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PYRANOSE OXIDASE HAVING A COVALENTLY BOUND FAD AS A COENZYME

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Flavin-peptides were purified from pyranose oxidase (EC 1.1.3.10) after tryptic-chymotryptic and tryptic digestion. The spectral and chromatographic properties of these flavin peptides showed that the FAD of pyranose oxidase appears to be bound, by way of the 8α-methylene group, to the N-1 position of the imidazole ring of the histidine. Automated sequence analysis showed that the amino acid sequence of the tryptic-chymotryptic flavin-peptide from pyranose oxidase is Ser-Thr-X-Trp and that of the tryptic flavin-peptide is Met-Ser-Thr-X-Trp. Upon changing the pH, the absorption spectrum of the flavin portion of pyranose oxidase was changed. The pK_a for the spectral changes of the first absorption band was found to be 5.66. Several carboxylates had an inhibition effect on the enzyme activity of pyranose oxidase. The absorption spectrum of pyranose oxidase has changed from the simple type to the partially resolved type by the addition of carboxylate such as acetate. A calculated single-proton dissociation curve for the $K_{d\ app}$ values determined at several pH showed that the ionizable group having a pK_a of 6.4 in the active site is involved in the formation of complex with acetate. The absorption spectral changes caused by 2-hydroxybenzoate showed a characteristic pH dependence. It suggests that a ionizable group of a pK_a of 6.4 in the active site of pyranose oxidase is involved in the formation of complex with 2-hydroxybenzoate.