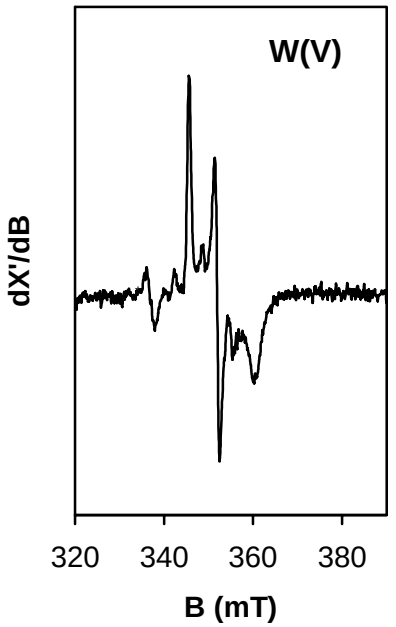
The tungsten containing aldehyde:ferredoxin oxidoreductase from *Pyrobaculum aerophilum*

Peter-Leon Hagedoorn[1], Tianhong Chen[1], Imke Schröder[2], Sander R. Piersma[3], Simon de Vries[1], Wilfred R. Hagen[1].

[1]*Department of Biotechnology, Delft University of Technology, Delft, The Netherlands,*
[2]*Department of Microbiology, Immunology and Molecular Genetics, UCLA, Los Angeles, USA,*
[3]*FOM Institute for Atomic and Molecular Physics, Amsterdam, The Netherlands* .

A tungsten-containing aldehyde:ferredoxin oxidoreductase (AOR) was purified to homogeneity from the hyperthermophilic denitrifier *Pyrobaculum aerophilum*[1]. The N-terminal sequence of the isolated enzyme matched a single open reading frame in the genome. Metal analysis and EPR spectroscopy indicated that the *P. aerophilum* AOR contains one tungsten center and one $[4Fe\text{-}4S]^{2+/1+}$ cluster per 68 kDa monomer. Native AOR was a homodimer. EPR spectroscopy of substrate reduced AOR revealed a W(V) species with g_{zyx}-values 1.952, 1.918, 1.872. The substrate reduced AOR also contained a $[4Fe\text{-}4S]^{1+}$ cluster with S = 3/2. Molybdenum was absent from the enzyme preparation. The *P. aerophilum* AOR lacks the amino acid sequence motif indicative for binding of mononuclear iron that is typically found in other AORs. Furthermore, the *P. aerophilum* AOR utilizes a 7Fe-ferredoxin as the putative physiological redox partner, instead of a 4Fe-ferredoxin as in *P. furiosus*. This 7Fe-ferredoxin has been purified from *P. aerophilum*, and the amino acid sequence has been identified using mass spectrometry.



1. Hagedoorn, P.L., Chen, T., Schröder, I., Piersma, S.R., de Vries, S., Hagen, W.R., *J. Biol. Inorg. Chem.*, in press.