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OxyR, a Redox-operated Switch

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Abbreviations:

AAPH, 2,2'=azobis hydrocholoride
ahpFC, Gene for NADPH-dependent alkyl hydroperoxidase
gorA, Gene for glutathione reductase
grxA, Gene for glutaredoxin
GSH, glutathione
GSSG, glutathione disulfide
H_2O_2, hydrogen peroxide
katG, Gene for catalase-hydroperoxidase
LDH, lactate dehydrogenase enzyme
ROS, reactive oxygen species
RSNO, S-nitrosothiol
t-BOOH, *tert*-butylhydroperoxide
TCA, tricarboxylic acid
trxA, Gene for thioredoxin

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Abstract

OxyR transcription factor is one of the redox-operated genetic switches. It can be activated in response to hydrogen peroxide, low GSH/GSSG (glutathione/glutathione disulfide) ratio, and nitrosothiols. Many OxyR-activated genes such as GSH (glutathione) reductase, glutaredoxin, are participating in antioxidant defense. It forms tetramers in the solution and contains a pair of cysteine residues (C-199 and C-208) that are redox-sensitive in each monomer. There are two forms of OxyR: the reduced form and the oxidized form. OxyR gets activated when these cysteines are oxidized to form intramolecular disulfide bonds in response to oxidative stress. There are two major pathways to activate OxyR transcription factor: (1) changed redox status and (2) the reaction with H_2O_2. The activated OxyR can be deactivated by enzymatic reduction with glutaredoxin or glutathione reductase.

Introduction

Damaging free radicals are generated in many biological processes, such as aerobic metabolism, inflammation, chronic diseases and cellular responses after ionizing irradiation treatment [1]. These generated free radicals may change the cellular redox status. The intracellular ROS (reactive oxygen species) levels are the main determinants of cellular redox homeostasis [2]. Excessive production of reactive oxygen species may lead to oxidative stress and disruption of redox homeostasis [1]. Among ROS, superoxide radical and hydrogen peroxide and RNS (reactive nitrogen species) are the key species. In bacteria *Escherichia coli* and *Salmonella typhimurium*, several important systems are formed to regulate gene expression in response to oxidative stress during evolution [1]. OxyR system is one of the genetic regulatory systems in bacteria. OxyR is a transcription factor that controls the expression of OxyR regulon that includes approximately 10 genes [1]. The OxyR transcription factor consists of 305 amino acids, and has DNA binding motif, regulatory domain, and oligomerization domain [2]. Hydrogen peroxide can activate OxyR by the formation of intramolecular disulfide bonds in *Escherichia coli* [2]. This mini review will focus on OxyR regulatory system and redox regulation of OxyR.

Structure of OxyR transcription factor

The OxyR protein contains 305 amino acid residues. It contains two domains: (1) the N-terminal domain that contains a helix-loop-helix DNA binding motif, (2) the C-terminal regulatory and oligomerization domain [2]. A flexible linker connects the N-terminal domain to the C-terminal domain. There are two important redox-active cysteines (Cys-199 and Cys-208) mediating the redox-dependent conformational changes in the C-terminal domain [2]. The OxyR monomer has a mass of 34 kDa [3]. The OxyR protein has two forms: the reduced form and the oxidized form. Figure 1 shows the reduced and oxidized forms of OxyR monomer [2].

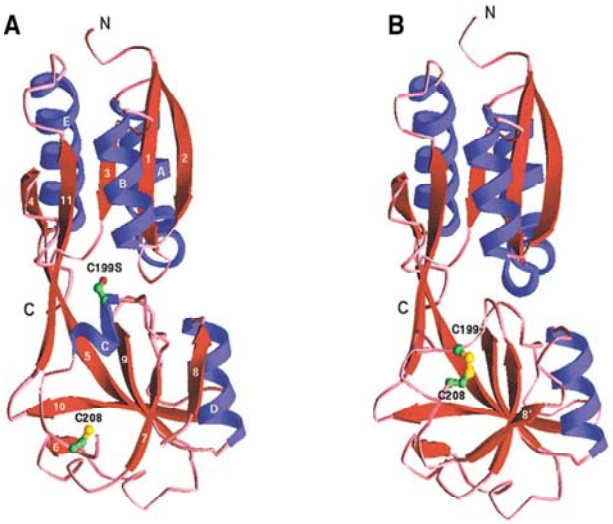


Figure 1: The schematic ribbon diagrams of the OxyR monomers in the reduced (A) and oxidized (B) forms are shown with the redox-active cysteines Cys-199 (C199S in the reduced form: cysteine was mutated to a serine for the reduced form crystal structure determination because the C199S mutation locks the protein in the reduced conformation) and Cys-208 in a ball-and-stick representation. In oxidized form, C199 and C208 forms a disulfide bond that leads to conformational changes of the OxyR overall structure. Adapted from [2].

