Abstract:

Elucidating coupling and reactivities in an ATPase driven reductase by vibrational spectroscopy and quantum mechanics/molecular mechanics computations

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In this project a combined vibrational spectroscopic (*Zebger*) and theoretical hybrid quantum mechanics/molecular mechanics (*Mroginski*) approach will be applied to elucidate the coupling of ATP-driven electron transfer to catalysis in an enzyme complex. The energy liberated from ATP hydrolysis in the associated, interacting metallo-ATPase can be utilized by a strong reductase to catalyze multiple reactions. In the present case, it is the double cubane-cluster protein, carrying an unusual [8Fe9S] cluster as active site, which employs ATP hydrolysis for reducing small molecules (acetylene, azide, hydrazine). Site directed mutagenesis of amino acid residues close to the active site is envisaged to promote new reactivities, such as Fischer-Tropsch-like formation of C2 and higher hydrocarbon compounds from CO, actually acting as a reversible inhibitor of the wildtype enzyme. In combination with crystallographic and additional EPR spectroscopic data provided by collaborative partners, the results of this project will allow to correlate the impact of specific amino acid residues on the substrate specificity of enzymes.

Extended description version of the project:

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1. Overall goal of the project

The general objective of the present proposal is to identify structural and electronic key parameters that control the catalytic specificity and the coupling mechanism of ATP-driven electron transfer in a strong reductase, catalyzing multiple reactions in a complex with its associated metallo-ATPase. Herein, the double cubane-cluster protein (DCCP), which harbors an unusual [8Fe9S] cluster as active site, and the interaction with its in ATP hydrolysis involved counterpart (DCCPR), carrying a cubane-type [4Fe4S] cluster, will be studied by multiple vibrational spectroscopic techniques in combination with hybrid quantum mechanics/molecular mechanics (QM/MM) computations. In such a way, we will elucidate the interplay and interdependence of observed conformational changes and the related (structural and electronic) modifications at the [8Fe9S] cluster upon ATP-hydrolysis and concomitant electron transfer within the DCCP/DCCPR complex. Furthermore, the impact of the cluster environment on the specific catalytic reactions will be investigated.

2. State of the art

ATP-driven electron transfer reactions, providing strong reducing power for a reduction or reductively coupling of unreactive molecules, such as N_2 or C_2H_2, are typically catalyzed by enzymes comprising two metalloenzymatic components. The first one is an ATPase containing an Fe/S-cluster on which the electron to be transferred is resting. The second one is the electron acceptor, which contain normally one (catalytic) metal center that cannot be reduced at physiological reduction potentials but by a highly energetic electron provided for enzymatic reactions, described above. The Dobbek group recently solved structures of such an enzyme complex. It consists of the double cubane-cluster protein (DCCP), that harbors an unusual [8Fe9S] cluster, consisting of two [4Fe4S] clusters bridged via a sulfido ligand, as active site, interacting with its corresponding homodimeric ATPase (DCCP-R). The latter carries a conventional [4Fe4S] cluster, which bridges the two monomers, each of which is equipped with an ATP binding site.[1,2] The [8Fe9S] contains potential coordination sites for small molecules and provide more than one electron for catalysis, as exemplified by the observed catalytic reduction of acetylene.[1] The most recent structure suggests the involvement of an hydrogen-bonding in the observed conformational changes propagating from the ATP-binding site to FeS centers and a CO binding site.[2] The Mroginski group developed a broken symmetry (BS)-DFT based approach for the prediction of vibrational spectra of coupled magnetic systems [4Fe4S] cluster in [8Fe9S].[3] Preliminary results indicated a marker band for the bridging sulfido ligand, which has been suggested to dissociate upon catalytic turnover based on studies of isolated inorganic [8Fe9S] models. Resonance Raman (RR) spectroscopy carried out in the Zebger/Hildebrandt groups is capable to monitor (un)usual FeS cluster modes in metalloenzymes, successfully assigned in combination with DFT or QM/MM calculation.[4] Indeed such band seems to be present in first RR spectra, which needs to be verified by complementary vibrational spectroscopy. (Surface sensitive) IR difference spectroscopy allows to monitor potential conformational changes as consequence of catalytic/redox reaction as well as binding of spectroscopic marker molecules such as CO and structural changes at the active site.[5-7] Based on the observed *Fischer Tropsch* like activity in nitrogenases,[8] a connatural enzyme class, such reaction appears possible in DCCP after site directed mutagenesis.

