Atomic description of inter-protein electron transfer reaction for biological nitrite reduction in the global nitrogen cycle

Protein electron transfer (ET) reactions play a critical role in biologically vital processes in living cells, most notably respiration and photosynthesis. The reactions occur between protein-bound prosthetic groups separated by long distances, often greater than 10 Å and display high efficiency and specificity. In addition to a dependence on factors inherent to the long-range ET processes, numerous studies have revealed that inter-molecular ET reactions requiring a balance of specific binding and fast dissociation are highly sensitive to protein association modes and their protein-protein interfaces. Hence, the conformational changes of the amino acid residues and the behavior of solvent molecules at the interface formed by redox-partner proteins have the potential to regulate inter-protein ET [1].

Recent earth science and geochemical studies have pointed out that the massive acceleration of the global nitrogen cycle as a result of the production and industrial use of artificial nitrogen fertilizers worldwide has led to a host of environmental problems, ranging from eutrophication of terrestrial and aquatic systems to global acidification. Denitrification is one of the biological processes contributing to the maintenance of the nitrogen balance on the earth. Dissimilatory copper-containing nitrite reductase (CuNIR) is a key enzyme in denitrification, catalyzing one-electron reduction of nitrite (NO_2^-) to nitrogen monoxide (NO). The reaction is specifically regulated by the efficient inter-protein ET reaction with a redox-partner protein. CuNIRs fold a trimeric structure with two distinct Cu sites per a *ca.* 37-kDa monomer unit. The type 1 Cu site (T1Cu) buried within each monomer relays an electron from the redox-partner protein to the catalytic type 2 Cu site (T2Cu), where NO_2^- is reduced to NO. Despite much effort by several groups, a crystal structure of the protein–protein complex between CuNIR and its redox-partner protein has not yet been determined. Here we have determined the binary complex of CuNIR with its redox partner cytochrome *c* (Cyt *c*) at 1.7 Å resolution by X-ray three-dimensional crystallographic analysis [2]. The data sets were collected at beamline **BL44XU**.

A model of the binary CuNIR:Cyt *c* complex, which consists of one Cyt *c* and one CuNIR molecule within an asymmetric unit, contains 8,242 protein atoms, one heme *c* group, six copper atoms, and 1,073 solvent molecules (Fig. 1). Only one Cyt *c* molecule is bound to one subunit (Sub-I) of trimeric CuNIR. The high-resolution crystal structure allows an accurate description of the complex interface between Sub-I and Cyt *c*. At the center of the interface, the Cyt *c* docking site near the T1Cu site of Sub-I and the heme *c* group are in close contact at a 3.5-Å distance between the Cε atom of Met87 in Sub-I and the edge CBC methyl carbon of the thioether-bonded substituent on heme *c*. At least 10 amino acid residues of CuNIR are associated with 11 amino acid residues and the heme *c* group of Cyt *c* at the interface. Interestingly, there is no salt bridge at the interface, and only three direct hydrogen bonds were observed. It was suggested that direct salt bridge and hydrogen bonds between the docking proteins are unfavorable for a transient ET complex because of energetically disadvantageous desolvation.

The building blocks of the interface between CuNIR and Cyt *c* are shown in Fig. 2. The interacting residues of CuNIR are localized both at the hydrophobic patch near the T1Cu site and at the "tower loop" region extending toward the T1Cu site in the CuNIR molecule. These primarily non-polar and neutral residues in both molecules indicate that hydrophobic and van der Waals interactions strongly contribute to complex formation. Contact between both hydrophobic patches brings the redox centers of heme *c* and T1Cu within 10.5 Å, which are close enough to allow for rapid

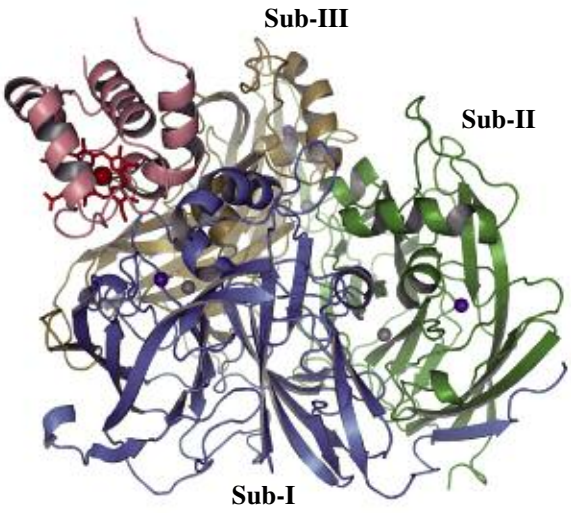

Fig. 1. Overall structure of the interprotein ET complex of CuNIR with Cyt *c*. The Cyt *c* molecule is represented as a pink-colored ribbon, and the Cyt *c*-docked subunit (Sub-I) of CuNIR is shown in sky blue, the undocked subunit (Sub-II) in green, and the other one (Sub-III) in sand color. The heme group (red), T1Cu (dark blue), and T2Cu (gray) are depicted as balls and sticks.

ET [3]. Furthermore, 25 water molecules are located at the docking interface. Eight waters bridging the two proteins through hydrogen bonds stabilize the partner proteins, and the remaining waters also provide stabilization through hydrogen bonds and van der Waals contacts to either Sub-I or Cyt *c* (Fig. 2). All of the water molecules form a characteristic semi-circle around the hydrophobic patch, and the non-polar core interface is sealed off from the aqueous environment.

