Anaerobic and Aerobic Structures of Ferredoxin II from *Desulfovibrio Gigas*

Microorganisms such as sulfate-reducing bacteria (SRBs) obtain energy for growth by transferring electrons from an electron donor to an electron acceptor, such as oxygen, nitrate, iron (III), manganese (IV), sulfate and carbon dioxide, during cellular respiration. During this process (electron transport chain), the electron acceptor is reduced and the electron donor is oxidized. Although they are still being considered as strictly anaerobes, when exposed to oxygen, SRBs are capable of surviving as well as taking advantage of the presence of oxygen in terms of energy conservation. In the presence of oxygen, a sulfate reducer, *Desulfovibrio gigas*, starts utilizing internal reserves of polyglucose, which is metabolized via the Embden-Meyerhof-Parnas pathway, thus generating NADH and ATP (Fig. 1) [1].

Ferredoxins (Fds), which are redox proteins containing iron and sulfur atoms, play a role in electron transfer processes related to the phosphoroclastic reaction and the reduction of sulfite by SRBs. These proteins of small molecular weight (~ 6 kDa) have low redox potentials, characteristic electronic spectra, and typical EPR signals. *D. gigas* has two forms of Fd, i.e., Fd I and Fd II. Fd I contains a single [4Fe-4S] cluster while Fd II contains one [3Fe-4S] cluster per molecule [2], and both proteins are composed of the same polypeptide chain of 58 amino acids. Fd I has a redox potential of 450 mV and Fd II exhibits an E_0' of -130 mV.

Most Fds exhibit the biological activity during the stimulation of hydrogen consumption with sulfite as a terminal electron acceptor or during hydrogen production from pyruvate. It has been shown that the tetraheme cytochrome c3 is an intermediate between hydrogenase and ferredoxin. In *D. gigas*, Fd I and Fd II function in different metabolic pathways. Fd I is required in the phosphoroclastic reaction in which hydrogen is evolved from the oxidation of pyruvate. Fd II also stimulates this phosphoroclastic reaction after a long lag phase when it is added to Fd-depleted *D. gigas* crude extract. Pyruvate can induce the conversion of the [3Fe-4S] cluster of *D. gigas* Fd II into the [3Fe-4S] cluster of Fd I in the presence of *D. gigas* crude cell extract. Purified Fd II can also be converted to Fd I following incubation with an excess amount of Fe^{2+} in the presence of dithiothreitol, demonstrating that the polypeptide chain of *D. gigas* Fd can accommodate both [3Fe-4S] and [4Fe-4S] clusters. The [3Fe-4S]/[4Fe-4S] interconversion previously found in *D. gigas* Fds indicated that transition metals, other than iron, could be incorporated into the center of the [3Fe-4S] cluster.

Both aerobic and anaerobic Fd II structures have been independently determined by the Fe-SAD method at beamline **BL12B2** and NSRRC [3]. The aerobic Fd II structure shows that the [3Fe-4S] cluster is bound to the polypeptide chain by three cysteinyl residues: Cys 8, Cys 14, and Cys 50. Residue Cys 11, a potential ligand for the fourth site of the [4Fe-4S] cluster, is twisted away from the cluster. A disulfide bridge between Cys 18 and Cys 42 is located where the second iron-sulfur cluster is found in other 2×[4Fe-4S] Fds. It was observed that this disulfide bridge can be opened during the reduction of Fd II. Furthermore, six additional zinc metal ions are observed at the highly

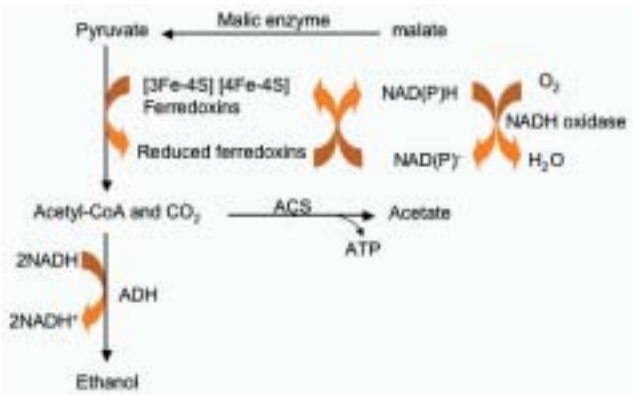

Fig. 1. Scheme of anaerobic metabolic pathways. The ferredoxins in *D. gigas* contain two cluster forms, [3Fe-4S] and [4Fe-4S].

