HYDROLYTIC ENZYMES AND THEIR INDUSTRIAL APPLICATIONS

By:

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Enzymes are proteins which catalyze, in highly specific way, chemical reactions taking place within the living cell without themselves suffering any overall change. Often a further, non-protein component, called a cofactor, is required before an enzyme has catalytic activity. Enzymes are synthesized by biological cells and, in all organisms, they are involved in chemical reactions related to metabolism (Belitz and Grosch, 1987; Palmer, 1991).

Enzymes have been used for many centuries, although their nature has only become known relatively recently, and they are still of great importance in scientific research, clinical diagnosis and industry (Palmer, 1991).

A great number and variety of enzyme-catalyzed reactions are known with their specific substrates. Each enzyme is quite specific in character, acting on a particular substrate(s) to produce a particular product(s). These include, for example, lipases and proteinases. Both are from the class of hydrolytic enzymes, the former are enzymes with lipid-base substrate(s) which cleave acyl lipids, and the latter are enzymes with protein-base substrate(s) which cleave proteins/peptides bonds other than terminal ones. Most of the enzymes used in the food industry are derived from this class (Belitz and Grosch, 1987; Palmer, 1991). These enzymes are widely distributed in animals, plants and microorganisms. Numerous published reports on the purifications and applications of these enzymes have indicated that the properties of purified enzymes, e.g. substrate specificity, thermostability, pH optimum and stability, activator requirements, etc., vary widely (Huang, 1984; Iwai and Tsujisaka, 1984; Sugiura, 1984; Verger, 1984; Antonian, 1988; Belitz and Grosch, 1987; Palmer, 1991).

Lipase is one of the most interesting pancreatic enzymes because of its known ability to act on emulsion or micellar substrates. There is little doubt that the origin of this ability lies in a special feature of the structure of the enzyme molecule. However, investigations concerning the structure of pancreatic lipase have been delayed by difficulties encountered during its purification.

Lipases or acylglycerol acylhydrolases (EC 3. 1. 1. 3) are defined as enzymes which hydrolyze esters of long-chain aliphatic acids from glycerol at

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ISSN-0854-9230

oil/water interfaces (Brockman, 1984; Brockman *et al.*, 1988; Brzozowski *et al.*, 1991). Although naturally occurring triglycerides are normally the preferred substrates, the enzyme can hydrolyze a wide range of insoluble fatty acid esters. It is well demonstrated that the reaction is reversible, and that the enzyme can catalyze ester synthesis from various alcohols and acids, and transesterification in reaction systems containing a low concentration of water, or often in nearly anhydrous organic solvents. In addition to the catalytic versatility, the enzyme can catalyze stereoselective as well as regioselective reaction (Akesson *et al.*, 1983; Macrae, 1983; Jones, 1986; Sonnet, 1988; Klibanov, 1990). Phospholipids and cholesterol esters are not included as substrates, although there are lipases which will hydrolyze acylglycerols and phospholipids or cholesteryl esters. Emulsion globules, fat bodies or lipoprotein particles usually provide the interface and these have been termed the supersubstrate.

Lipases have many industrial uses, such as in dairy and/or marine food industries. In the dairy industry, for example, the enzymes involve in the production of the characteristic flavors of cheese and cheese products. In the marine food industry, lipases have an important role in the production of fish oil containing high levels of polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), the omega-3 (w-3) fatty acids that are not present in other foods. EPA and DHA are rare fatty acids of potential pharmaceutical value. These two fatty acids have been shown to cause significant biochemical and physiological changes in the body. The three main effects on body function and maintenance associated with these fatty acids are: they accumulate in the eye, brain, testes and placenta; they lower blood lipid concentrations, especially cholesterol and triglycerides; they serve as precursors that mediate biochemical and physiological actions (Svennerholm, 1968; Bernsohn and Spitz, 1974; Ross and Glomset, 1976; Brown and Goldstein, 1986; Ross, 1986; Gordon and Ratliff, 1992).

EPA was shown to be effective in preventing blood platelet aggregation and to be useful for blood cholesterol reduction, thus reducing the risks of atherosclerosis, i.e., an accumulation of cholesterol along with a deposit of other metabolic materials on and in the vessel walls surrounding the heart. This chronic accumulation of lipids and other circulating materials leads to a chronic disease. EPA serves as a precursor to a large group of lipid mediators, the eicosanoids, which include the families of compounds called prostaglandins, thromboxanes, leukotrienes and hydroxy fatty acids. These lipid mediators are associated with a number of different physiological actions and counteractions in various organs of the body. Through these effects on the body, these lipid mediators derived from the w-3 fatty acids have been suggested to have beneficial effects in the prevention and/or treatment of major diseases affecting human health: coronary heart disease, cancer, diabetes, high blood pressure and autoimmune diseases (Ross and Glomset, 1976; Brown and Goldstein, 1986; Ross, 1986; Gordon and Ratliff, 1992).

