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Characteristics of Proteases from Stomachless Aquatic Organisms

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Enzymes have been used in practical applications as diverse as brewing and industrial fermentations, detergent, analytical determinations and recombinant DAN technology. As processing aids, enzymes have been used in the manufacture of food products to improve their quality, solubility and stability for centuries. About 50% of the enzyme used as industrial processing aids are proteases which have been used in a number of industrial applications including laundry detergents, chill proofing, meat tenderizing, fermented sauces, and the production of pharmaceuticals. Usually enzymes are derived from living organisms such as plant, animal and microorganisms. Recently, interest has developed in the proteases found in aquatic organisms, especially stomachless aquatic organisms. In stomachless aquatic organisms, the digestion of proteins in the absence of acid denaturation and gastric proteases is compensated for by intestinal enzymes that are more efficient in digesting native proteins.

Proteases were isolated and characterized from stomachless aquatic organisms. Four proteases from hepatopancreas of crawfish (*Procambarus clarkii*) were considered as trypsin-like. The apparent molecular weights were determined to be 35.0, 41.2, 37.9 and 39.5 kDa by SDS-PAGE. The proteases had optimal esterase activity at pH 8.0-8.5 and at temperature between 60-70℃. The proteases were rich in acidic amino acids. Activation energies for hydrolysis of tosyl arginine methyl ester by crawfish proteases were 6.98-8.34 kcal/mole. Unlike other serine proteases, the activities of two of four crawfish proteases were activated by mercury chloride ($HgCl_2$) while the other two proteases were inhibited. Immunological study showed that the four crawfish proteases are crossreactive, therefore, they have shared structural components. The peptide fragmentation of pectate lyase c generated by crawfish proteases was determined by MALDI-TOF, indicating that cleavage site in native substrate were at lysine residue. The partially purified protease from small intestine and pyrolic ceca of mullet (*Mugil cephalus*) and the four crawfish proteases were able to inactivate pectiensterase which is the cause of the cloud loss in fresh orange juice. These enzymes could be used in inactivating the deleterious enzymes in food processing without any heat treatments that often cause undesirable changes.