2013B 期　採択長期利用課題の紹介

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2013B 期は4件の長期利用課題の応募があり、3件が採択されました。採択された課題の審査結果および実験責任者による研究概要を以下に示します。

－ 採択課題1 －

課題名	Application Development of Nuclear Resonance Vibrational Spectroscopy (NRVS) and Synchrotron Mössbauer Spectroscopy of Multinuclear Iron Proteins
実験責任者名(所属)	Stephen Cramer (University of California, Davis)
採択時の課題番号	2013B0103
ビームライン	BL09XU
審査結果	採択する

［審査コメント］

The principal investigator (PI) developed the technique of NRVS (Nuclear Resonance Vibrational Spectroscopy) at beamline BL09XU and has been applying it to iron atoms in metalloproteins. This achievement should be highly appreciated. SPring-8 was featured on the cover of Angewante Chemie in which a recent paper from the PI's group was published. Currently, the PI is working on several iron-containing proteins with NRVS. However, in this new long-term proposal, the focus of the entire study was not clear to the committee. Since the PI made significant contribution for development of nuclear resonance scattering, the committee expects him to expand this technique further. For instance, the spectral range may be widened. Thus, the committee suggests the PI to use the reduced shifts on technical development to make NRVS more widely utilized in protein chemistry. Accordingly, the title should be changed to "Application Development of Nuclear Resonance Vibrational Spectroscopy (NRVS) and Synchrotron Mössbauer Spectroscopy of Multinuclear Iron Proteins". For application to individual proteins, the PI should consider submitting a regular proposal.

［実験責任者による研究概要］

This proposal aims to use the technique of nuclear resonance vibrational spectroscopy (NRVS) to address issues about the structure and dynamics of Fe-S proteins. The NRVS technique involves scanning a highly monochromatic (~1 meV) beam of x-rays through a nuclear resonance (in our case ^{57}Fe) and monitoring transitions that correspond to vibrational modes. NRVS is proving to be a valuable complement to more established techniques such as protein crystallography, EXAFS, resonance Raman, and other methods. NRVS yields a vibrational spectrum that is selective for normal modes involving motion of Fe sites.

Fe-S proteins serve a wide variety of essential tasks in living systems, including electron transfer within proteins, catalysis of chemical reactions, sensing of the chemical environment, regulation of DNA expression, repair of damaged DNA, and maintenance of molecular structure[1]. Our research in this long term proposal focuses on three critical reactions that are handled by Fe-S proteins: the production and consumption of hydrogen (H_2) by the NiFe and FeFe classes of hydrogenase (H_2ase) enzymes[2], (2) the production of ammonia (NH_3) and hydrocarbons by the MoFe and VFe versions of the enzyme called nitrogenase (N_2ase)[3,4], and (3) the sensing of nitric oxide (NO) by the Fe-S cluster proteins[5] WhiD from the tuberculosis causing bacteria *M. tuberculosis*[6] and NsrR from *E. coli*[7].

Our program aims to use NRVS to answer structural and dynamic issues about these proteins. Based on the advice of the review committee, we will also work to improve the biological NRVS technique. In particular,

we will attempt to (1) develop a cryostat that can more efficiently capture the Fe K-fluorescence from our samples, (2) further lower the background count rate to allow observation of weaker signals, and (3) develop new scanning algorithms to optimize our use of beam time. We hope that our contributions will make biological NRVS an even more popular technique at SPring-8.

References

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［2］"Studies on hydrogenase", Yagi, T.; Higuchi, Y. *Proc. Japan Acad. Series B*, **2013**, *89*, 16-33.

［3］"Nitrogenase reduction of carbon-containing compounds", Seefeldt, L. C.; Yang, Z.-Y.; Duval, S.; Dean, D. R. *Biochim. Biophys. Acta*, **2013**.

［4］"Nitrogenase: A Draft Mechanism", Hoffman, B. M.; Lukoyanov, D.; Dean, D. R.; Seefeldt, L. C. *Acct. Chem. Res.*, **2013**, *46*, 587-595.

［5］"Bacterial Iron-Sulfur Regulatory Proteins As Biological Sensor-Switches", Crack, J. C.; Green, J.; Hutchings, M. I.; Thomson, A. J.; Le Brun, N. E. *Antioxidants Redox Sig.*, **2012**, *17*, 1215-1231.

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［7］"In vivo Fe-S cluster acquisition by IscR and NsrR, two stress regulators in Escherichia coli", Vinella, D.; Loiseau, L.; de Choudens, S. O.; Fontecave, M.; Barras, F. *mol. microbiol.*, **2013**, *87*, 493-508.