OxyR binds DNA either as dimers or tetramers using a helix-turn-helix motif with each monomer binding on an adjacent part of the DNA duplex [4]. Figure 2 and 3 show the OxyR dimer and tetramer in reduced form and oxidized form [2].

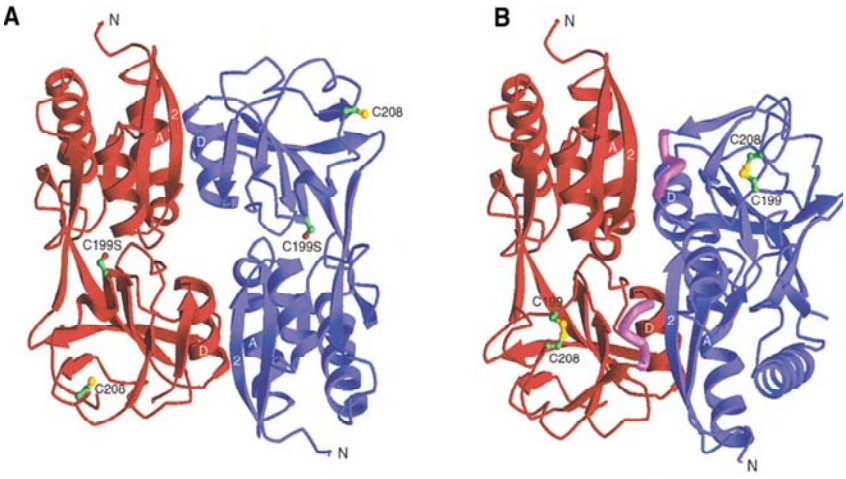


Figure 2: Dimeric structure of the reduced (A) and oxidized (B) forms. In the oxidized form, the disulfide bond between C199 and C208 is formed. The left-hand side monomers (red) in the reduced and oxidized forms are presented in a similar orientation to show clearly the relative monomeric rotation of the right-hand side monomer (blue) in the oxidized form. Adapted from [2].

The different structures of OxyR dimer and tetramer between reduced and oxidized forms imply the different DNA binding and transcription activating activities [2]. As in Figure 3A, the reduced form of tetramer could be aligned with a DNA stretch of five helical turns which contains

a bend in the third turn. In this complex, two pairs of major grooves in DNA are positioned above the regulatory domain in a direction parallel to the monomeric long axis [2]. In Figure 3B, the oxidized tetramer could be aligned with a DNA stretch of four consecutive helical turns. In the oxidized form of the complex, the DNA runs diagonally to the monomeric long axis. Thus, the switch from the reduced to oxidized form of the tetramer seems to be accomplished by a roughly 90° rotation of the right-hand side dimer in the reduced form, facilitating the occupation of different binding sites in DNA [2]. The two forms, oxidized and reduced forms, contribute to OxyR's transactivation functions.