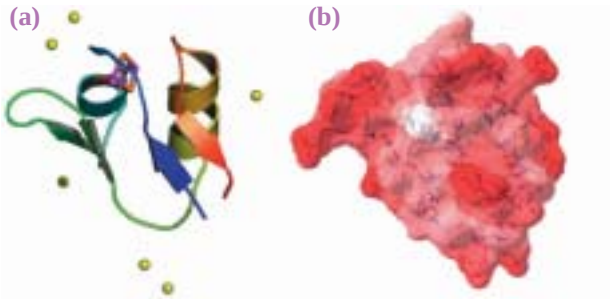

Fig. 2. **(a)** Overall structure of ferredoxin II. Beside the cluster, there are six Zn^{2+} ions bound around the structure. **(b)** The electrostatic potential of ferredoxin II shows negatively charged residues (deep red regions) predominating on the protein surface, which is important for metal binding.

negatively charged surface area ligated with Glu and Asp residues, implying the potential metal-binding sites for iron and other possible heterometal-containing cluster formation and conversion in the reduced state under the metal-abundant environment (Figs. 2(a,b)). In particular, two metal sites with the binding residues Asp6, Asp7, Glu12 and Glu16 are relatively close to the existing [3Fe-4S] cluster. Moreover, an additional [2Zn-2S] cluster bound by residues Glu26, Glu27, Asn35 and Asp37 is found (Fig. 3). Hence, two clusters form the electron transfer pathway with one [3Fe-4S] cluster inside and the other [2Zn-2S] cluster outside the protein.

The superimposition of aerobic and anaerobic Fd II structures shows an overall r.m.s. deviation of 1.01 Å over 58 amino acids with the most significant main-chain structural variations occurring at residues 25-28. The tide disulfide bond (Cys18-Cys42) existing in the aerobic structure shows the double conformations, which can be interpreted as the opening up of the covalent disulfide bond. Moreover, the iron-sulfur cluster geometries in the anaerobic structure are much different from those in the aerobic structure with the maximum increasing and decreasing changes of Fe-S bond distances by 0.15 Å and 0.14 Å, respectively. Hence, the longest and shortest interatomic distances of Fe-S bonds in the cluster are 2.18 Å and 2.36 Å, respectively, which is meaningful for high-resolution data (Figs. 4(a,b)). The influence of these bond variations might play a role in determining the electronic configuration of the reduced cluster. Thus, a comparison between aerobic and anaerobic structures at a high resolution provides valuable insights into different iron-sulfur cluster conversions and disulfide bridge conformations, which may reveal the unique iron storage function and electron transfer mechanism of Fd II from *D. gigas*.

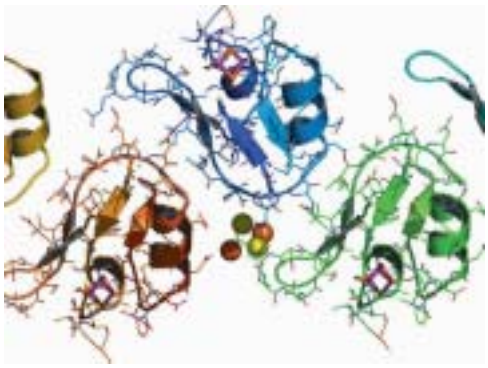

Fig. 3. An additional [2Zn-2S] cluster is bound by residues Glu26, Glu27, Asn35 and Asp37, which may involve the electron transfer pathway with the other existing [3Fe-4S] cluster inside.

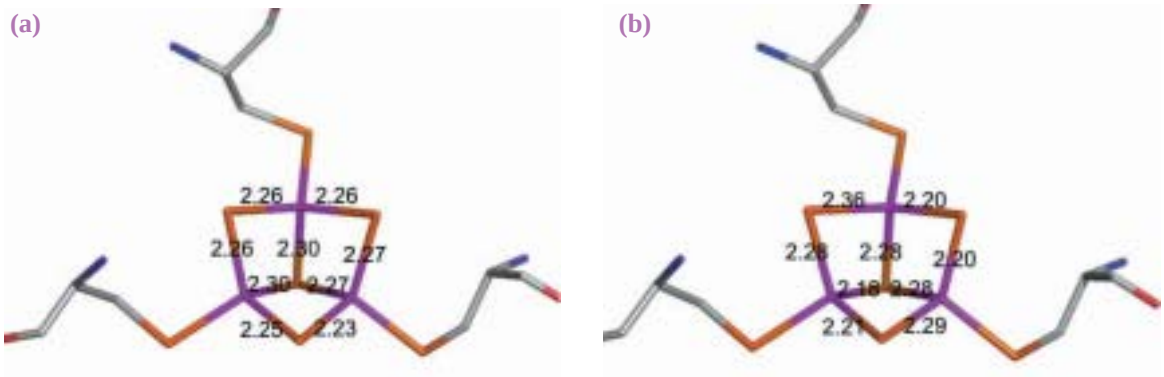

Fig. 4. (a) The iron-sulfur cluster geometries in the aerobic structure show similar Fe-S bond distances with the maximum deviation of 0.7 Å. (b) The anaerobic cluster geometries, however, are much different especially in the longest and shortest distances of 2.18 Å and 2.36 Å, respectively.

Yin-Cheng Hsieh[a,b], Ming-Yih Liu[a] and Chun-Jung Chen[a,c,*]

(a) Biology Group, National Synchrotron Radiation Research Center, Taiwan
(b) Department of Bioinformatics and Structural Biology, National Tsing-Hua University, Taiwan
(c) Department of Physics, National Tsing-Hua University, Taiwan

*E-mail: cjchen@nsrrc.org.tw

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