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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 8α 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 Ka values of 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+. NADPH
inhibits the enzyme activity competitively with NADP+ 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-.