Molecular Dynamics Simulation of the NF-kB Transcription Factor

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Motivation

Transcription factors are proteins which regulate gene expression by promoting or inhibiting the transcription process by binding to target DNA sequences. The Rel/NF-kB proteins belong to one of the most studied transcription factor families.

These proteins are involved in the control of a large number of normal cellular and organism processes, such as immune and inflammatory responses, development, cellular growth and apoptosis [1]. It is known that the activity of NF-kB is regulated primarily by interaction with inhibitory IkB proteins through the formation of a stable IkB/NF-kB complex [1-2]. One of the best-characterized and most prevalent members of NF-kB family is a heterodimer composed of p50 and p65 subunits and the most studied NF-kB-IkB interaction is that with IkBalpha. Different X-ray crystallographic structures of both NF-kB p50-p65/ DNA and NF-kB p50-p65/IkBalpha complexes have been solved and deposited in the Brookhaven Protein Databank. The three dimensional structures of the complexes suggest that the presence of IkBalpha allow a strong conformational change of the DNA-bound open conformation of p50-p65 heterodimer with the adoption in the resting state of a IkB-bound closed conformation. The aim of this work was to investigate the dynamic properties and the conformational behaviour of the NF-kB p50-p65/IkBalpha system by molecular dynamics simulation.

Methods

The protein complex simulated consists of the NF-kB p50/p65 heterodimer in the open conformation and the IkBalpha inhibitor. The crystal structure of the NF-kB p50/p65-IkBalpha (1IKN) complex shows the NF-kB protein in a closed conformation, but it is not completely determined, with the N-terminal part of subunit p50 of NF-kB missing. Therefore, we started our study from the DNA complexed p50/p65 heterodimer structure (1LE9), where the crystal is completely resolved and shows the transcription factor in an open conformation. We then removed the DNA from the NF-kB N-terminal domain and added the IkBalpha inhibitor to the NF-kB C-terminal domain. A 13 ns molecular dynamics simulation of this complex was performed using the NAMD program with the following parameters: CHARMm 27 force field, explicit solvent, 300 K, and Particle Mesh Ewald for electrostatic force calculation. The calculations were run in parallel on the IBM Linux cluster installed at CINECA. On this machine 1ns took about 800 CPU hours using 16 processors. The trajectories were analyzed by tools available with the GROMACS package.

Results

A plot of the root mean square deviation calculated from 0 to13 ns for the NF-kB protein (without inhibitor) alpha-carbon shows a plateau region from 7 ns with a value of about 0.5 nm. It was possible to characterize the essential protein rotation motion that happens during the first 7 ns by using an essential dynamics approach.

Fig. 1 shows a concerted rotation motion along the first eingenvector of the p50/p65 subunits at the NF-kB protein N-terminal domain from the beginning of the simulation (marked in green) to the end (marked in red). In addition, the root mean square fluctuation RMSF calculated for the NF-kB protein alpha-carbon chain shows zones of high fluctuation, probably involved in rotational motion. Further evidence of the rotation comes from analysis of the secondary structure which shows a transient increase in the protein secondary structure for 10 amino-acids in the interval 2.5 ns to 6.0 ns. To summarise we have shown that in the presence of the inhibitor and without DNA the NF-kB open conformation begins to rotate to the closed conformation at the NF-kB N-terminal domain p50/p65 subunits level and the NF-kB structure increases. We are currently investigating whether the observed conformation change is due to the removal of the DNA or the presence of the IkBalpha. We will also characterize the aminoacids of NF-kB protein involved in the secondary structure increase.

1.Karin, M.,et al. (2000) Annu. Rev. Immunol. 18, 621-663 2.Verma, I. M., et al. (1995) Genes Dev. 9, 2723-2735

Image: http://www.biogate.it/nfkb/image/figure1NFkB.html

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