BL-5A, BL-17A/2013G122

High-resolution crystal structures of the solubilized domain of porcine cytochrome b_5

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1 Introduction

Mammalian cytochrome b_5 has multiple electron-transfer partners that function in various electron-transfer reactions. Extensive mutational studies have been reported based on the crystal structure of bovine cytochrome b_5. However, high-resolution structures have not been reported for both the oxidized and reduced states of cytochrome b_5. We have determined four crystal structures at sub-angstrom resolutions in two crystal forms for both the oxidized and reduced states [1].

2 Experiment

Two crystal forms (form 1 and 2) were obtained under the conditions containing polyethylene glycol as a precipitant. Crystals of the oxidized form were obtained under an aerobic condition and crystals of the reduced form were obtained under an anaerobic condition. The concentrations of the polyethylene glycol were increased to collect diffraction data at 100 K. The crystals of the oxidized form were flash-cooled in a nitrogen-gas stream. The crystals of the reduced form were cooled in liquid nitrogen under an anaerobic condition.

Diffraction data sets were collected on the BL-5A and BL-17A beamlines. Two data sets for high-resolution and low-resolution were separately collected from a single crystal at different positions. For the high-resolution data sets, the positions at which the crystals were exposed to X-rays were changed in order to restrict the maximum dose for each exposure position. For the low-resolution data sets, the crystals were exposed to attenuated X-rays to avoid overloading the CCD detectors.

3 Results and Discussion

The crystal structures were determined at resolutions of 0.83 Å (oxi, form1), 0.93 Å (oxi, form 2), 0.76 Å (red, form1) and 0.95 Å (red, form 2) (Table 1). The final models contained 20-28 multiple conformations of amino-acid residues. 17-48% of the hydrogen atoms in the amino-acid residues could be included in the models (Fig. 1a).

Unrestrained refinement for bond angles and lengths indicated that the 7-propionate group of the heme was possibly protonated in the reduced form 2 structure. Form 2 crystals were obtained at pH 5.5, which is lower than the experimentally measured pK_a value of 5.9. On the other hand, the 7-propionate group was in a charged state in the oxidized form 2 structure. The protonation states in the form 2 structures are consistent with electrostatics calculations, in which heme oxidation has been reported to be coupled to some proton loss.

Structural changes depending on the redox states were observed in the hydrogen bond distances from the heme axial ligand His68 to Phe63 between the form 1 structures (Fig. 1b). In addition, water molecules form hydrogen-bonds to both Phe63 and His68. The hydrogen-bond distances of the water molecules are different between the oxidized and reduced forms of the form 1 structures. These results indicate that the hydrogen-bond network around His68 might be involved in regulating the redox state of the heme.

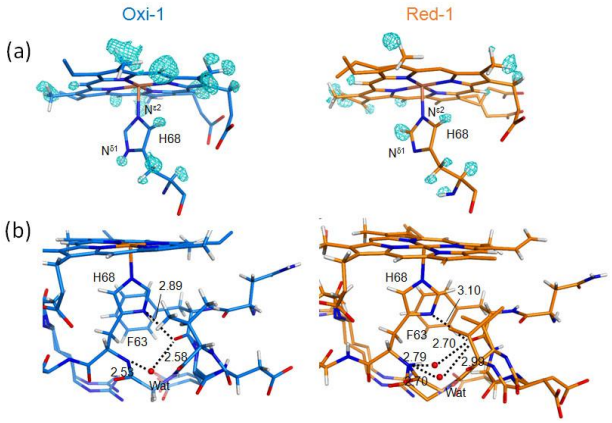

Fig. 1: (a) Electron densities (hydrogen omit maps, 1.5 σ) and (b) hydrogen bonds around the heme axial ligand His 68.

Table 1: Diffraction data statistics

	Oxi-1	Oxi-2	Red-1	Red-2
Beamline	BL-17A	BL-17A	BL-5A	BL-5A
Wavelength (Å)	0.91	0.91	0.80	0.90
Resolution (Å)	50-0.83 (0.84-0.83)	50-0.93 (0.95-0.93)	50-0.76 (0.77-0.76)	50-0.95 (0.97-0.95)
Unique reflections	72,789	63,824	99,196	58,020
Completeness (%)	98.2 (82.3)	98.8 (96.7)	99.8 (97.3)	95.8 (87.0)
$R_{r.i.m.}$ (%)	8.9 (37.4)	6.8 (43.5)	7.1 (38.6)	8.4 (43.9)
$\langle I \rangle / \langle \sigma(I) \rangle$	59.8 (7.6)	50.9 (3.5)	67.4 (4.1)	54.8 (2.3)

Values in parentheses represent the highest resolution shells.

References

[1] Y. Hirano, S. Kimura and T. Tamada, *Acta Cryst.* **D71**, 1572 (2015).

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