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# X-ray structure of the direct electron transfer-type FAD glucose dehydrogenase catalytic subunit complexed with a small subunit

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## 1 Introduction

Various sugar oxidoreductases (dehydrogenases) have been reported to be inherently capable of direct electron transfer to electrodes composed of carbon materials or to gold electrodes. These dehydrogenases harbor an electron-transfer domain or subunit, together with a catalytic domain or subunit. The bacterial flavin adenine dinucleotide (FAD)-dependent glucose dehydrogenase complex derived from *Burkholderia cepacia* (BcGDH) is a representative molecule of direct electron transfer-type FAD-dependent dehydrogenase complexes. These complexes are composed of a catalytic subunit with FAD (α-subunit), an electron-transfer subunit containing three heme c moieties (β-subunit) and a small subunit (γ-subunit). In this study, we determined the X-ray structure of BcGDHγα harboring iron-sulfur cluster [1].

## 2 Experiment

The recombinant BcGDHγα was used for crystallization. Crystals were obtained in a droplet containing a mixture of 1.5 μl protein solution (5.7 mg/ml in Milli-Q water) and 0.75 μl reservoir solution (59.9-60.2% Tacsimate pH 7.0) in a well containing 50 μl reservoir solution using the sitting-drop method at 293 K. To determine the initial phases of wild-type BcGDHγα, selenomethionine derivative (SeMet BcGDHγα) was prepared. Some crystals of SeMet BcGDHγα appeared in a droplet consisting of 1.0 μl protein solution (10.2 mg/ml in Milli-Q water), 0.2 μl of a 0.1 mg/ml subtilisin solution and 1.0 μl reservoir solution (60% Tacsimate pH 7.0) in a well containing 50 ml reservoir solution using the sitting-drop method at 293 K.

X-ray diffraction data of wild-type BcGDHγα and SeMet BcGDHγα were collected on the PF-AR NE3A in the KEK, and processed using the programs HKL-2000 and the CCP4 suite. The initial phases of SeMet BcGDHγα were obtained using the single-wavelength anomalous dispersion (SAD) method with the AutoSol program, and the model of BcGDHγα was constructed using AutoBuild in the PHENIX system. The anomalous dispersion of Fe atoms was utilized in order to determine the number and the positions of Fe atoms in the iron-sulfur cluster. A SAD data set from a crystal of wild-type BcGDHγα was collected to 1.74086 Å resolution on beamline PF-AR NW12A at KEK.

## 3 Results and Discussion

The overall structure of the complex of the γ- and α-subunits of BcGDH was determined and shown in Fig.1. The γ-subunit consists of five α-helices (colored red). The overall structure of the α-subunit comprises 15 α-helices (colored blue) and 17 β-strands and adopts an FAD-binding fold. The additional domain contains a six-stranded antiparallel β-sheet surrounded by six α-helices and a protruding loop including two α-helices (α11 and α12) facing towards the γ-subunit. Two distinguishing long loop regions are located between β2 and β3 and between β4 and α8. The former loop (Ala39-Leu83) contains a unique α-helix (α2) that is located in close proximity to the γ-subunit at the center of the γα-subunit complex and above the iron–sulfur cluster shown in the red circle. The latter loop (Glu197-Asn229) surrounds the iron–sulfur cluster and contains a cysteine cluster (Cys212, Cys213, Cys218 and Cys222); three of the four cysteines are involved in forming the 3Fe-4S cluster.

The locations of Fe atoms were identified using the anomalous dispersion method and were observed in the expected positions of the iron–sulfur cluster. Three Fe-atom sites were identified in the iron–sulfur cluster of each molecule (Figs. 2(a, b)). The simulated-annealing OMIT maps of sulfur ions in the iron-sulfur cluster and the disulfide bond indicated that this iron-sulfur cluster is a 3Fe-4S cluster coordinated by Cys212, Cys218 and Cys222 of the α-subunit. A neighboring cysteine, Cys213, in the α-subunit forms a disulfide bond with Cys152 of the γ-subunit. The unique cysteine cluster of BcGDH γα contributes to the formation of the 3Fe-4S cluster for electron transfer and the disulfide bond for stabilization of the structure of the γα complex.

Our previous report revealed the presence of a 3Fe-4S cluster in BcGDH [2]. The X-ray structure of the catalytic subunit clearly indicated the position of the 3Fe-4S cluster, which is located on the surface of the catalytic subunit. The distance between N5 of FAD and the 3Fe-4S cluster is about 12-13 Å, which is an adequate distance for electron transfer. These results support our hypothesis that the 3Fe-

4S cluster functions in the intramolecular electron transfer from FAD and mediates intermolecular electron transfer from the 3Fe-4S cluster to the electron-transfer subunit (β-subunit). The 3Fe-4S cluster is responsible for electron transfer and interacts with the multi-heme c electron-transfer subunit. To elucidate the electron transfer mechanism of BcGDH, the X-ray structure of the complex of the γ-, α- and β-subunits of BcGDH is needed.

