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Iron(II) triggered conformational changes in *Escherichia coli* fur upon DNA binding: a study using molecular modeling

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Abstract

In order to identify the Fur dimerization domain, a three-dimensional structure of the ferric uptake regulation protein from *Escherichia coli* (Fur EC) was determined using homology modeling and energy minimization. The Fur monomer consists of turn- helix -turn motif on the N-terminal domain, followed by another helix–turn–helix–turn motif, and two β-strands separated by a turn which forms the wing. The C-terminal domain, separated by a long coil from the N-terminal, and consisting of two anti parallel β strands, and a turn–helix–turn–helix–turn motif.

Residues in central domain were found to aid the dimer formation, residues 45–70 as evident in the calculated distances; this region is rich in hydrophobic residues. Most interactions occur between residues Val55, Leu53, Gln52, Glu49 and Tyr56 with closest contacts occurring at residues 49–56. These residues are part of an α-helix (α_4) near the N-terminal.

Upon raising the Fe^{2+} concentration the binding of Fur dimer to DNA was enhanced, this was evident when, the Fur EC dimer was docked onto DNA ''iron box'' (it was found to bind the AT-rich region) and upon addition of Fe^{2+} the helices near the N-terminal bound to the major groove of the DNA. Addition of high Fe^{2+} concentration triggered further conformational changes in the Fur dimer as was measured by distances between the two subunits, Fe^{2+} mediated the Fur binding to DNA by attaching itself to the DNA. At the same time DNA changed conformation as was evident in the distortion in the backbone and the shrinking of major groove distance from 11.4 to 9.3 Å.

Two major Fe^{2+} sites were observed on the C-terminal domain: site 1, the traditional Zn site, the cavity contains the residues Cys92, Cys95, Asp137, Asp141, Arg139, Glu 140, His 145 and His 143 at distances range from 1.3 to 2.2 Å. Site 2 enclave consists of His71, Ile50, Asn72, Gly97, Asp105 and Ala109 at very close proximity to Fe^{2+}.

The closest contacts between Fur dimer and DNA at the AT-rich region were at residues Ala11, Gly12, Leu13, Pro18 and Arg19 mostly hydrophobic residues near the N-terminal domain. Close contacts repeated at His87, His88 and Arg112, and a third region near the C-terminal at Asn137, Arg 139, Glu140, Asn141, His143, Asn141 and His145. Fur dimer has three major contact regions with DNA, the first on the N-terminal domain, a second smaller region at His87, His88 and Arg112 mediated by Fe^{2+} ions, and a third region on the C-terminal domain consisting mainly of hydrophobic contacts and mediated by Fe^{2+} ions at high concentration.

Keywords: Ferric; Uptake; Repressor; Protein; Corepressor; Regulation; DNA

1. Introduction

Fur protein from *Escherichia coli* K12 (Fur EC) is a 17 KDa, 148 amino acid residues protein [1]. Fur EC has attracted much attention in recent years [1–7] and it has been extensively studied as a repressor protein which uses Fe^{2+} as co-repressor to bind specifically to DNA [2–6], it was especially studied with the 19 bp iron box (5′-GAT AAT GAT AATC ATT ATC-3′) [2,8–13]. Other divalent transition metal ions such as Mn^{2+}, Co^{2+} were found to activate Fur both in vitro and in vivo with varying degrees, while Zn^{2+}, Cd^{2+} and Cu^{2+} were found to bind Fur strongly and activated Fur in vitro only [1,5,18]. The X-ray structure of Fur protein from *E. coli* is still not resolved; the NMR studies gave insight about the structure of FurEC and its relation to the Fur function [13–15]. An X-ray structure on a member of the Fur family from *Rhizobium leguminosarum* was reported [16]. The first crystal structure of Fur from *P. aeruginosa* in complex with Zn^{2+} was determined at a resolution of 1.8 Å [17]. X-ray absorption spectroscopic measurements and micro PIXE analysis were also performed

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[17,45] in order to characterize the distinct iron binding sites in solution and it was found to bind four Zn^{2+} ions per Fur dimer with N/O ligands at an average metal–ligand distance of 2.1 Å.

