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論　文　内　容　の　要　旨

氏　名　（　Ju Yaen Kim　）	
論文題名	Interaction of Sulfite Reductase with Ferredoxin and its Regulation to Enzymatic Activity (フェレドキシンと亜硫酸還元酵素間の分子間相互作用と酵素活性の関係性に関する研究)

Sulfite reductase (SiR) plays a critical role in sulfur assimilation by reducing sulfite to sulfide. SiR uses six electrons transferred from ferredoxin (Fd) which is a physiological electron donor. Electrons are intermolecularly transferred from the [2Fe-2S] cluster of Fd to the [4Fe-4S] cluster of SiR and then flow intramolecularly to siroheme. Further sequential catalytic reactions by other enzymes produce amino acids such as methionine and cysteine using sulfide. Thus, interprotein interactions between Fd and SiR have been suggested to be essential for overall activity of SiR.

Although intermolecular electrostatic interactions between negatively charged Fd and positively charged SiR have been shown to be important for SiR function, natures of the interprotein which may control the formation of the electron transfer complex for SiR activity still remain to be clear. In order to address this issue, I performed in-depth investigation on interactions between Fd and SiR and their relations to SiR activity using various biochemical and biophysical approaches. The analyses of the crystal structure of the Fd:SiR complex and site-directed mutagenesis based on the structure, multiple SiR activity assays, binding thermodynamics of isothermal titration calorimetry (ITC), and solution-state NMR spectroscopy at atomic resolution revealed that interfacial non-covalent interactions are important for the interprotein affinity and modulation of the Fd:SiR complex thereby affecting Fd-dependent SiR activity.

The structure of the complex of maize SiR and Fd has been determined by X-ray crystallography and three possible structures of the complex were dissolved. Although topological relationship of SiR and Fd varied in each of the structures, common characteristics were found both in the electrostatic intermolecular interactions and the positional arrangements of the redox centers in a close proximity with the shortest distance around 12 Å. This indicated that the three complexes would be functionally competent. Mutational analysis of basic residues of SiR distributed widely at the interfaces of the three structures showed their importance for Fd-dependent SiR activity and contribution for a strong affinity of SiR with Fd. Based on these combined results, I suggest that the electron transfer complex of SiR and Fd could be formed though multi-intermolecular interaction processes. This implication is discussed in terms of the multi-functionality of Fd in various redox metabolisms.

Furthermore, I examined how interfacial mutagenesis of SiR influences its activity and binding ability for Fd in combination of SiR activity assays with three types of substrates, NMR, and ITC. SiR activity assays revealed that disruption of interprotein electrostatic interactions by neutralizing positive charges of SiR decreased mostly SiR activity without remarkable activity changes depending on substrates. ITC and NMR results showed abolishment of binding ability of a K582Q/K584Q double mutant for Fd. Meanwhile, SiR mutants, which hydrophobicity decreased, showed the substrate-dependent changes in activity. ITC results showed that thermodynamic parameters of complex formation of Q504G SiR with Fd were similar to those of wild type SiR. Similar but distinct binding sites of ^{15}N-labeled Fd for wild type and Q504G SiRs were revealed by the chemical shift difference and distinct direction of NMR peak shifts. These findings demonstrate that intermolecular electrostatic interactions are fundamental for SiR activity by forming the productive electron transfer complex and hydrophobic interactions play an important role for substrate preference using subtle differences in configuration of the Fd:SiR complex. I suggest that enzymes may evolve to use skillfully these non-covalent interprotein forces for biological efficiency.

I also investigated Fd:SiR complex formation and SiR activity over NaCl concentrations between 0 and 400 mM with considering physiological conditions. Fd-dependent SiR activity assays and Michaelis-Menten kinetics revealed a bell-shaped activity curve with a maximum around 40-70 mM NaCl and a reverse bell-shaped dependence of affinity (Michaelis constant). Calorimetric analyses showed the monotonic increase in the dissociation constant on increasing NaCl concentrations, distinguished from the biphasic change in the Michaelis constant. The results further revealed that Fd:SiR complex formation and interprotein affinity were thermodynamically adjusted by both enthalpy and entropy through electrostatic and non-electrostatic interactions. A residue-based NMR investigation on addition of SiR to ^{15}N-labeled Fd also demonstrated that a combination of both non-covalent forces stabilized the complex with similar interfaces and modulated binding affinity and mode depending on NaCl concentrations. These findings elucidate that non-electrostatic forces are also essential for complex formation and modulation. I suggest that a complex configuration optimized for maximum enzymatic activity near physiological conditions is achieved by structural rearrangement through controlled non-covalent interprotein interactions.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

亜硫酸還元酵素は、電子伝達蛋白質であるフェレドキシンから供与される還元力に依存して触媒機能を発揮する。この際、両者は電子伝達複合体を形成し、効率の良い分子間電子伝達反応が進行する。本学位論文では、両者の複合体のｘ線結晶構造とその知見に基づいた亜硫酸還元酵素の変異体の解析、及び複合体形成の溶液状態でのNMRや等温滴定熱測定による解析を行い、複合体形成の構造生物学と熱力学的特性を明らかにするとともに、亜硫酸還元酵素の酵素活性が分子間相互作用の制御下で調節されることを提唱した。

本研究は、蛋白質・蛋白質の弱い相互作用の実体とその生物学的意義を明らかにした点で重要性と新規性があり、関連分野に波及する成果を提出しているものである。よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。