

Molecular Modeling of Full-length OxyR from *Shewanella oneidensis* MR-1 and Molecular Dynamics Studies of the Activation Domain

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Abstract

OxyR is a transcriptional factor, which activates transcription of antioxidant genes. In this study, we constructed a structural model for the full-length OxyR from *Shewanella oneidensis* MR-1 using threading and comparative modeling techniques. To further study the conformational changes, we also performed molecular dynamics simulations on the activation domain of OxyR. Molecular dynamics simulations were performed using GROMACS force field under periodic boundary conditions. The Particle Mesh Ewald (PME) method was used to treat long-range electrostatic interactions. The simulation results show that the oxidized form is very stable while the reduced form is quite flexible. Our results suggest that the reduced form provides structural flexibility for disulfide bond formation and which in turn regulates its function.

1. Introduction

The OxyR protein, first identified as a key regulator of the peroxide stress response in *Salmonella typhimurium*, is found in many prokaryotic organisms [1]. OxyR belongs to one of the largest families of prokaryotic DNA binding proteins, the LysR-type transcriptional regulators (LTTRs). LTTR family proteins have an N-terminal DNA binding domain and C-terminal activation domain. OxyR is referred as an archetypal example of the redox regulatory protein. It is activated by oxidation of H₂O₂ and then induces the transcription of genes necessary for the bacterial defense against oxidative stress. In the oxidized form, an intramolecular disulfide bond between Cys-199 and Cys-208 is formed after activation by H₂O₂. Although OxyR has been studied extensively for many years, the

exact mechanism is still not clear. The structure of the cofactor binding domain of *E. coli* OxyR has been solved. However, there is no experimentally determined structure of the N-terminal DNA binding domain and the full-length OxyR. In this study, we used threading method to construct a model for full-length OxyR from *Shewanella oneidensis* MR-1. Molecular dynamic studies showed the possible conformational changes between the reduced form and oxidized form.

2. Methods

Threading analysis: Protein threading was carried out using our in-house program PROSPECT [2,3]. PROSPECT employs a knowledge-based energy function and it guarantees to find the globally optimal sequence-structure alignment under the given energy function. The template is CbnR (PDB: 1IXC), the first crystal structure of a full-length LTTR.

Molecular Dynamics Simulations: Molecular dynamics simulations were performed using GROMACS (version 3.1). The simulations employed the GROMACS force field under periodic boundary conditions. The Particle Mesh Ewald (PME) method and a distance cutoff of 10 Å were used for long-range electrostatic interactions and van der Waals interactions, respectively. The time step was 2 femtoseconds (fs). The simulations were carried out under NPT conditions (constant number of particle, pressure and temperature) at 300 K. A constant pressure of 1 bar in all three directions was used with a coupling constant of $\tau_p = 1.0$ picoseconds (ps). The system was energy minimized, followed by equilibration for 30 ps. The simulation was carried out for 4 nanoseconds (ns).

3. Results and Discussions

3.1 The structural model of the full-length OxyR

To construct the full-length model, we first generated an alignment between the OxyR sequence and the structural templates 1IZ1, 1I6Aa (oxidized form), and 1I69a (reduced form). Figure 1 shows the full-length model in reduced form with a DNA binding domain and an activation domain.

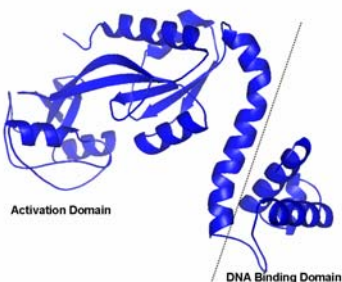


Figure 1. Cartoon representation of the full-length OxyR structure

3.2 Molecular dynamics simulations

The structure of the cofactor binding domain of *E. Coli* OxyR revealed the conformational difference between the reduced form and the oxidized form that has a disulfide bond between residues 199 and 208. However, the mechanism of this conformational change is not clear. Results from molecular dynamics

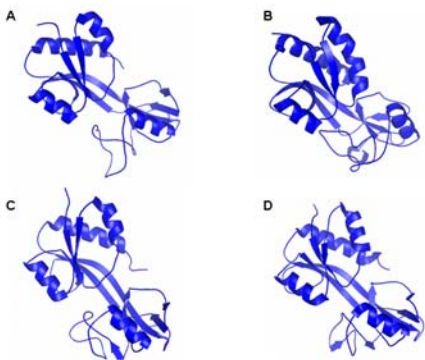


Figure 2. The structures of the activation domain before and after 4ns simulation (A: starting model, reduced form; B: ending structure, reduced form; C: starting model, oxidized form; D: ending structure, oxidized form).

simulation studies showed that the loop region between residues 196 and 211 is very flexible in the reduced form, but not in the oxidized form (Figure 2, and Figure 3), which provides a structural basis for disulfide bond formation between two Cysteine residues C199 and C208.

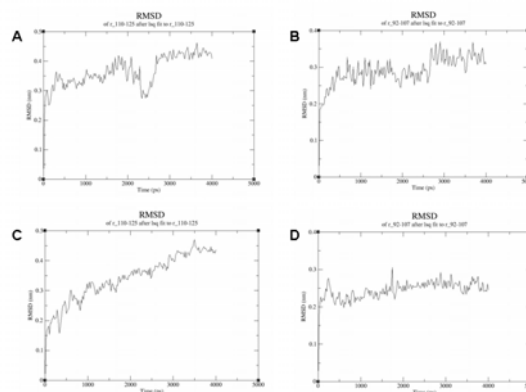


Figure 3. Root mean square deviation of the backbone atoms relative to the starting structure during 4ns simulation. A: residue 110-125, oxidized form. B: residue 92-107, oxidized form. C: residue 110-125, reduced form. D: residue 92-107, reduced form.

Our simulation results also show that the oxidized form is very stable even though we mutated C199→S199 and C208→S208, suggesting that the structural stability is very important for OxyR to exert its function as a regulator.

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References

- [1] Paget MS, Buttner MJ. "Thiol-based regulatory switches". *Annu Rev Genet.* 2003;37:91-121.
- [2] Xu Y, Xu D. "Protein threading using PROSPECT: design and evaluation". *Proteins* 2000 Aug 15;40(3):343-54.
- [3] Kim D, Xu D, Guo JT, Ellrott K, Xu Y. "PROSPECT II: protein structure prediction program for genome-scale applications". *Protein Eng.* 2003 Sep;16(9):641-50.
- [4] GROMACS: <http://www.gromacs.org>.