Pulse EPR studies on the molybdenum center of polysulfide reductase from *Wolinella Succinogenes*

S. Lyubenova [a], F. MacMillan [a], O. Klimmek [b] and T. Prisner [a]

[a] *Institute for Physical and Theoretical Chemistry,* [b] *Institute for Microbiology*
J. W. Goethe University, Frankfurt am Main, Germany, E-mail:sevdalina@prisner.de

Polysulfide reductase (Psr) from *Wolinella succinogenes* is involved in anaerobic respiration and catalyzes the reduction of polysulfide to sulfide. The enzyme consists of three different subunits PsrA, PsrB and PsrC, where the catalytic subunit PsrA contains a molybdenum ion coordinated by two molybdopterin guanine dinucleotides (MGD) and a cysteine amino acid residue [1].

Pulse electron paramagnetic resonance (EPR) techniques are used to study in details the coordination sphere of the molybdenum ion in its paramagnetic Mo^V state. Earlier, based on the unusual high G-tensor values of one of the observed Mo^V paramagnetic states (very high G-state) we proposed that this state is the catalytically active state in the polysulfide reduction where the substrate is directly bound to the metal ion [2]. Both hyperfine spectroscopy (ESEEM and HYSCORE) experiments with ^{33}S-labeled polysulfide confirmed this hypothesis. A weak effect was observed in the 1D- ESEEM, although difficult to separate from the additional nitrogen frequencies in the same region. The 2D-HYSCORE experiment allowed unambiguous detection and separation of the ^{33}S-coupling to the substrate. Orientation selective studies elucidated the anisotropy of the observed hyperfine and quadrupole couplings. By S- and X-band HYSCORE experiments the ^1H hyperfine coupling constants to the molybdenum were measured as well. Analysis of the obtained HYSCORE pattern allow determination of the substrate position and show that the polysulfide substrate is indeed directly bound to the Mo ion in the very high G-state.

[1] R. Hedderich, O. Klimmek, A. Kröger, R. Dirmeier, M. Keller, K. O. Stetter, *FEMS Microbiol. Rev.*, **22,** 353 (1999).
[2] T. Prisner, S. Lyubenova, Y. Atabay, F. MacMillan, A. Kröger, O. Klimmek, *J. Biol. Inorg. Chem.*, **8**, 419 (2003).