MS01.P26

Acta Cryst. (2011) A**67,** C224

Structure of [NiFe] hydrogenase from *Allochromatium vinosum* in its Ni-A state

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The crystal structure of the membrane-associated [NiFe] hydrogenase from the photosynthetic bacterium *Allochromatium vinosum* (*A. vinosum*) has been determined at 2.1 Å resolution [1]. The oxidation state (Ni-A) of the enzyme was confirmed by EPR and FTIR on dissolved crystals. The overall structure of the *A. vinosum* [NiFe] hydrogenase resembles that of the [NiFe] hydrogenase structures derived from the sulfate-reducing bacteria, *Desulfovibrio* species [2].

The [NiFe] hydrogenases are composedof 2 subunits (the molecular mass of the large and the small subunits are 60 and 30 kDa, respectively). The [NiFe] active site is located in the large subunit and possesses three diatomic non-protein ligands (2 CN^-, 1 CO) coordinated to the Fe^{2+} ion. They were assigned from the results of the FTIR studies and the environment of the active site. Ni is five-coordinated in the oxidized state. It is bound to 4 cysteine residues, two of them also bridge the Ni and Fe metals. One of the bridging cysteines (Cys64) exhibits a modified thiolate in part of the sample. In the Ni-A state, a mono-oxo bridging ligand was assigned between the Ni^{3+} and Fe^{2+} ions. This is in contrast to a proposal for *Desulfovibrio* sp. hydrogenases that show a di-oxo species in this position for the Ni-A state.

3 FeS clusters (2 $[Fe_4S_4]$ and 1 $[Fe_3S_4]$) were found in the small subunit. They comprise the electron-transfer chain and they are located within distances of ~12 Å. The anomalous-difference Fourier map shows a distorted proximal $[Fe_4S_4]$ cluster. This altered proximal $[Fe_4S_4]$ cluster is supposed to be paramagnetic and is exchange-coupled to the Ni^{3+} ion and the medial $[Fe_3S_4]^+$ cluster that are both EPR active (S = 1/2 species). This finding of a distorted proximal FeS cluster might explain the earlier observation of split EPR signals [3].

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Keywords: hydrogenase, FeS cluster, NiFe active site

MS01.P27

Acta Cryst. (2011) A**67,** C224

Crystal structure of SNX11 PX domain reveals a dimer formation

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Sorting nexins (SNXs) are a large group of proteins containing PX (Phox homology) domain and play critical roles in endosome trafficking. The PX domain, which specifically binds to phosphoinositides and mediates SNXs targeting to endosome, is essential for SNXs function. SNX10 is involved in the regulation of endosome morphogenesis; both PX domain and CD1 domain are required for SNX10 function. SNX11 is another member of SNXs, which contain PX domain at the N terminal followed by a non-conserved C-terminal region, has about 55% sequence identity with SNX10. Here we report the crystal structure of SNX11 PX domain in a dimeric form. Further biochemical experiments confirmed the dimer formation of SNX11 PX domain.

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Keywords: SNX11, PX domain, sorting nexins

MS01.P28

Acta Cryst. (2011) A**67,** C224

Structure determination of a KSR protein domain

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Kinase Suppressor of Ras (KSR) proteins are scaffolds that regulate the mitogen activated protein kinase (MAPK) ERK. These multidomain proteins bind all three components of the ERK MAPK module—RAF, MEK and ERK—thereby driving efficient ERK activation. Additionally, recent evidence suggests that KSR1 plays a direct, non-catalytic role in the activation of RAF that is aided by the scaffolding function of Connector Enhancer of KSR (CNK) [1]. Of the three structured domains of KSR1, the function of only the CA1 domain remains poorly characterized. This unique region is highly conserved across almost all KSR proteins and studies have shown that it is necessary for the ability of KSR to immunoprecipitate RAF [2], [3]. Recently, we identified an additional structured region immediately adjacent to the CA1 region that is also unique to KSRs, which we have named CA1α. Although the CA1-CA1α domain is predicted to be folded, its sequence does not resemble that of any other known protein. Until now, the role of the CA1-CA1α domain in KSR-mediated ERK activation was unknown.

We determined the structure of the KSR1 CA1-CA1α domain. First, the structure of this domain reveals that the CA1 domain forms a coiled-coil (CC) while, unexpectedly, the CA1α region adopts a sterile alpha motif (SAM) fold. SAM domains are widely occurring protein-protein interaction motifs that bind RNA, other SAM domains and also polymerize. Second, our structure shows that the CA1-CC and CA1α-SAM domains interact extensively, burying nearly 2000 $Å^2$ of solvent accessible surface area (18% of the total), and thus form a single structured domain. This demonstrates that the KSR1 CC-SAM domain constitutes a novel SAM-containing fold. Third, our structure reveals that the CA1 residues originally proposed to play a role in RAF binding (L56, R57) are buried, suggesting that CA1-CA1α does not interact directly with RAF. Collectively, these studies expand our knowledge of the versatility in the protein interaction properties of the SAM domain family and, more importantly, reveal the role of the CC-SAM domain in KSR1 scaffolding.

This work was supported by an American Cancer Research Scholar award to RP (RSG-08-067-01-LIB).

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Keywords: structure, signaling, novel