

## E305

### Active site of D-arabinono-1,4-lactone oxidase from *Candida albicans*

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D-Arabinono-1,4-lactone oxidase contained FAD, covalently-linked to the protein. A flavin peptide, isolated from tryptic-chymotryptic digests of the enzyme and hydrolyzed to the FMN level, showed a pH-dependent fluorescence yield being maximal at pH 3.5 and decreasing over 90% at pH 7.5. Absorption spectral data showed the covalent substituent to be at the 8 $\alpha$  position of the flavin moiety. The flavin peptide was not reduced with borohydride and relatively resistant to the storage. It is therefore concluded that the FAD moiety of D-arabinono-1,4-lactone oxidase is covalently linked *via* the 8-methylene group to the N(3) position of the imidazole ring of histidine. Chemical modification studies showed that one Cys, one His and one Trp residues are at or near the active site of D-arabinono-1,4-lactone oxidase.

## E306

### Purification and characterization of D-arabinose dehydrogenase from *Candida albicans*

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D-Arabinose dehydrogenase was purified 2,500-fold to apparent homogeneity with an overall yield of 3.1% from *Candida albicans*, through a purification procedure of ammonium sulfate precipitation, anion-exchange, gel filtration, dye-ligand and hydrophobic interaction chromatography. The purified enzyme was a monomeric protein with a molecular mass of 42 kDa as determined by SDS/PAGE and gel filtration chromatography. The optimal pH for the enzyme activity was pH 8.0 and the pI value of the enzyme was 5.0. The enzyme catalyzed the oxidation of D-arabinose, L-fucose, L-xylose and L-galactose with apparent  $K_m$  values of 18.8, 17.7, 23.0 and 86.1 mM at pH 8.0, respectively. All active sugars have the same configuration of hydroxyl groups at C2 and C3. The purified enzyme was specific for NADP<sup>+</sup>. NADPH inhibits the enzyme activity competitively with NADP<sup>+</sup> and noncompetitively with D-arabinose. The N-terminal sequence of the enzyme was Met-X-Leu-Ala-X-Glu-Ile-Asp-Phe-X-Leu-Leu-Asn-Gly-.