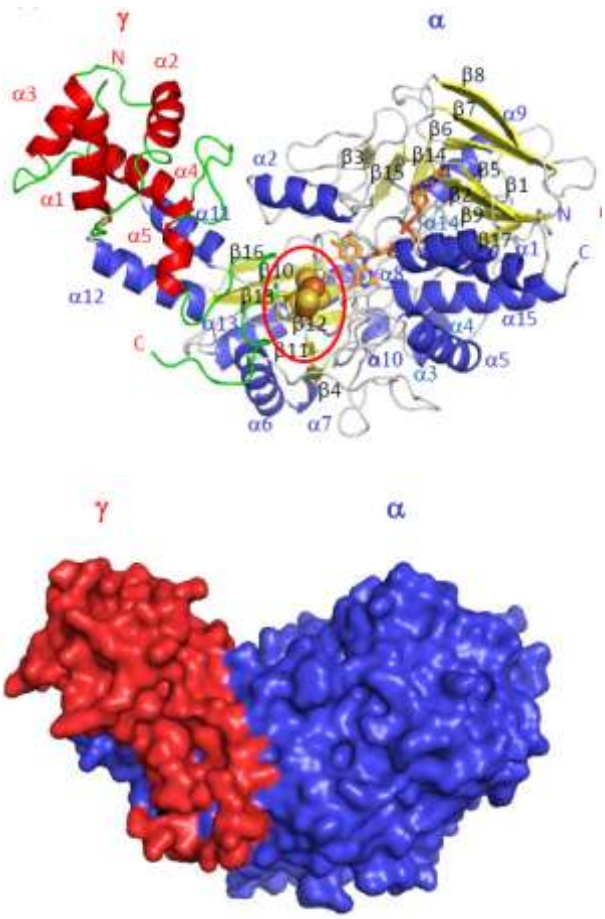


Fig. 1: Overall structure of BcGDHγα.
In the cartoon model (top), the γ-subunit is shown as red α-helices and green loop regions, and the α-subunit is represented with blue α-helices, yellow β-strands and gray loop regions. In the surface model (down), the γ- and α-subunits are shown in red and blue, respectively, and are tightly bound to each other. The bound FAD is represented as an orange stick model. The cysteine cluster indicated with a red circle includes the iron-sulfur cluster shown as spheres.

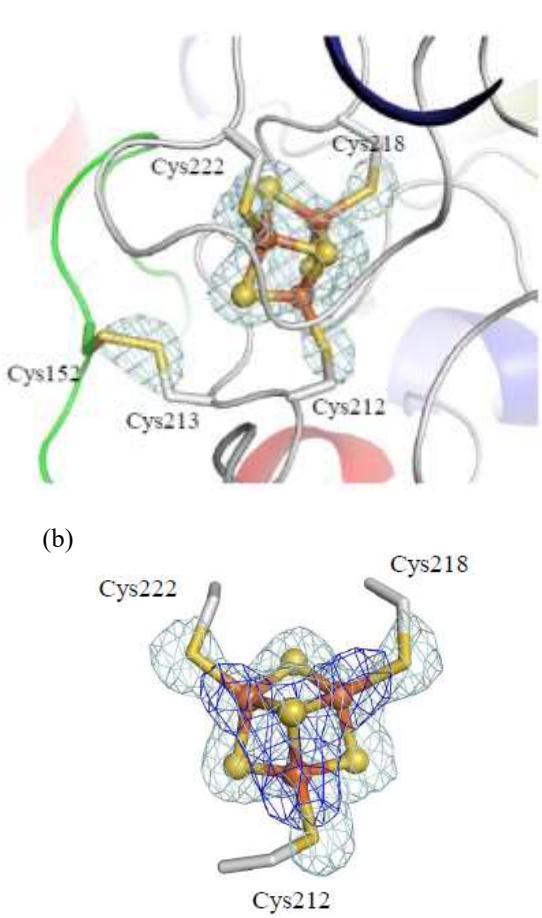


Fig. 2: Cysteine cluster of BcGDHγα.
A unique cysteine cluster is formed by one cysteine from the γ-subunit and four cysteines from the α-subunit. Cys152 and Cys213 form a disulfide bond between the γ- and α-subunits. Cys212, Cys218 and Cys222 are involved in the iron–sulfur cluster (3Fe-4S). The identified positions of the 3Fe-4S cluster and the disulfide bond between the γ- and α-subunits are shown. Simulated-annealing OMIT maps of the 3Fe-4S iron-sulfur cluster and five cysteine residues (Cys152, Cys212, Cys213, Cys218 and Cys222) in wild-type BcGDHγα contoured at 5σ are shown in light blue in Fig. 2. The 2*F*o - *F*c electron-density maps using the merged data sets of wild-type BcGDHγα collected at wavelengths of 1.0 Å and 1.74086 Å are shown in blue and contoured at 5σ in Fig. 2(b).

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References
[1] Yoshida, H. et al., *Acta Cryst*. D**75**, 841-851 (2019).
[2] Shiota, M. et al., *Bioelectrochemistry*, **112**, 178–183 (2016).

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