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Purification and Characterizaton of Sulfide Quinone Oxidoreductase from *Chlorobium tepidum*

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Sulfide quinone oxidoreductase (SQR) was purified 150-fold to an electrophoretic homogeneity from the cytoplasmic membrane of the green sulfur bacterium, *Chlorobium tepidum*. The specific activity of the purified enzyme was 678 units per mg of protein as determined by an assay based on the sulfide dependent reduction of decyl-plastoquinone. The activity of the enzyme was detected in the native gel electrophoresis by the modified NADPH diaphorase staining method. The enzyme was composed of a single polypeptide of about 48 kDa. The UV and visible absorption and fluorescence spectra of the purified enzyme suggested that SQR is a flavoprotein.

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Amino acid residues involved in the catalytic activity of glyoxalase II purified from *Trimorphomyces papilionaceus* 31

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Gyloxalase II hydrolyzes S-2-hydroxyacylglutathione releasing 2-hydroxyacid and reduced glutathione. The enzyme does not contain any metal at the active site, and was not inactivated by serine- nor cysteine-directed inhibitors unlike other well-known hydrolases. Glyoxalase II from *Trimorphomyces papilionaceus* 31 was inactivated irreversibly by diethylpyrocarbonate (DEPC) in a pH-dependent manner, which was partially overcome by a competitive inhibitor of the enzyme, S-carbobenzoxy-glutathione (S-CBG). DEPC-modified enzyme showed an increased absorption near 240 nm compared with normal one. This suggests that glyoxalase II has a histidyl residue in its active site. Besides it, catalysis-related tyrosyl residue was identified. The enzyme was inactivated by tetranitromethane (TNM), and fully protected by S-CBG. TNM-modified enzyme gave a specific nitrotyrosine spectrum in uv-visible region. Glyoxalase II has a residue affecting substrate-binding affinity and whose pKa is 8.3. This value shifted to 8.6 in 25% ethanolic solution suggesting that it's neither sulfhydryl nor amino group, but the hydroxyl gorup of tyrosine residue.