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Atomic Resolution X-ray Crystal Structure Analysis of Pseudoazurin

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1 Introduction

Type 1 copper site of blue copper protein functions as electron carriers in many biological electron-transfer systems. Therefore, the electronic structure of the copper site has been studied extensively to elucidate the correlation between the structure, unique spectroscopic properties, and the Cu^{2+}/Cu^{+} reduction potential. Up to now, the distortion model describing the presence of axial or rhombic sites invoked a single copper site. However, the possibility for the simultaneous presence of different copper sites in the same protein environment has already been suggested by early resonance Raman measurements and computational modelling.

Pseudoazurin from *Achromobacter cycloclastes* (*Ac*PAz) is an electron donor to nitrite reductase and nitrous oxide reductase in the denitrification process. The previous comprehensive spectroscopic observations indicated the simultaneous presence of two different Cu^{2+} sites, and non-covalent weak interaction significantly influence the ratio. In this experiment, the improvement in crystallization conditions and developments of synchrotron beamlines and software evidence the two positions for the Cu site in *Ac*PAz. The atomic resolution crystallographic analysis also enabled us to correlate with the precise computational modelling with our result of spectroscopy[1, 2].

2 Experiment

The protein of *Ac*PAz was expressed by the recombinant *E. coli* and purified using ion-exchange chromatography. The crystal of *Ac*PAz was ontained by the hanging drop vapor diffusion method. The single crystal X-ray diffraction data for the *Ac*PAz crystal were collected at beam line NW 12A (PF-AR). The temperature of crystal was maintained at 100 K during measurement. The *Ac*PAz crystal belong to the *I222* space group with cell parameters a = 56.1 Å, b = 63.4 Å, c = 67.2 Å. The structure of *Ac*PAz was refined at 1.10 Å resolution. (PDB ID: 4YL4)

3 Results and Discussion

The structure of *Ac*PAz at 1.10 Å resolution demonstrated the co-existence of two Cu position in the same crystal (Figure 1). The occupancy of Cu positions was found to be consistent with the population of axial and rhombic sites from spectroscopic measurements.

Computational modellings using an approximately 6 Å protein environment around the Cu site for both the oxidized and reduced forms showed that a small scale inner sphere rearrangement can account for the co-existence of two different redox active sites for a mononuclear cupredoxin independently from the employed density functionals. The structural optimizations resulted in an energetically less favourable axial site by 20 kJ mol^{-1} than the rhombic, which is in qualitative agreement with the axial site being the minor and the rhombic site being the major component in *Ac*PAz.

The above discussion for the crystallographic results and computational modelling led the conclusion that the dual positions of Cu atom in *Ac*PAz can be explained by the presence of two energetically comparable minima on the potential energy surface within a highly similar ligand environment[3]. The small energetic differnece of these minima indicates the importantce of non-covalent weak interaction from outer sphere (2nd coordination sphere) in type 1 Cu site, or other active sites in metalloproteins.

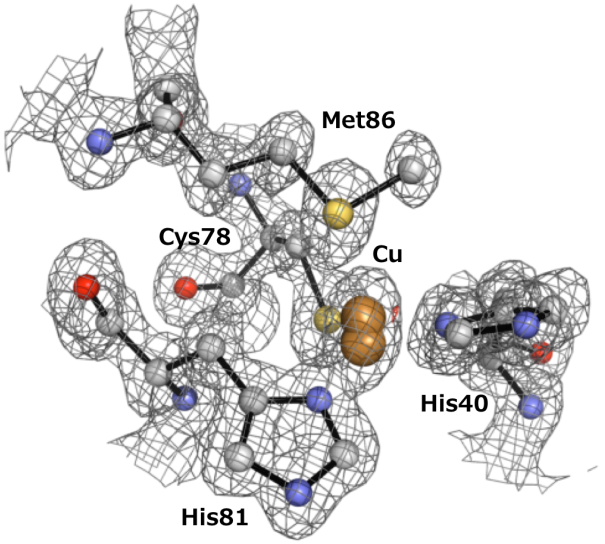

Figure 1. The structure of type 1 copper site of pseudoazurin with $2F_o$-F_c (gray at 1.5 σ level) and F_o-F_c(green and red at ± 4.0 σ level).

References

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