PATHWAY [4,5] analysis of the CuNIR:Cyt *c* complex was performed to determine the most efficient predicted ET pathway from heme *c* to T1Cu. The predicted pathway through the entry/exit port inside the hydrophobic patches of the interfaces is exhibited in Fig. 3. An electron that leaves iron via the exposed CBC methyl group in the CuNIR:Cyt *c* complex is directly transferred to the Cδ atom of Pro88 of CuNIR by a through-space jump and then shifts from Pro88 to T1Cu through the His89 ligand. The ET pathway represents the most favorable route between the redox centers in the core of the hydrophobic interface.

Recognition and interaction between the protein surfaces, as observed in the transient donor-acceptor (Cyt *c*-CuNIR) complex structure occur through sufficient specificity of polar and non-polar interactions, providing a minimal site at the core of the protein–protein interface that ensures the geometry suited for ET reaction. It is particularly important for a deeper understanding of biological ET processes to explore how interface constructions for efficient ET reaction vary with protein–protein shape complementarity, surface charge and polarity, and dynamic fluctuations of the proteins and the organized water molecules at the interface.

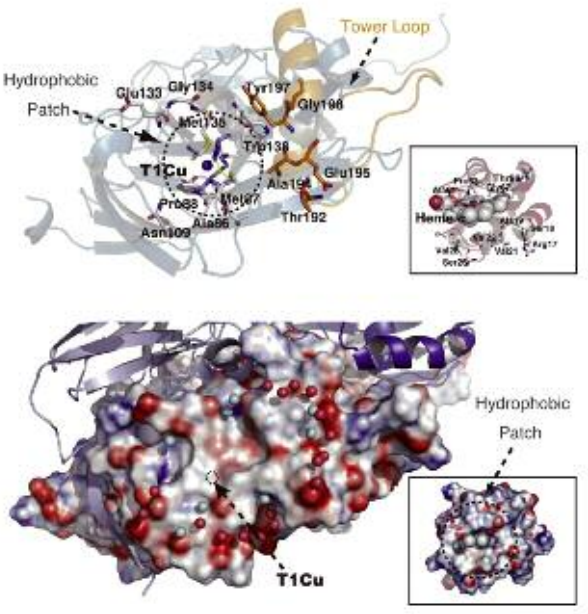

Fig. 2. Docking interface of the CuNIR:Cyt *c* complex. **(a)** The building block of the interface. The amino acid residues at the protein interface and the T1Cu ligands are represented as sticks. The T1Cu ligands are colored in blue, the residues involved in the hydrophobic patch in gray, and the residues of the "tower loop" in orange. Inset: the residues around the heme group in Cyt *c*. The heme group is shown as spheres. **(b)** Electrostatic potentials of contact protein surfaces. Twenty-five water molecules at the interface are represented as spheres. Eight water molecules bridging between the partner proteins through hydrogen bonds are colored in cyan and the other waters binding to Sub-I or Cyt *c* in red.

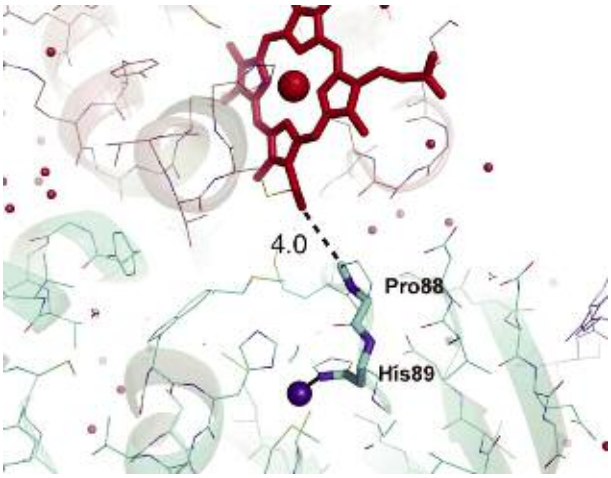

Fig. 3. Theoretically dominant ET pathway between heme *c* and T1Cu. Interprotein ET pathway in the CuNIR:Cyt *c* complex. The best pathway is shown as a broken-line (through-space process) and sticks (through-bond process). The distance of through-space jump between the CBC methyl group and the Cδ atom of Pro88 is given in angstrom. The heme group (red), the T1Cu atom (dark blue), and waters (red) are depicted as balls and sticks.

Masaki Nojiri

Department of Chemistry, Osaka University

E-mail: nojiri@ch.wani.oaka-u.ac.jp

References

[1] C.C. Page *et al.*: Curr. Opin. Chem. Biol. **7** (2003) 551.
[2] *M. Nojiri, H. Koteishi, T. Nakagami, K. Kobayashi, T. Inoue, K. Yamaguchi and S. Suzuki: Nature* ***462*** *(2009) 117.*
[3] R.A. Marcus *et al.*: Biochim. Biophys. Acta **811** (1985) 265.
[4] C.C. Page *et al.*: Nature **402** (1999) 47.
[5] J.N. Onuchic *et al.*: Annu. Rev. Biophys. Biomol. Struct. **21** (1992) 349.