3. Specific aims and how they may be reached

The project will be carried out in tight collaboration with the group of H. Dobbek (HU Berlin) who will provide enzymes samples and share kinetic as well as crystallographic data. To complement already available and future crystallographic/kinetic investigations of the enzymatic (DCCP/DCCP-R) system, the underlying catalytic, redox and coupling processes in the double-cubane cluster protein DCCP during the interaction with its ATP-ase partner, DCCP-R under (non)turnover conditions will be studied by vibrational spectroscopic approaches in combination with (broken-symmetry) BS-QM/MM studies on possible reaction intermediates, bound to the catalytically active [8F9S] cluster. Thereby, IR, RR spectroscopy and ^{57}Fe NRVS, a synchrotron-based Mößbauer-technique[9] that can selectively probe iron-specific normal mode, are complementary methods, which provide in combination a powerful tool to probe the backbone, catalytic sites and cofactors of metalloenzymes in (frozen) solution, or if accessible in the crystalline state or immobilized on surfaces.[4-7,9] Since the proteins under investigations are O_2-sensitive, sample preparation and spectroscopic investigations under anaerobic conditions are mandatory. The expected results will be complemented with other EPR (Teutloff/FUB/UniSysCat) and Mößbauer (Schünemann/TU Kaiserslautern/SPP 1927) spectroscopic techniques on the different FeS clusters to gain a comprehensive structural and electronic description of the (intermediary) cofactors states and involved structural rearrangements during the different processes. The utilization of BS-QM/MM predictive calculations of the respective cofactor spectra in various (intermediary) states and in combination with specific isotope labeling will contribute to an adequate (band) assignment of the involved redox species and thus to a deeper mechanistic understanding of the various enzymatic reactivities depending on the active site environment. Initial vibrational spectroscopic investigations on the wildtype enzymes without substrate turnover proved already the feasibility of our approach, therefore, we will extend our studies with different substrates (e.g. C_2H_2, CO) to the wildtype and variants with new reactivities.

Work package 1 Characterization of the iron-sulfur cofactors in DCCP-R, DCCP (FeS vibrations and ligation of substrate intermediates) by RR spectroscopic studies in frozen solution and on single crystals of isolated enzymes and their complex, in the presence of ATP with and with out substrates. The experiments can be complemented by orientation-dependent studies in the crystalline phase or after immobilization on Ag surface for a selective enhancement of the individual [8Fe9S] and [4Fe4S] cluster RR signals.[10] In addition, we plan time-resolved *in crystallo* measurements via freeze quenching crystals of the enzyme complexes after defined reactions times to monitor reaction intermediates bound to the catalytically active [8Fe9S] cluster (collaboration with the Dobbek group). Furthermore, NRVS will be used to resolve the spectroscopic fingerprints of the two individual FeS cluster types by their selective ^{57}Fe labeling within the single enzyme, which will be firstly studied in their as-isolated form and subsequently in the complex with its NRVS silent counterpart, comprising only natural abundant ^{56}Fe.

Work package 2 Computation of structural and vibrational properties of the [8Fe9S] cluster of DCCP and the [4Fe4S] cluster at DCCP-R using broken symmetry DFT embedded in a classical MM point charges environment (BS-QM/MM). The focus lies on the computation of IR, Raman as well as NRVS spectra. The effect of redox changes of the FeS centers as well as mutations of the protein environment will be investigated *in silico*.

Work package 3 Surface-sensitive and bulk infrared spectroscopy to monitor concomitant conformation changes in the protein backbone during ATP hydrolyses and catalytic turnover and of IR marker band like CO. Time resolved *in situ* studies +/- substrates will be triggered by light induced release of “caged” ATP, visualizing spectral changes relative to initial state by difference spectra.

References

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