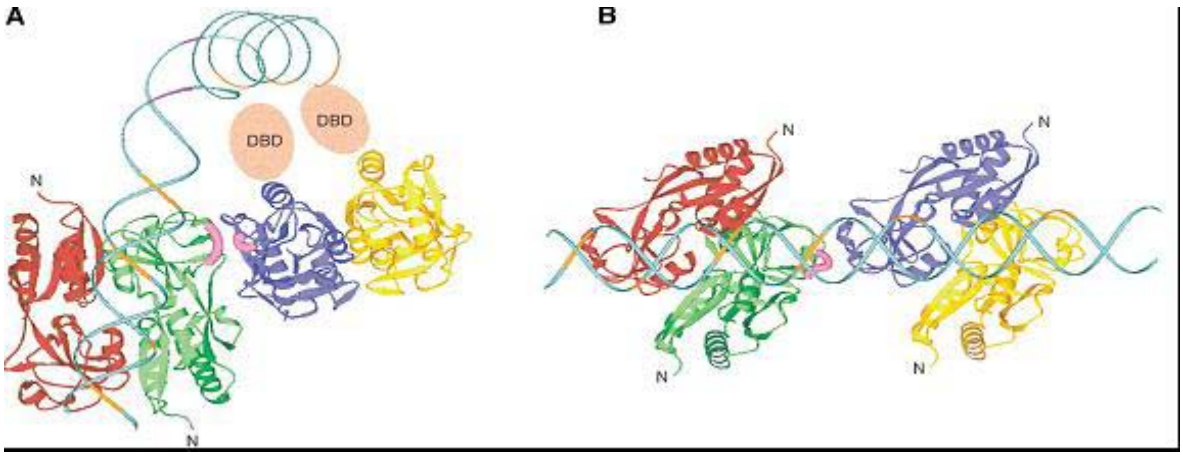


Figure 3: The tetramers observed in crystals of the reduced (A) and oxidized (B) forms are shown with the DNA positioned on the tetramers with plausible orientations. The DNA binding domains of the left-hand side dimer in the reduced form and both dimers in the oxidized form are likely to be located behind the DNA in the space between the DNA and the regulatory domain dimers. The redox loop regions (residues 204–208) of two molecules (molecules colored green and blue) in the reduced form, which play an important role in the tetrameric interaction, are shown as thick, violet lines. The structurally switched redox loop region of the oxidized form makes completely different tetrameric crystal contacts. Adapted from [2].

Role of OxyR in bacteria

OxyR protein belongs to a family known as the LysR type of DNA binding proteins [4]. This family has the feature to positively regulate its target genes and negatively regulate its own genes [4]. The OxyR protein is very sensitive to oxidation, and only oxidized OxyR is capable of

activating transcription [5]. The OxyR protein is the sensor and direct transcriptional activator of the OxyR regulon [6, 7]. It regulates about 10 genes that encode catalase-hydroperoxidase I (*katG*), NADPH-dependent alkyl hydroperoxidase (*ahpFC*), glutathione reductase (*gorA*), glutaredoxin (*grxA*), a protective DNA binding protein (Dps), and several other genes [6]. All of these genes are hydrogenperoxide-inducible genes [7]. Upon activation, OxyR also can increase the expression of *oxyS* (a small, nontranslated regulatory RNA) that is usually used as an indicator of OxyR transcription activity [9]. Figure 4 is an example of *ahp*FC up-regulation by OxyR overexpression.

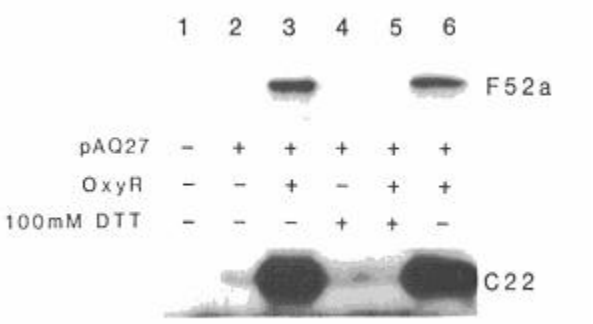


Figure 4: Effect of OxyR on the expression of the components (F52A and C22 encoded by *pAQ27*) comprising *ahpFC*, the alkyl hydroperoxide reductase. In lane 3 and 6, OxyR overexpression increases the expression of the two proteins. In lane 5, this ability can be inhibited by DTT, a reducing agent. Adapted from [7].

After *E. coli* and *S. typhimurium* are treated with low dose of H_2O_2, they become resistant to another large dose H_2O_2 treatment; but in *E. coli* and *S. typhimurium* without OxyR gene, they are very sensitive to H_2O_2, and unable to induce the hydrogenperoxide- inducible genes [7]. Catalase-hydroperoxidase, alkyl hydroperoxidase, and glutathione reductase are all the proteins and enzymes to protect against oxidation. *ahpFC* converts organic peroxides to less reactive alcohols and glutathione reductase restores a key antioxidant. So OxyR plays an important role to prevent oxidative damage that usually occurs in bacteria when they are growing in aerobic conditions [7].

