Crystal Structure of Adenylylsulfate Reductase from *Desulfovibrio gigas* Suggests a Potential Self-regulation Mechanism Involving the C-terminus of the β-subunit

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Adenylylsulfate reductase (adenosine 5'-phosphosulfate reductase, APS reductase or APSR) plays a key role in catalyzing APS to sulfite in dissimilatory sulfate reduction. Here, we report the crystal structure of APSR from *Desulfovibrio gigas* at 3.1 Å resolution. Different from the $\alpha_2\beta_2$ heterotetramer of the *A. fulgidus*, the overall structure of APSR from *D. gigas* comprises six αβ-heterodimers that form a hexameric structure. The flavin adenine dinucleotide (FAD) is non-covalently attached to the α-subunit, and two [4Fe-4S] clusters are enveloped by cluster-binding motifs. The substrate-binding channel in *D. gigas* is wider compared to *A. fulgidus* because of shifts in the loop (326-332) and the α-helix (289-299) in the α-subunit. The positively charged residue Arg160 in the structure of *D. gigas* likely replaces the role of Arg83 of *A. fulgidus* for the recognition of substrates. The C-terminal segment of the β-subunit wraps around the α-subunit to form a functional unit, with the C-terminal loop inserted into the active-site channel of the α-subunit from another αβ-heterodimer. Electrostatic interactions between the substrate-binding residue Arg282 in the α-subunit and Asp159 on the C-terminus of the β-subunit affect the binding of the substrate. Alignment of the APSR sequences from *D. gigas* and *A. fulgidus* shows the largest differences toward the C-terminal of the β-subunit, and structural comparison reveals the notable differences at the C-terminal, activity site and other regions. The disulfide Cys156–Cys162 stabilizes the C-terminal loop of the β-subunit and is crucial for oligomerization. Dynamic light scattering and ultracentrifugation measurements reveal multiple forms of the APSR upon the addition of adenosine monophosphate (AMP), indicating that AMP binding dissociates the inactive hexamer into functional dimers presumably by switching C-terminus of β-subunit away from the active site. The crystal structure of APSR, together with its oligomerization properties, suggests that APSR from sulfate-reducing bacteria might self-regulate its activity through the C-terminus of the β-subunit [1].

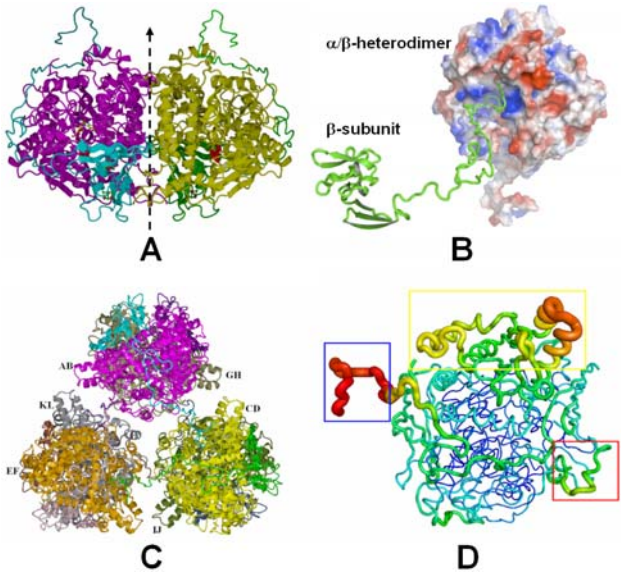

Fig. 1: Structure of APS reductase hexamer from *D. gigas*. (A) The structure of $\alpha_2\beta_2$ heterotetramer from *D. gigas*. The αβ-heterodimer performs a rotation about a 2-fold axis with another αβ-heterodimer to form a tightly contacted $\alpha_2\beta_2$ heterotetramer. (B) Interactions between the αβ-heterodimers and C-terminus of another β-subunit. The long C-terminal loop of the β-subunit (shown in ribbon; green) plugs into the active channel of another αβ-heterodimer (shown in electrostatic surface). (C) A view of the hexamer structure. Three $\alpha_2\beta_2$ heterotetramers contact each other through the C-terminii of the β-subunits to form a hexamer containing six αβ-heterodimers. (D) The *B*-factor labeled structure of αβ-heterodimer. The structure exhibits a high *B*-factor with red color and the larger caliber. The blue box shows the high *B*-factor at the C-terminus of the β-subunit. The electron-accepting site on the β-subunit that is suggested to interact with an unknown electron donor shows a higher *B*-factor in the red box. The capping domain in the α-subunit also shows a higher *B*-factor in the yellow box.

Reference

[1] Y.-L. Chiang, Y.-C. Hsieh, J.-Y. Fang, E.-H. Liu, Y.-C. Huang, P. Chuankhayan, J. Jeyaraman, M.-Y. Liu, S. I. Chan, and C.-J. Chen, J. Bacteriol. **191**, 7597 (2009).