

442d Characterization and Comparison of Alkyl Hydroperoxide Reductase and Water-Forming Nadh Oxidase

Rongrong Jiang, Bettina R. Riebel, William B. Wellborn, and Andreas S. Bommarius

NADH oxidases are useful biocatalysts for regenerating nicotinamide cofactors of many biological redox reactions. In this presentation, we compare the alkyl hydroperoxide reductase (AhpR) and the H₂O-forming enzyme (nox-2) from *Lactococcus lactis* (*L. lactis*), as well as the H₂O-former from *Lactobacillus sanfranciscensis* (*L. sanfranciscensis*). AhpR is composed of H₂O₂-forming NADH oxidase (nox-1) and peroxidase. The net reaction of AhpR is the same as nox-2. In this work, both nox-1 and nox-2 are found to be flavoproteins. We found a considerably lower maximum specific activity of nox-1 from *L. lactis* compared to its nox-2 counterpart or nox-2 from *L. sanfranciscensis*. Both nox-1 and nox-2 are turnover-limited, as expected for enzymes with labile, redox-active thiols in the active site. In the absence of exogenously added thiols, both nox-1 and nox-1/peroxidase are considerably more stable against overoxidation than nox-2. We will investigate the possibility of using the *E. coli*-based whole cell system for the oxidative biocatalysis reactions with carbonyl reductase and these flavoproteins.