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Crystal structure of Group I truncated hemoglobin from *Tetrahymena pyriformis*

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Introduction

A wide diversity in both structure and function has been discovered in the study of hemoglobins (Hbs) from many species. Hemoglobins play oxygen transport in red blood cells in higher organisms. Even though oxygen molecule can diffuse into the cell, unicellular organisms also have hemoglobin-like molecules [1].

Truncated hemoglobins (trHbs) are distributed from bacteria to unicellular eukaryotes and play a role in oxygen transport or nitric oxide detoxification. It has been known that trHbs exist in the ciliates, *Tetrahymena*, but its function and structure was unknown [1]. To understand the function in relation to stability of bound oxygen and its structure, we measured the oxygen binding kinetics of *T. pyriformis* trHb (*Tp* trHb), and solved the crystal structure.

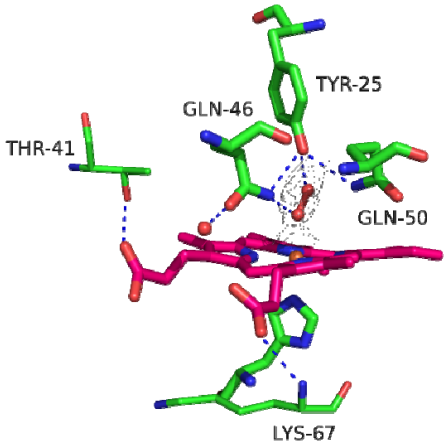
In the present study, we measured optical absorption spectra, O_2-binding property and stability. In addition, crystal structure of Fe(II)-O_2 complex was solved.

Materials and Methods

Recombinant protein was prepared as described [2, 3]. The crystal of Fe(II)-O_2 complex was formed using sitting drop vapor diffusion method, 1.9-2.1 M ammonium citrate, pH 7.0 as a precipitant for 3-4 days at 20 °C. Crystals grew in space group $P6_522$ with unit cell dimensions $a = b = 69.4$ Å, $c = 353.8$ Å, with two molecules per asymmetric unit and a solvent content of 71%. The structure of *Tp* trHb was solved using the molecular replacement method.

Results

O_2 association and dissociation rate constants of *Tp* trHb were determined to 5.5 $\mu M^{-1}s^{-1}$, and 0.18 s^{-1}, respectively. The K_d value of *Tp* trHb was 33 nM. The autoxidation rate was determined to 3.8×10^{-3} h^{-1}. These values are comparable to those of HbN from *Mycobacterium tuberculosis*. To understand the molecular mechanism of O_2 stability, we solved the crystal structure of Fe(II)-O_2 complex of *Tp* trHb. There were two molecules of *Tp* trHb in an asymmetric unit. *Tp* trHb showed typical 2/2 α-helical sandwich fold. N-terminal 4 residues were not modeled because of poor electron density. Heme was coordinated to His73, and further stabilized with hydrogen bond between Thr41 OG1 and heme propionate O2A, and main chain nitrogen of Lys67 and heme propionate O1D (Figure 1).



Figrue 1: Active site structures of Fe(II)-O_2 complex of *Tp* trHb. The F_o–F_c omit map contoured at 3.0σ for oxygen molecule is also shown. Hydrogen bonds are shown by dashed lines. The atomic color scheme for amino acids is: carbon, green; nitrogen, blue; and oxygen, red.

In the Fe(II)-O_2 complex of *Tp* trHb, O_2 molecule is tilted by 135° (average of molecule A and B), relative to Fe axial bond, and is oriented towards to the rear end of the heme crevice. The oxygen molecule formed two hydrogen bonds with the protein molecule. One hydrogen bond was from the hydroxyl of Tyr25 to distal oxygen (O2) with a hydrogen bond distance of 3.1 Å. The other hydrogen bond was from Gln46 of Nε to the proximal oxygen (O1) with a distance of 3.1 Å. Furthermore, the hydroxyl of Tyr25 was hydrogen-bonded from Gln46 and Gln50 of Nε with distances of 2.8 and 3.0 Å, respectively. Although Tyr25 was conserved and hydrogen-bonded to the bound ligand in Group I truncated hemoglobin, strong hydrogen bond between Gln46 and Tyr25 was found only in *Tp* trHb.

References

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