# Redox Potentials of Bovine Adrenodoxins: Quantifying the Effects of Mutations

J. Riehm[1], M.C. Hutter[1], T. Sagadin[2], F. Hannemann[2], R. Bernhardt[2]

*1 Center for Bioinformatics, Saarland University, Campus E2.1, 66123 Saarbrücken*

*2 Department of Biochemistry, Saarland University, Campus B2.2, 66123 Saarbrücken*

Adrenodoxin (Adx) belongs to the family of vertebrate [2Fe2S]-cluster ferredoxins and plays an essential role in the steroid biosynthesis. Its task is to supply electrons from a NADPH-dependent Adx reductase (Adr) to Cytochrome P450 enzymes. The next step includes activation of molecular oxygen whereby one oxygen atom is introduced into the substrate and the other one is reduced to water [1]. The efficiency of the electron transfer also depends on the redox potential of the Adx. In the past years many different Adx mutations from bovine have been established and quantified [2]. To elucidate the effects of a certain mutation at the protein level we investigated the set of Adx mutants listed in [2] and computed the individual energy contributions to the redox potentials. Solvation effects were obtained via the Poisson-Boltzmann theory by applying the APBS program [3]. The electronic reorganization energy that comes along with the reduction of the Adx [2Fe2S]-cluster was derived from density functional theory calculations on $[Fe_2S_2(SC_2H_5)_4]$ model compounds at B3LYP/cc-pVTZ level of theory. Surface analysis of the homology models of the Adx mutants was performed via the VADAR webserver [4]. Our findings show that the energy gap between the reduced and the oxidized state of the [2Fe2S]-cluster is caused by the different orientations of the ligating sulfur atoms. This was clearly shown when comparing the geometrical non-constraint energy optimization of the $[Fe_2S_2(SC_2H_5)_4]$ model compounds vs. the constraint ones. Also the local protein environment plays an important role. Investigation of the T49 deletion mutant showed a substantially lower redox potential compared to the wildtype, essentially due to the rearrangement of the [2Fe2S]-cluster and the surrounding geometry, ending up in a change of 0.08 eV of the adiabatic ionization potential.

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