－ 採択課題２ －

課題名	放射光メスバウア法とＸ線粉末回折による下部マントルおよび核構成物質の高温高圧物性の研究
実験責任者名(所属)	大谷　栄治（東北大学）
採択時の課題番号	2013B0104
ビームライン	BL10XU
審査結果	採択する

［審査コメント］

本課題は、高温度・高圧力環境下における放射光メスバウア分光とＸ線粉末回折の同時測定により、地球中心部の物質構成のモデル構築を目指すものである。地震学の観測によって得られた情報を説明するモデル構築は、現代地球科学のフロンティア研究としてインパクトのあるテーマである。本課題は、１)地球内部に存在する金属軽元素合金、ケイ酸塩や酸化物の高温度・高圧力下での結晶構造、融点、電子状態、磁性の解明、２)地球内物質の溶融状態の解明の２項目を具体的なテーマとして設定している。これらは、これまでの長期利用課題で構築した極限環境下複合測定システムを活用するものであり、SPring-8の計測基盤を最大限に活用する研究である。

これまでのすぐれた成果を踏まえた上で、明確な目標とそれを実施するための適切な研究計画が立てられており、今後も大きな成果が期待できるので、本申請課題を長期利用課題として採択するものとする。

［実験責任者による研究概要］

本長期利用課題は、大谷による「放射光Ｘ線回折法およびスペクトロスコピーを併用した地球中心部の総合的解明」(長期利用課題2009B0028）の発展を目指すものである。この長期利用課題において、我々は二つの大きな技術開発を行った。その一つは、放射光メスバウア分光法を高温高圧粉末Ｘ線ビームライン（BL10XU）に導入することによって、メスバウア分光法と同じ光学系でＸ線粉末回折実験を行うシステムを導入し、ルーチンでの同時測定に成功した。このシステムに外熱ダイヤモンドアンビル高圧装置を設置することによって、1000 Ｋまでの高温での測定が可能である。そして、地球の核・マントルを構成する代表的な物質であるマグネシオブスタイト (Mg, Fe) O およびペロブスカイト (Mg, Fe) SiO_3 の圧縮実験とともに、放射光メスバウア分光測定によって、これらに含まれる鉄のスピン状態を高圧下で明らかにした。第二の技術開発は、様々なビームラインにおいて、ダイヤモンドアンビルを用いた高圧発生実験に際して、2000 Ｋを超える高温を発生し温度測定を可能にするポータブルレーザー加熱測温システムを開発し、150 GPa 以上の高圧のもとで2300 Ｋで30時間の安定な加熱を可能にした。

この長期利用課題においては、これらの我々が新

たに開発した技術を用いて、BL10XU ビームラインにおいて、外熱法およびレーザー加熱法を用いて室温から2000 K を超える広い温度領域において、X線粉末回折法と放射光メスバウア分光の同時測定を行う。具体的には、これらの方法を併用して、地球核を構成すると考えられている鉄・軽元素合金や下部マントルを作るケイ酸塩や酸化物の高温高圧下でのスピン状態、磁性、電子物性、結晶構造を解明したい。

さらに、これまで実験を継続している金属鉄軽元素系の溶融状態の解明を進める。これまでに、ダイヤモンドアンビルの試料部を工夫することによって、30 GPa、2500 K において Fe-S-O 融体からのX線散漫散乱の測定に成功している。今後、試料構成をさらに工夫すること、より細いX線ビーム径を用いるなど、放射光X線回折技術をさらに改良・開発することによって①金属鉄軽元素系やマントルを構成するケイ酸塩系の溶融関係、融点の決定、②融体の密度、構造の解明を行いたい。

以上の実験によって、地震学観測によって得られた情報を説明する外核の実態と温度構造を解明することなど、地球中心部の物質構成のモデルを構築したい。

－ 採択課題３ －

課題名	NRVS of mononuclear and binuclear non-heme iron enzyme intermediates and related model complexes
実験責任者名(所属)	Edward Solomon (Stanford University)
採択時の課題番号	2013B0105
ビームライン	BL09XU
審査結果	採択する

［審査コメント］

This group have been applying NRVS (Nuclear Resonance Vibrational Spectroscopy) to a few classes of non-heme iron-containing proteins. They observed low-frequency vibrational modes of high-valent diiron complexes involving Fe motion and its spin state, and assigned them using DFT (Density Functional Theory) calculations. Such studies have been published on several high-impact journals. The group are now focusing on several classes of iron-containing protein to understand the common function of iron atoms in each class of proteins. To achieve this goal, a substantial amount of beam time is necessary. Through this long-term project, the committee expects them to develop a unified understanding of function of non-heme iron atoms.

