2010B採択長期利用課題の紹介

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2010B期は２件の長期利用課題の応募があり、うち１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。

課題名：Nuclear Resonance Vibrational Spectroscopy (NRVS) of Iron-Based Enzymes for Hydrogen Metabolism, Nitrogen Fixation, Small Molecule Sensing, DNA Repair, Photosynthesis, and Iron Storage

実験責任者名	Stephen P. Cramer （University of California - Davis）
採択時の課題番号	2010B0032
ビームライン	BL09XU
審査結果	採択する

〔審査コメント〕

The method adopted in this long term project is known as Nuclear Resonance Vibrational Spectroscopy (NRVS). NRVS has an expectation by the analytical chemistry experts to have a potential to answer structural and dynamical issues about metalloproteins that are left unanswered by protein crystallography, such as “How does the structure change during the catalytic cycle?” or “What are the unidentified ligand atoms?” The present status of NRVS for biological specimens, however, may be described that it has a potential but not fully proven yet. This proposal is one of the challenging researches to tackle this kind of issues.

They have already carried out two successive long term projects, which had basically the same aim. It is reasonable to regard this proposal as the third one along this line, though each one is independent in a rigorous sense. This situation was taken into account when reviewing the present proposal.

The committee admits that Cramer’s group has made a reasonable progress and produced some good results as shown in publication lists. For example, during the second long term period, they published 8 good papers. In addition, they have established the experimental technique of NRVS for biological specimens, which had been developed in the previous long term period. It is anticipated that more results with higher biological significance will be obtained in future using this technique.

Under these circumstances, the committee decided to approve the new proposal by Stephen P. Cramer not as a continuation of the previous projects but as a challenge for an epoch-making application of NRVS in the field of biology. Such a work should prove that NRVS can really answer the above mentioned biological issues. It is also naturally expected by the committee that such a work will be published in highly prestigious journals. In this context, the proposed study on Gd should be excluded from this proposal.

〔実験責任者による研究概要〕

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 focuses on two critical iron-sulfur enzymes - nitrogenase (N_2ase) and hydrogenase (H_2ase). N_2ase catalyzes the reduction of dinitrogen to ammonia and this biological ammonia synthesis is responsible for about half of the protein available for human consumption. It is estimated that about 2% of world energy consumption goes toward artificial nitrogen fixation, so any contribution to this area could have enormous impact. H_2ase catalyzes the evolution (or consumption) of dihydrogen. H_2 catalysis is crucial for

the metabolism of many anaerobic organisms, and knowledge about the mechanism of H_2 evolution may prove critical for a future hydrogen economy. Many groups are trying to make synthetic catalysts analogous to hydrogenases, or to improve hydrogenases themselves for biological H_2 production.

Our program aims to use nuclear resonance vibrational spectroscopy (NRVS) to answer structural and dynamic issues about these proteins that are beyond the reach of protein crystallography and other methods. NRVS is proving to be a valuable probe of these enzymes, yielding detailed vibrational spectra of the enzyme Fe sites. The broad questions we seek to address are: How does structure change during the catalytic cycle? Where do substrates and inhibitors bind? What are the undefined light atoms? In short - how do these exquisite catalysts work?

The NRVS technique involves scanning monochromatic (~1 meV) x-rays through a nuclear resonance (in this case ^{57}Fe) and monitoring transitions that correspond to vibrational modes. We are the first group to report NRVS spectra of Fe-S proteins. During the next phase of this program, we will use *in situ* photolysis to monitor changes in the NRVS. This should allow detection of small molecules (CO, N_2, H_2) in the midst of the Fe-S cluster background signals. We anticipate that this work will yield better understanding of (a) the structure and dynamics of N_2ase and H_2ase, (b) how these enzymes are biosynthesized and ultimately (c) their molecular mechanism of catalysis.

[1] "Structure, function, and formation of biological iron-sulfur clusters.", Johnson, D. C.; Dean, D. R.; Smith, A. D.; Johnson, M. K. *Ann. Rev. Biochem.* **74** (2005) 247-281.