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NEW ASPECTS OF THE STRUCTURAL AND SPECTROSCOPIC CHEMISTRY ON [NiFe]hydrogenase

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Hydrogenases catalyze the reversible oxidation of molecular hydrogen and play a key role in hydrogen metabolism in various bacteria. Hydrogenases are classified into [non-metal], [FeFe], [NiFe], and [NiFeSe] hydrogenases according to the metal composition of their active sites. The [NiFe]hydrogenase from *D. vulgaris Miyazaki F* is composed of a heterodimer with a total molecular mass of 91 kDa. The active site of the [NiFe] hydrogenases in the oxidized form is composed of Ni and Fe atoms with four cysteinyl sulfur and four non-protein ligands (S/O, CO, CN or SO). Carbon monoxide (CO) was known as a reversible inhibitor of hydrogenases. The carbon monoxide complex of [NiFe]hydrogenase from *D. v. Miyazaki* has been prepared and characterized by X-ray crystallography, and absorption and Raman spectroscopy. The exogenously added CO was assigned to be bound to the Ni atom at the Ni-Fe active site. Distinct changes were observed in the electron density distribution of the Ni and Sγ (Cys546) atoms between the CO-bound and CO-liberated structures for all the crystals tested. One of the CO-bound structures showed an additional electron density peak between the CO and Sγ (Cys546), suggesting that an intermediate of the catalytic reaction was trapped at the active site. The novel structural features found near the Ni and Sγ (Cys546) atoms suggest that these two atoms at the Ni-Fe active site play a key role during the initial process to bind H_2.

Keywords: HYDROGENASE NI-FE ACTIVE SITE CO COMPLEX

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HYBRID CLUSTER PROTEIN (HCP) FROM HYBRID CLUSTER PROTEIN (HCP) FROM DESULFOVIBRIO VULGARIS AND DESULFOVIBRIO DESULFURICANS: AEROBIC, ANAEROBIC AND REDUCED STRUCTURAL STUDIES

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A novel fe-s cluster was first identified in protein referred to as hybrid cluster protein (hcp) purified from the strictly anaerobic sulphate reducing bacterium *desulfovibrio vulgaris*1,2. The crystal structure to 1.6 Å resolution showed the protein to contain two fe-s clusters, one conventional 4fe-4s cubane cluster and the novel cluster, a hybrid combining iron-sulphide and iron-oxo substructures[3]. The anomalous magnetic properties and the very high-spin epr signal of this protein made it of special and unique interest. Both the reduced and as-isolated anaerobic *desulfovibrio desulfuricans* hcp structures have been solved to 1.25 Å resolution5, and confirm the presence of both the cubane and the hybrid clusters found in the *d. Vulgaris* protein. *Desulfovibrio vulgaris* hcp structures reduced and anaerobic isolated were also solved recently up to 1.35a resolution. Sequence alignments with co-dehydrogenases revealed the conservation of almost all fe-s cluster binding residues, raising the possibility of their functions being related.

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CRYSTAL STRUCTURE AND X-RAY ABSORPTION SPECTROSCOPIC ANALYSIS OF THE FERRIC UPTAKE REGULATOR

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The dynamic control of intracellular iron concentrations is paramount to all biological systems. Consequently, all living organisms have evolved mechanisms to acquire this nutrient from the environment. On the other hand an excess of intracellular iron will catalyze the generation of highly reactive oxygen radicals that may damage many biological macromolecules.

In many bacteria including the opportunistic human pathogen *Pseudomonas aeruginosa* the ferric uptake regulator (Fur) controls a wide variety of basic physiological processes including the iron-uptake system and the production of the potent Exotoxin A (ETA). Here, we present the first crystal structure of a member of this family of iron-dependent repressors. The structure of Fur from *P. aeruginosa* crystallized in the presence of Zn(II) was determined by MAD methods at a resolution of 1.8 Å. In addition, we performed X-ray absorption spectroscopic measurements of PA-Fur with Fe(II) in order to further characterize the two metal binding sites. The combination of these complementary techniques enabled us to present a model for the activation mechanism and DNA-binding.

Keywords: REGULATOR, DNA BINDING, PROTEIN CRYSTALLOGRAPHY

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MINERALOMIMETIC SYSTEMS USING CADMIUM CYANIDE CLATHRATES

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The mineralomimetic systems using cadmium cyanide clathrates have been interested in the crystal engineering associated with supuramolecular assemblies. The cadmium cyanide and the silicon dioxide frameworks have the AB_2 composition, A takes a tetrahedral position, B bridges between a pair of A. Among natural fields of crystalline silicon dioxide minerals, the tetrahedral units are linked so every oxygen atom is shared between two tetrahedra, giving the composition SiO_2. Likewise, the mineralomimetic cadmium cyanide host frameworks in the clathrates are topologically similar to the polymorphism forms of silicon dioxide. The cadmium cyanide host frameworks depend on guest molecules. The H-cristobalite, L-cristobalite and H-tridymite-like hosts of $Cd(CN)_2$ were obtained using guest molecules of different properties, sizes, shapes and symmetries. For example, 1,1,2,2-tetrachloroethanel, 1,1,2-trichloroethane, and dibutylether, produce a H-cristobalite-like cadmium cyanide host framework (*Fd*-3*m*), a L-cristobalite-like species ($P4_12_12$) and a H-tridymite-like species (*P6₃/mmc*), respectively. Selecting guest molecules provides the mineralomimetic cadmium cyanide host frameworks with a body-centered tetragonal $I4_1/amd$ lattice between the H-cristobalite like species (space group *Fd*-3*m*) and the L-cristobalite-like species (space group $P4_12_12$).

Seven types of cadmium cyanide host frameworks at least have been obtained. These mineralomimetic system using cadmium cyanide may be of significant interest as a crystalline model compounds for studies of the transformation of silicon dioxide and nanoscale control of host frameworks.

Keywords: SUPURAMOLECULE CLATHRATE CADMIUM CYANIDE