［実験責任者による研究概要］

Mononuclear and binuclear non-heme iron (NHFe) enzymes have a wide range of activity and play crucial roles in health and in bioremediation. The goal of this research is to use Nuclear Resonance Vibrational Spectroscopy (NRVS) to characterize oxygen intermediates of these enzymes for mechanistic elucidation. NRVS is ideal for these studies because the vibrational information on the enzyme intermediates gives selective information on the Fe active site and therefore provides insight into the geometric and electronic structures of the oxygen intermediates and their contributions to enzyme reaction mechanism. The intermediates we plan to study are summarized below.

The key reactive intermediate of many mononuclear NHFe enzymes that catalyze a wide variety of oxidative reactions, including halogenation, hydroxylation, ring closure, desaturation and aromatic ring cleavage, is a high-spin (*S*=2) Fe(IV)=O species. A Cl-/Br--ligating *S*=2 Fe(IV)=O intermediate of the aKG-dependent halogenase SyrB2, which chlorinates the native L-threonine (Thr) through activation of the aliphatic C-H bond during syringomycin E biosynthesis, was recently trapped (with an inert substrate analog, Cpg) and structurally characterized by NRVS. We would like to extend these studies to elucidate the geometric structure of the *S*=2 (SyrB2)Fe(IV)=O intermediate in the presence of native (Thr) and non-native L-norvaline (Nva) substrates, which are halogenated and hydroxylated, respectively, and to the *S*=2 Fe(IV)=O intermediate of taurine dioxygenase (TauD), a prototypical aKG-dependent mononuclear NHFe enzyme which has a facial triad carboxylate rather than the halide that can both H atom abstract and perform electrophilic aromatic substitution (EAS).

Rieske dioxygenases (RDO) are mononuclear non-heme iron enzymes that catalyze the stereo- and regio-specific addition of dioxygen to unreactive aromatic substrates to produce *cis*-dihydrodiols, the first step in the bioremediation of aromatic compounds in the environment. A molecular understanding of the detailed mechanism by which these enzymes activate O_2 for

incorporation in organic substrates will aid in the development of bioinspired catalysts and bioremediation systems. Benzoate 1,2-dioxygenase (BZDO) is our benchmark RDO enzyme. The key intermediate in its catalytic cycle is proposed to be a side-on (hydro) peroxo–Fe(III) species BZDOp, and two pathways have been proposed for the reaction of this intermediate with benzoate. Understanding the protonation state and binding mode is key to distinguish these proposed reaction pathways, and we will obtain that information through NRVS.

HPCD is a member of the class of extradiol dioxygenases that utilize a mononuclear NHFe active site and play an important role in the bioremediation of aromatic carbon sources in soil bacteria. The extradiol enzymes are unusual among mononuclear NHFe enzymes in that the reaction they catalyze is believed to proceed through a peroxy-bridged intermediate. HPCD is an ideal enzyme for exploring reactivity of the extradiol dioxygenases, as several oxygenated intermediates in mutant forms have been trapped, and intermediates in the wild-type enzyme have been crystallized. Applying NRVS to this enzyme will help determine the geometric nature of the oxygenated intermediates in the extradiol reaction pathway, and coupling NRVS data to DFT calculations will lead to the experimentally-calibrated elucidation of the extradiol reaction mechanism.

Binuclear NHFe enzymes utilize a diiron cofactor to activate O_2 for a wide range of reactions. In general, their active resting states bind O_2 to form peroxy biferric intermediates which can be classified into two groups: P and P'. Elucidating the structural differences between P and P' is essential to understand their different reactivities: P needs to be activated for enzyme reactivity, and in wild-type ribonucleotide reductase (RR), conversion from P to P' is necessary for the reaction to proceed. P' is also demonstrated to be key in EAS reactions of binuclear NHFe enzymes. For this conversion, computational studies have proposed several possible active-site structural changes. To distinguish among the possibilities, experimental data on P and P' are necessary, and NRVS is the most suitable technique for this system, as P' is inaccessible to the generally employed spectroscopies.

Two different high-valent intermediates have been observed in the binuclear NHFe enzymes. One is an Fe(III)Fe(IV) intermediate, X, in RR that is generated from P' after one-electron reduction, and the other is an Fe(IV)Fe(IV) intermediate, Q, in methane monooxygenase (MMO) that is generated from a P-type intermediate without accepting external electrons. X is capable of H-atom abstraction from a neighboring Y122, while Q abstracts an H atom from the substrate CH_4. These reactive high-valent intermediates have not been structurally characterized, making NRVS the key method to elucidate these intermediates and thus their reactivities.