripts "out" and "in" denote the outer (positively charged) and the inner (negatively charged) side of the membrane, respectively.

NORMAL MODES

A normal mode vector describes directions that each atom moves and how far it moves relatively to the other atoms. Nevertheless, a normal mode vector does not describe an absolute amount of displacement for any atom. Additional information (e.g. the temperature) is required for fixing the global amplitude of the atomic displacement.

The important contribution of normal modes is the identification and characterization of the low-frequency motions. On the other hand, the vibration frequency obtained are of little physical relevance, because the real frequencies are strongly influenced by anharmonic effects and solvent damping. In fact, low-frequency motions in a realistic environment are overdamped, so for this reason they are not vibration at all [7, 8, 9, 10].

In this article we use the following models:

Brownian modes.- Brownian Modes are used for low-frequency motions only. The model consists only of calpha atoms, which are assigned the masses of the whole residues that they represent. An harmonic potential is used that is given by [11]:

$$U(\mathbf{R}_1...\mathbf{R}_N) = \Sigma_{all\ pairs\ i,j}\, U_{ij}(\mathbf{R}_i - \mathbf{R}_j) \qquad (2)$$

with the pair potential

$$U_{ij}(\mathbf{r}) = k(\mathbf{R}_{ij}^{(0)})(|\mathbf{r}| - |\mathbf{R}_{ij}^{(0)}|)^2 \qquad (3)$$

and

$$k(\mathbf{r}) = -c\ .exp\ (|\mathbf{r}| / r_0^2) \qquad (4)$$

where $\mathbf{r}$ is a vector representing the current conformation and $\mathbf{R}_{ij}^{(0)}$ is the pair distance vector $\mathbf{R}_i - \mathbf{R}_j$ in the input configuration. To obtain the relaxation times and configuration respectively we need to resolve.

$$\bar{K} = \sqrt{\Gamma}^{-1}\ .K.\ \sqrt{\Gamma}^{-1} \qquad (5)$$

Where K is a 3N x 3N (N is the number of atoms) force constant matrix which is the second derivative of the potential. The Matrix Γ, called friction matrix, is diagonal because the simplifying assumption that each particle of the protein has a independent friction constant

Calpha modes.- In this case we use an harmonic Force Field such as in Brownian modes, where we have one point mass per residue, located at the Carbon alpha position.

Simplified potential modes.- This model uses a simplified potential derived from AMBER 94 [12,13], where electrostatic and Lennard-Jones interactions are replaced by an harmonic deformation in order to save CPU time and memory space. This potential is used for over the whole frequency range.

The vibration frequency and configurations are obtained calculating the eigenvalues and eigenvectors of the matrix

$$\bar{K} = \sqrt{M}^{-1}.K.\ \sqrt{M}^{-1} \qquad (6)$$

Where the Matrix M is a 3N x 3N diagonal matrix which contains the masses of the atoms on its diagonal, each mass being repeated thre times, once for each of the tree Cartesian coordinates.

SOFTWARE EMPLOYED

All the programs used in this study run on Linux. Normal modes are calculated employing the Molecular Modeling Tool Kit (MMTK) [14] which is partly based on Python and C [15]. To visualize the results is used Visual Molecular Dynamics (VMD) [16].

Fig. 2 displays the F_1-ATPase highlighting the Gamma subunit. Fig. 3 shows the Gamma subunit and the C-terminal helix isolated.

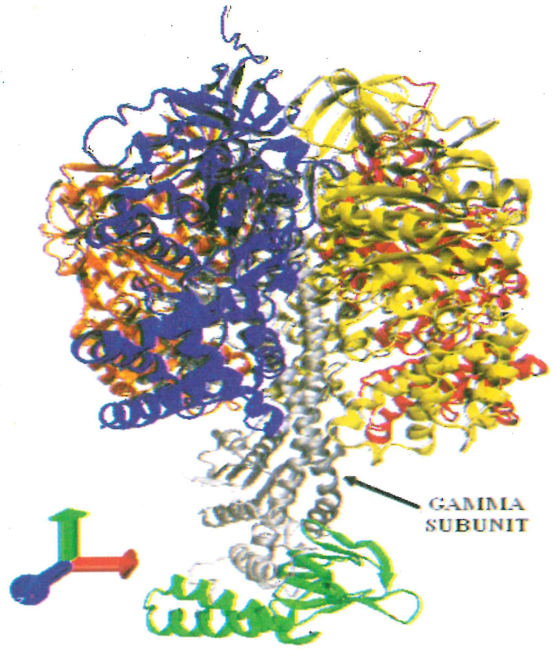

Fig. 2 F_1-ATPase showing the gamma subunit.

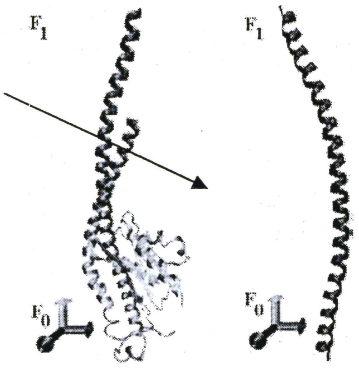

Fig. 3 Left: Gamma Subunit and right: C terminal Helix.

RESULTS

Calculations were performed on a desktop computer equipped with an Athlon xp 3000+ processor and 512 MB of memory RAM. The CPU time spent on Brownian, Calpha and simplified potential modes were 31, 29 and 3060 seconds respectively.

