バイオサイエンス研究科　博士論文要旨

所　属 (主指導教官)	生体高分子構造学講座　(箱嶋敏雄)		
氏　名	広津晶子	提　出	平成　12年　1月　6日
題　目	Crystal structure of a multifunctional 2-Cys peorxiredoxin heme-binding protein 23kDa / proliferation-associated gene product. (多機能性の2-Cys型ペルオキシレドキシンであるヘム結合蛋白質２３ｋＤａ／ＰＡＧの結晶構造)		

Heme-binding protein 23kDa (HBP23), a rat isoform of human proliferation-associated gene product (PAG), is a member of a recently identified peroxidase family referred to as peroxiredoxins (Prxs) [1]. To date, more than 40 Prxs were isolated from several sources including bacteria, yeast, plant, and mammalia. They regulate the intracellular concentration of H_2O_2 by reducing it in the presence of an appropriate electron donor. Recently, H_2O_2 has been implicated as an intracellular messenger that affects cellular processes, and Prxs have been reported to reduce such H_2O_2. In addition, HBP23/PAG has been reported to directly interact with the signaling protein, c-Abl, and to inhibit its tyrosine kinase activity . HBP23/PAG also exhibits specific binding to heme. Prxs have catalytically active cysteine at the N-terminal region, and most Prxs including HBP23/PAG have additional conserved cysteine at the C-terminal region . The catalytic cycle of these Prxs (2-Cys Prxs) has been appeared to link to the thioredoxin reductase system, which is one of two major intracellular redox systems. To clarify the reaction mechanism of a 2-Cys Prx and to obtain some information about the binding surface of c-Abl and heme, the author determined a 2.6Å resolution crystal structure of rat HBP23 in oxidized form[2,3].

The structure revealed an unusual dimer structure in which the active residue Cys52 forms a disulfide bond with conserved Cys173 from another subunit by C-terminal tail swapping. The active site is largely hydrophobic with partially exposed Cys173. In site-directed mutagenesis studies, the replacement of Cys173 with serine was found to abolish the peroxidase activity with a coupled thioredoxin reductase system. The molecular surfaces around the active residues, thioredoxin Cys32 and HBP23 Cys173, show a general shape match, suggesting a reduction mechanism of oxidized HBP23 by thioredoxin. Thus, the unusual cysteine disulfide bond is involved in peroxidation catalysis by using

thioredoxin as the source of reducing equivalents. In addition, significant local structural changes around the active site of HBP23 in reduced form are proposed. The structure also provides a clue to possible interaction surfaces for c-Abl and heme. Several significant structural differences, especially around the active site, have been found from a 1-Cys Prx, ORF6 [4], which lacks the C-terminal conserved cysteine corresponding to Cys173 of HBP23.

[1] Claiborne, A., Yeh, J. I., Mallett, T. C., Luba, J., Crane, E. J., Charrier, V., and Parsonage, D. (1999). Protein-sulfenic acid: diverse roles for an unlikely player in enzyme catalysis and redox regulation. *Biochemistry*, **38**, 15407-15416.

[2] Hirotsu, S., Abe, Y., Nagahara, N., Hori, H., Nishino., T., Okada, K., and Hakoshima, T. (1999). Crystallographic characterization of a stress-induced multifunctional protein, rat HBP23. *J. Struct. Biol.* **126**, 80-83.

[3] Hirotsu, S., Abe, Y., Okada, K., Nagahara, N., Hori, H., Nishino, T., and Hakoshima, T. (1999). Crystal structure of a multifunctional 2-Cys peroxiredoxin heme-binding protein 23kDa/proliferation-associated gene product. *Proc. Natl. Acad. Sci. U S A.* **96** 12333-12338.

[4] Choi, H., Kang, S. W., Yang, C., Rhee, S. G., and Ryu, S. (1998). Crystal structure of a novel human peroxidase enzyme at 2.0Å resolution. *Nat. Struct. Biol.* **5**, 400-406.

論文審査結果の要旨

申請者氏名　広津　昌子

平成１２年１月６日に提出された論文は、多機能性の2-Cys Prc型ペルオキシレドキシンであるヘム結合蛋白質23kDa（HBP23）/PAGのＸ線結晶解析の方法を用いた三次元構造決定とその構造に基づいた分子機能のメカニズムの解明からなる。

三次元構造決定では、試料の充分な精製、良質な結晶の調製、高分解能の観測強度データの収集、多重重原子同型換法による位相決定、精度の高い三次元構造の精密化がなされており、技術的信頼性は高い。蛋白質のＸ線による原子レベルの三次元構造決定とは、蛋白質の発現や精製などの生化学実験から、Ｘ線強度データ収集などの物理実験、そして位相決定や構造解析における数値計算を含んでいるが、それらの全ての方法について十分な実力を有するものと判断した。

分子機能とそのメカニズムについては、その精度の高い三次元構造に基づいて詳細な構造学的な議論と、多くの関連した生化学的データを引用した機能についての慎重な考察の結果として結論されており、充分な妥当性が認められる。これらは、HBP23の活性部位の同定、ペルオキシダーゼ活性の触媒メカニズムの提唱、チオレドキシンとの反応の構造的妥当性の検証、触媒反応における蛋白質構造変化、ヘム結合部位、チロシンキナーゼc-Ablの結合部位の検討したことである。
また、論文全般におりて、記述の明解さも水準に達していると判断された。

以上のように、本論文は酸化ストレス応答蛋白質の構造生物学に貴重な基礎データを提供するもので、学術上、応用上貢献するところが少なくない。よって審査委員一同は、本論文が博士（バイオサイエンス）の学位論文として価値あるものと認めた。