Multiheme (type *c*) membrane bound nitrite reductase from *Desulfovibrio desulfuricans* ATCC 27774: The relevance of the two calcium sites in the catalytic subunit.

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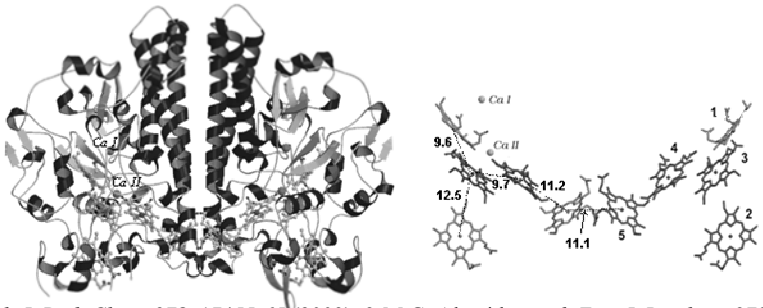
The crystal structure of cytochrome *c* nitrite reductase (NrfA) from *D. desulfuricans* ATCC 27774 was determined to 2.3 Å resolution [1]. In solution the protein was purified as a hetero-oligomer of two different subunits, the catalytic subunit NrfA (62 kDa) and a smaller subunit (19 kDa) that corresponds to the membrane-bound subunit NrfH [2]. NrfA possesses five *c*-type hemes per monomer and catalyses the six-electron dissimilatory conversion of nitrite to ammonia. Biochemical studies suggest that the homodimer is the functional form of the catalytic unit. The active site is localized at heme 1, an unusual lysine coordinated heme with the distal coordination position free to accommodate the substrate molecule. In comparison to homologous structures, NrfA presents structural differences mainly located at the regions close to the substrate inlet and product outlet, and includes a well defined second calcium site, coordinated to propionates of hemes 3 and 4, and caged by a loop non-existent in the previous structures. The highly negative electrostatic potential in the environment around hemes 3 and 4 suggests that the main role of this calcium ion may not be electrostatic but structural, namely in the stabilization of the conformation of the additional loop that cages it and influences the solvent accessibility of heme 4. The NrfA active site (heme 1) is similar to that of mono-heme peroxidases with a nearby calcium site at the distal side nearly in the same location as occurs in the class II and class III peroxidases. This fact suggests that the calcium ion at the distal side of the active site in the NrfA enzymes may have a similar physiological role to that reported for peroxidases.



1-Cunha, C.A. *et al*.,*J. Biol. Chem.* **278**, 17455-65 (2003); 2-M.G. Almeida. *et al*.,*Eur. J. Biochem*. **270**, 3904-15 (2003)