E331 Purification and Characterization of Glutaredoxin-like Protein from *Dictyostelium discoideum*

Chang-Hoon Choi, Jae-Yeoul Kwon, Chang-Hun Lee and Sa-Ouk Kang Department of Microbiology, College of Natural Sciences, and Research Center for Molecular Microbiology, Seoul National University

A glutaredoxin-like protein was purified 50-fold to homogeneity from *Dictyostelium discoideum* based on its activity as glutathione-disulfide oxidoreductase. The protein has a molecular mass of 29.3 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. When the initial velocity of the reaction was plotted against the concentration of hydroxyethyl disulfide, the reaction of glutaredoxin did not obey Michaelis-Menten kinetics. The enzyme had an optimal pH at about 8.4 and was stable in the pH range of 5 to 10. Amino acid sequence analysis of the enzyme indicated that N-terminus might be blocked. The activity of the enzyme increased during early stages and reached the maximal level after 16 h of development which corresponds to the early culmination stage, proposing that it plays a role in the development of *D. discoideum*.

E332 Purification and characterization of a quinone reducing enzyme from Streptomyces seoulensis

Dong Won Kim, Hwan Youn, Jin Won Lee, and Sa-Ouk Kang*
Department of Microbiology, College of Natural Sciences, and
Research Center for Molecular Microbiology, Seoul National
University

Three types of NADPH:quinone reducing enzymes were identified from the cytosolic fraction of *Streptomyces seoulensis*. Among them, the most abundant one was purified to apparent homogeniety with a molecular weight of 35 kDa, as judged by SDS-PAGE. The enzyme had an absorption peaks at 272, 383, and 457 nm. resembling those of many other flavoproteins. The flavin of the enzyme was identified as FAD, which was noncovalently associated with its apoenzyme. The enzyme could reduce a variety of quinone compounds with NADPH as electron donor, although it could use NADH at a reduced rate. The enzyme showed the highest activity with 1,4-benzoquinone as electron acceptor. The K_m values for 1,4-benzoquinone and NADPH were measured as 188 μ M and 80 μ M, respectively. The enzyme could produce 1,4-naphtosemiquinone radical anion which was detected by EPR spectroscopy.