It was found that OxyR overexpression mutant strain had less sensitivity to the toxic effects of lipid peroxidation than OxyR deletion mutant strain [8]. Figure 5 shows that TA4110 (OxyR overexpression mutant strain) and TA4112 (OxyR deletion mutant strain) have different viability when exposed to *t*-BOOH (*tert*-butylhydroperoxide) and AAPH (2,2'=azobis hydrocholoride),

which can induce lipid peroxidation in membranes [8]. Moreover, inactivation of SOD by lipid peroxidation products can be reduced in OxyR overexpression strain [8]. All in all, OxyR has apparent roles in defending against oxidative damage.

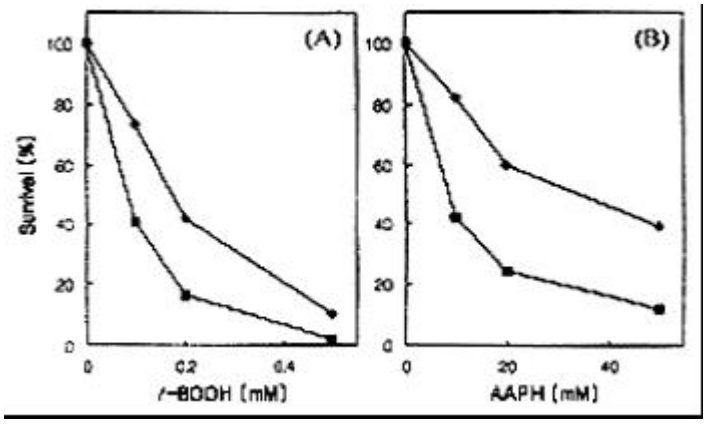


Figure5: TA4112 strain showed more cell survival than TA 4110 strain after exposure to *t*-BOOH (*tert*-butylhydroperoxide) and AAPH (2,2'=azobis hydrocholoride) respectively. ♦TA4110; ■ TA4112.

Redox regulation of OxyR

OxyR transcription factor can response to oxidation and activate the expression of a series of antioxidant genes [5, 9]. OxyR oxidation and activation can be achieved in two possible ways: (1) by a shift of the redox status of the cell or (2) through the high reactivity of OxyR with hydrogen peroxide [5]. Moreover, nitrosative stress can activate OxyR protein [10].

It was found that the *trxA*$^{-}$ (thioredoxin mutants) and *gorA*$^{-}$ (glutathione reductase mutants) strains expressed constitutive more OxyR even in absence of H_2O_2 [5]. This phenomenon may due to decrease in the thiol-disulfide ratio that could directly change the redox status of the cell, because GSH/GSSG ratios and H_2O_2 were measured and there were no differences between wild type and the two mutant strains [5]. In another experiment (Figure 6), OxyR was incubated with defined concentrations of GSH/GSSG and then the relative amount of oxidized (activated) OxyR was measured by *in vitro* transcription assays. When the GSH/GSSG ratio in the buffer exceeded 5:1 (Figure 6, between lane 4 and 6), there was a sudden and substantial drop in transcription activity [9]. The intensities of the *oxyS* and *bla* bands in figure 6 were measured by a

PhosporImager. It theoretically fits the equation (1). These data were consistent with the fact that OxyR is a tetramer in solution. It was inferred that the molecular event of redox signaling by OxyR was disulfide bond reduction and formation [9]. The extracted equilibrium constant for the equation was used to calculate the redox potential of OxyR and the redox potential of -185 ± 5 mV was derived from three independent experiments. The derived value of -185 ± 5 mV is about 90 mV higher than the estimated redox potential of the *E. coli* cytosol (-280 mV), providing a thermodynamic basis for the observation that OxyR is predominantly reduced (deactivated) under normal conditions [9].

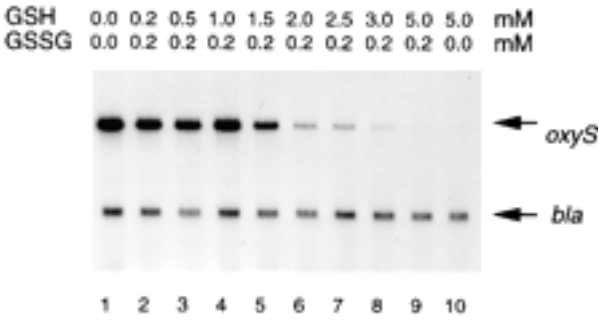


Figure 6: The indicated amounts of GSH and GSSG were incubated with 0.8 μM OxyR for at least 72 hours. The samples were then added to RNA polymerase and assayed by *in vitro* transcription. All steps were carried out anaerobically. We can see the change of OxyR transcription activity according to different GSH/GSSG ratio. Adapted from [9].

$$\text{OxyR (ox)} + 8\text{GSH} \leftrightarrow \text{OxyR (red)} + 4\ \text{GSSG} \qquad (1)$$

There is some evidence showing that certain S-nitrosothiols (RSNOs) can lower intracellular thiol levels and inhibit the transcriptional activation of OxyR by S-nitrosylation [10].