The first six eigenvalues obtained are zero because they describe the six rigid-body movements of the protein (translation along three independent axes plus rotation around three independent axes) [7]. Another fact to ponder is that recently normal mode studies have confirmed that large-scale conformational changes are dominated by the two to five lowest frequency modes [8]. For this reason we show modes corresponding to the four lowest non-zero frequency.

According to the results, Brownian and simplified potential modes show similar configuration, excepting their first mode. Brownian modes show clearer vector displacement trends than simplified potential modes, this is because Brownian modes are employed only for large-scales motions [7,11]. On the other hand, the simplified potential model is used to obtain configurations over the whole frequency range [12,13] and therefore it is more general. The third mode obtained from both models are apparently opposite but they are physically equivalent because the motion is considered harmonic. In the case of calpha modes only the second and forth mode show clear information. The following figures display from left to right Brownian, calpha and simplified potential configurations.

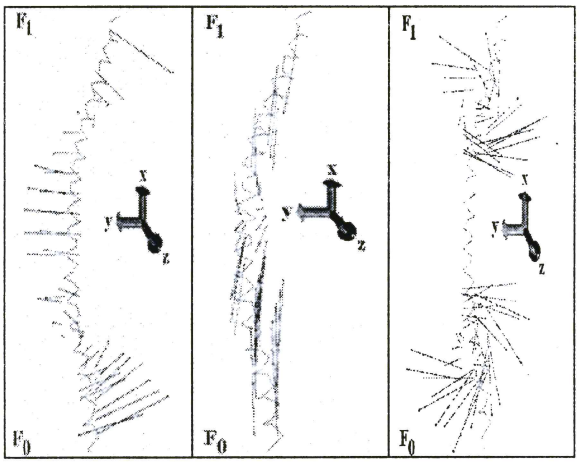

Fig. 4 First mode.

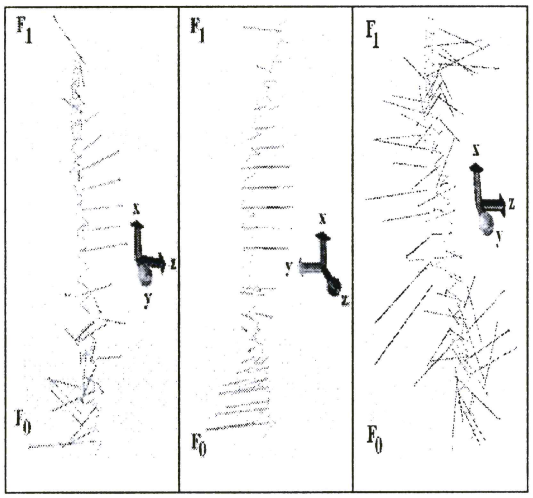

Fig. 5 Second mode.

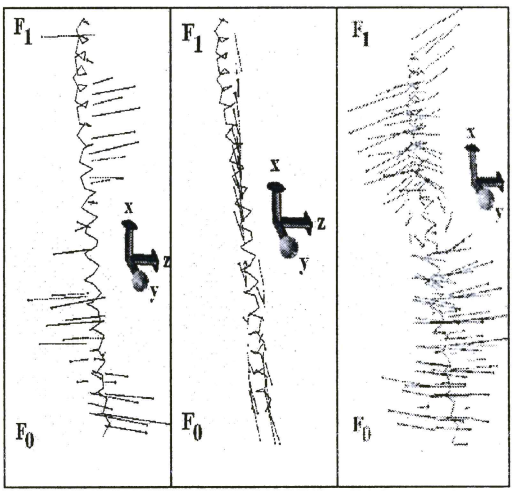

Fig. 6 Third mode.

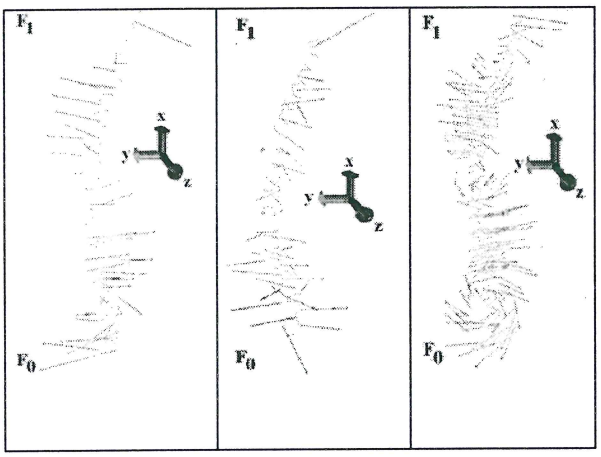

Fig.7 Fourth mode.

CONCLUSION

The first four configuration modes obtained with the three methods used show, first the F_1 end of the helix presents small displacements. These observations are coherent with recent studies where is proposed that the F_1 end of the helix acts as a crankshaft, converting the bending and unbending of the Beta subunit into rotational motion.

Second, results along the helix also show flexible lengths followed by rigid lengths. This feature may serve to smooth out the torque produced at the F_1 end, delivering a nearly constant torque necessary to achieve the highest efficiency possible.

Finally, the first and second modes show that helix on the x-y plane is more flexible than on the x-z plane. This fact is in accordance with the geometric properties of the helix.

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