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Molecular mechanism of the redox-dependent interaction between NADH-dependent ferredoxin reductase and Rieske-type ferredoxin

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Introduction

Redox-dependent affinity regulation is critical to fast and efficient electron transfer (ET) between ET proteins. The molecular mechanism of the affinity regulation, however, remains elusive due to the lack of tertiary structures of the ET proteins in every redox state relevant to the ET reaction. BphA4 and BphA3 are, respectively, an FAD-containing NADH-dependent ferredoxin reductase and a Rieske-type [2Fe-2S] ferredoxin from a biphenyl dioxygenase BphA derived from *Acidovorax* sp. strain KKS102. Our biochemical study showed that the reduction of the FAD in BphA4 increases the affinity between BphA3 and BphA4 approximately 20-fold [2]. In order to reveal the molecular mechanism of this redox-dependent affinity regulation, we determined the crystal structure of BphA4 in oxidized, hydroquinone, semiquinone, and reoxidized forms; the crystal structure of BphA3 in oxidized and reduced forms; and the crystal structure of the ET complex of BphA3 and BphA4 [2]. Here, we report the molecular mechanism of the redox-dependent interaction between BphA3 and BphA4.

Methods

Crystallization of the reduced BphA3 and preparation of the two-electron reduced BphA4 were carried out under anaerobic conditions [2, 4]. Crystallization of the BphA3-BphA4 complex was also performed under anaerobic conditions [3]. For anaerobic crystallization, we developed an anaerobic chamber system [4].

Results

Data collection of the reaction intermediate crystals were carried out on the beam lines BL-5A, BL-6A, BL-17A and NW12A. The diffraction data were processed using an XDS or HKL2000 program. In the crystal of the BphA3-BphA4 complex, BphA3 binds to one subunit of homo-dimeric BphA4 (Figure 1, [2]). A comparative analysis of the seven crystal structures obtained revealed that the conformational changes of BphA4 upon reduction of FAD are required for the formation of the high-affinity BphA3-binding site in BphA4. The BphA3-binding site of BphA4 is composed of FAD-binding and C-terminal (CT) domains (Figure 2-1). The reduction of BphA4 induces the rotation of the NADH/CT domain (Figure 2-2), which causes a conformational change of the BphA3-binding site through the reorientation of the CT domain with respect to the FAD-binding domain. Then, BphA3 binds BphA4. In this stage, $Glu47_{BphA3}$ and $Arg327_{BphA4}$ seem to form an electrostatic interaction (Figure 2-3). After accepting an electron, BphA3 is likely to undergo a flip of the peptide bond between Gly46 and Glu47 (Figure 2-4). This peptide flip seems to break the electrostatic interaction between $Glu47_{BphA3}$ and $Arg327_{BphA4}$, inducing the dissociation of BphA3 from BphA4 (Figure 2-5). The interplay of ET and induced conformational changes seems to be critical to the sequential reaction of the ET from NADH to BphA3 (Figure 2, [1, 2]).

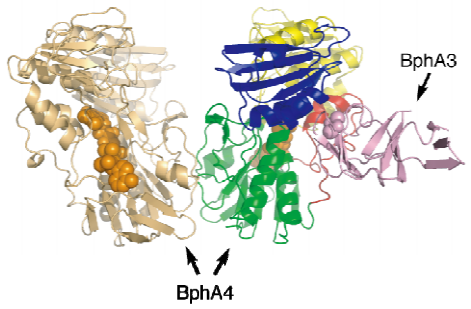

Figure 1 Crystal structure of the BphA3-BphA4 complex

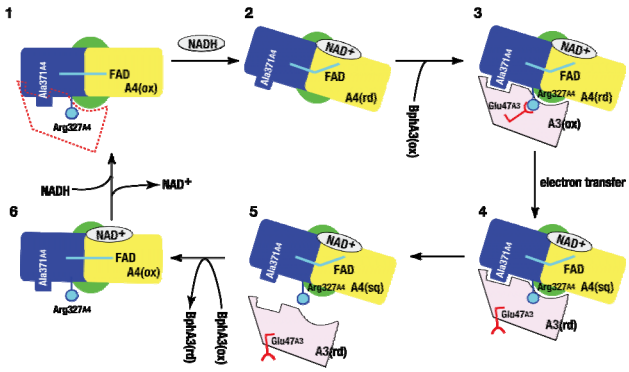

Figure 2 Proposed mechanism of the redox-dependent affinity regulation. The FAD-binding, NADH-binding, and C-terminal domains of BphA4 are shown in green, yellow, and blue, respectively; BphA3 is shown in pink.

References

[1] T. Senda *et al.*, *Antioxid. Redox Signal*. In press.
[2] M. Senda *et al.*, *J. Mol. Biol.*, **373**, 382-400 (2007).
[3] M. Senda *et al.*, *Acta Crystallog. Sect. F*, **63**, 520-523 (2007).
[4] M. Senda *et al.*, *Acta Crystallog. Sect. F*, **63**, 311-314 (2007).

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