In wild-type cells, OxyR can also be activated by hydrogen peroxide [5]. Figure 7 shows activation of OxyR by H_2O_2. However the total protein level does not change when cells are treated with H_2O_2 [3]. It was reported that activated OxyR stimulates transcription by interacting with carboxyl-terminal domain of the α-subunit of RNA polymerase [3].

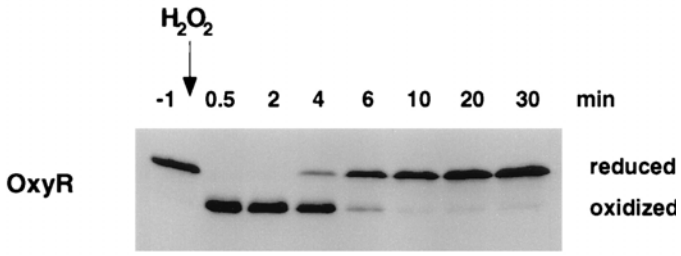


Figure 7: Transient activation of OxyR by H_2O_2 (western blot). After treatment of H_2O_2, OxyR was oxidized in 30 s. After 6 minutes, reduced form became dominant. Adapted from [5].

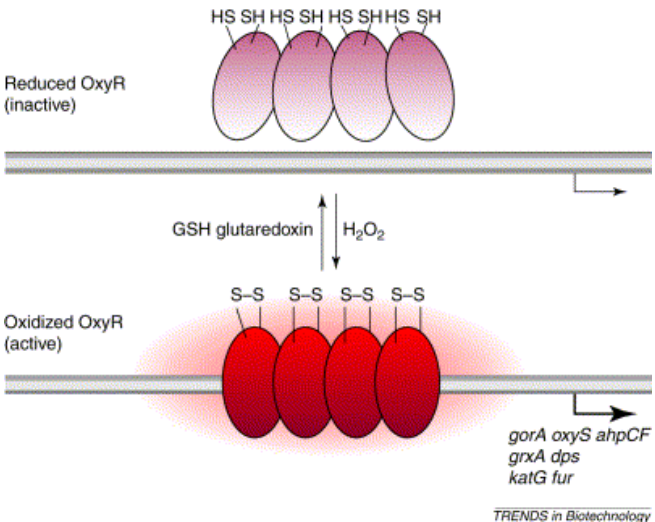
So there are two pathways to bring about oxidation and reduction of OxyR. As shown in reaction 2 and 3 [5], two different reactions can determine the redox status of OxyR. Under normal conditions, hydrogen peroxide concentrations are low; then the first pathway plays the major role and the reduced OxyR dominates. However, if there is a low GSH/GSSG ratio, oxidized OxyR is formed. After exposure to high hydrogen peroxide, the second pathway dominates and oxidized OxyR becomes the major species [5].

(1) $OxyR_{reduced} + GSSG \rightleftharpoons OxyR_{oxidized} + 2\,GSH$ (2)

(2) $OxyR_{reduced} + H_2O_2 \longrightarrow OxyR_{oxidized} + 2\,H_2O$ (3)

Summary

OxyR protein is a transcription factor of many genes in bacteria. OxyR protein's activation depends on the redox status and its exposure to H_2O_2. Figure 8 summarizes the function and redox regulation of OxyR protein. The OxyR protein is produced constitutively and forms reduced tetramers in solution under normal conditions. Each subunit of the OxyR tetramer contains two cysteine residues that form intramolecular disulfide bonds upon exposure to H_2O_2. It can be oxidized by hydrogen peroxide (H_2O_2) and



a low GSH/GSSG ratio. The oxidized form of OxyR binds to promoter regions of target genes and activates transcription. OxyR-activated genes have direct and indirect antioxidant functions. The disulfide bonds are reduced by glutaredoxin and glutathione, which in turn is re-reduced by glutathione reductase. Glutaredoxin and glutathione reductase are the gene products under the transcriptional control of OxyR. Thus, the response is self-regulated [3].

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