Experimental work revealed many aspects about the Fur structure-function relationship. The HTH motif near the N-terminus was suggested to play the DNA binding role similar to other repressor proteins (λ repressor, DtxR, lac repressor [20–22] and IdeR [24]. Other reports provided insight on the metal ion-binding sites provided by Fur and the role of metal ion in the DNA binding process [25]. Indeed, previous work based on thermodynamic equilibrium gave evidence that Fe^{2+}, Mn^{2+}, and Co^{2+} ions are weakly bound to Fur and ^{57}Fe Mössbauer study showed that Fe^{2+} is present in an axially distorted octahedral environment with $\partial = 1.3\ mms^{-1}$ $\Delta = 1.3\ mms^{-1}$ [5,7,26]. These values, when compared with reported values for Fe^{2+} sites, indicated a moderately bound Fe^{2+} to oxygen and/or nitrogen ligands [23]. This is consistent with the reversible metal ion-binding (K_d value 55 μM [5]) which agrees well with the role of Fur protein as metal ion sensor. Site multiplicity and flexibility was not ruled out as more than one ion was found to bind per Fur [5]. Other metal ions could replace Fe^{2+} as co-repressors and was active in various degrees [1].The proposed role of metal ion was interpreted as to trigger conformational changes in the Fur protein dimer and consequently facilitate DNA binding. Coy [12], basing his study on proteolytic enzymatic cleavage suggested that the metal ion role was to induce conformational changes, and also proposed that both DNA binding and N-terminal sensitivity of Fur were dependent on the metal ion concentration. He also suggested that the C-terminal was responsible for metal ion binding [12]. Most workers [12,17,24,25] tend to agree that Fur has three major domains based on its function; an N-terminal which is responsible for the DNA binding process, a middle domain which plays a role in the dimerization of Fur and the C-terminal which contains the metal ion-binding sites. C-terminal plays the role of metal ion concentration sensing and binding. In this work, the three-dimensional structure of Fur was built using molecular dynamics. The dimerization of Fur was performed in water to produce the Fur dimer. The dimer was studied in the presence of DNA with and without the presence of Fe^{2+} ion. The effect of metal ion on the conformational changes of Fur and how does this act to enhance the DNA binding process at elevated Fe^{2+} concentration and the unbinding of Fur dimer to DNA at reduced Fe^{2+} concentration [44].

In this work, computational methods were used to establish the structure function relationship of Fur protein and to give insight on the mechanism of repressor activity of the Fur dimer upon varying the concentration of the co-repressor (Fe^{2+}). The effect of metal ion on the protein and DNA conformations is established. The most pronounced effect of metal ion at elevated concentrations is the observed distortions took place in DNA which would translate into decreased synthesis of bacterial mRNA.

1.1. Computations and homology modeling

All the molecular dynamics (MD) simulations were performed using AMBER7 molecular simulation package [27,28]. An AMBER force field was used for molecular minimization and molecular dynamics. The analyses of MD trajectories were also preformed by AMBER7. Pymol molecular viewer package was used for visualization [29]. All other calculations were performed on a single-CPU Pentium III machine with Linux platform.

1.2. Homology modeling of Fur protein

The known Fur sequence (from *E. coli*) was submitted to different modeler servers in order to predict the three-dimensional structure. SWISS-MODEL [30], PHD, 3DPSSM [31] and VADAR servers were used to align the Fur sequence with similar known proteins Data Bank. Several templates for Fur protein were generated while the sequence with high similarity served as a reference sequence. The superposition of each atom was optimized by maximizing C_α in the common core while minimizing their relative mean square value deviation (RMSD) at the same time. Spare part algorithm was used to search for fragments that can be accommodated into the framework of the Brookhaven Protein Data Bank (PDB). The coordinates of central backbone atoms (N, O and C) were averaged, and then added to the target model. The side chains were added according to the sequence identity between the model and the template sequence. AMBER7 was used to idealize the geometry for bonds and also to remove any unfavorable non-bonded contacts. This was done by minimizing the energy. All hydrogen atoms were added and the apoFur structure was subjected to a refinement protocol with constraints on the Fur structure gradually removed. 100 steps of steepest descent, followed by 300 steps of conjugate gradient algorithm were applied during energy minimization. The energy minimization process on the apoFur model was performed, first in vacuum and second in H_2O as solvent, nine Na^+ ions were added to the model to neutralize the system.

1.3. Building the Fur dimer

AUTODOCK 2.4 [32] was used to generate the apoFur dimer. Two molecules of the previously determined structure for the apoFur monomer were docked on each other, and the best docking sites were predicted. Monte Carlo (MC) simulated annealing (SA) algorithm was used for exploring the Fur configuration by a rapid energy evaluation technique using a grid-based molecular affinity potential. The energy of interaction, affinity and the grid for electrostatic potential were evaluated using the Poisson–Boltzmann finite difference method and were assigned to each atom.

1.4. Docking of the apoFur dimer onto a 19 bp fragment representing the DNA

Nucgen suite program (part of the AMBER7 package [28]) was used to build the Cartesian coordinates for canonical B- model of the iron box (a 19-bp inverted repeat sequence designated the iron box (5′ GATAATGATAATCATTATC 3′); the proposed recognition site of Fur on the DNA. The