ISSN-0854-9230

DHA plays very important role in the nervous system; this fatty acid is incorporated abundantly into phosphatidylethanolamine or phosphatidyl-serine. The amount of DHA increased according to the growth of organs. A large amount of DHA is found in the brain of rat born from a mother fed with a-linolenic acid. The role of DHA and its physiological function in the brain has not been clearly understand, but it is assumed that DHA plays an important part in the structural role of the brain, specifically in learning and memory systems. DHA has also been shown to be essential for the proper functioning of the eye (Svennerholm, 1968; Bernsohn and Spitz, 1974; Gordon and Ratliff, 1992).

Recently, various lipases, alone or in conjunction with other enzymes, have been widely used as hydrolytic reagents in the manual and automated determination of triglyceride (TG). The hydrolytic system must be able to digest completely all of the acylglycerols present to glycerol. The glycerol is determined by coupled enzymatic reactions and gives an indirect estimate of the acylglycerol content of the material.

Some new potential applications of lipases are hydrolysis of TG under mild condition, synthesis of new TG by interesterification and hydrolysis of other compounds. However, as the application increases, the requirement for bulk amounts of enzymes becomes a limiting factor. Immobilization of lipase is expected to allow reuse of the enzyme, and longer usage might be possible. For any of these applications, the enzyme being studied must first be identified as a lipase and then the activity be determined under nearly optimal conditions.

As aforementioned, most enzymes used in the food industry are derived from the class of hydrolytic enzymes. The next enzymes belong to this class, besides lipases, are proteinases, i.e., the enzymes with protein-base substrate(s).

Processes involving proteolysis (because of the action of peptide hydrolases or proteolytic enzymes) play an important role in the production of many foods. Proteolysis can be used to increase the solubility and sometimes the foaming and emulsifying properties and stabilities of proteins in water, butter and/or formulated foods. Proteolysis is used to change the physical properties of proteins by largely increasing or decreasing the solubility of proteins. Proteolysis can occur as a result of proteinases in the food itself, e.g. autolytic reactions in meat and fish, or with commercial proteinases (as used in the food industry), such as the addition of pure cultures of selected microorganisms during the production of plant and animal cheeses, dough modification, chill proofing beer, fortification of juices and soft drinks, solubilization of protein concentrates, removal of undesired flavors, pigments and toxic compounds, covalent addition of important constituents and modification of functional properties, etc. (Belitz and Grosch, 1987; Chobert *et al.*, 1992).

The group of proteolytic enzymes is divided into two subgroups, i.e., peptidases (exopeptidases) and proteinases (endopeptidases). Peptidases cleave amino acids or dipeptides stepwise from the terminal ends of proteins.

This subgroup consisted of a-aminoacylpeptide hydrolases, dipeptide hydrolases, dipeptidylpeptide hydrolases, peptidyldipeptide hydrolases, serinecarboxypeptidases, metalocarboxypeptidases and cysteinecarboxy-peptidases. Proteinases hydrolyze the linkages within the peptide chain, but not attacking the terminal peptide bonds. This subgroup includes the most important types of proteolytic enzymes, i.e., serine proteinases, cysteine proteinases, aspartic proteinases and metaloproteinases (Belitz and Grosch, 1987). The mixture of proteolytic enzymes used in the food industry contains primarily endopeptidases. Examples of the utilization of these enzymes are described in the following paragraphs.

In the dairy industry the formation of casein curd is achieved with chymosin or rennin. Rennin is essentially free of other undesirable proteinases and is, therefore, especially suitable for cheese-making. However, there is a shortage of rennin since it is isolated from the stomach of a suckling calf. Hence, substitutes have been sought in recent decades. Microbial proteinases, especially those of *Mucor* spp., e.g. *Mucor pusillus,* appear to meet the demand. Proteinases are also added to wheat flour in the production of some bakery products to modify rheological properties of dough and, thus, the firmness of the end-product. Plant proteinases and also those of microorganisms are utilized for ripening and tenderizing meat. In the beer industry, a so-called cold turbidity sometimes occurred due to protein sedimentation. This can be eliminated by hydrolysis of protein by plant proteinases such as papain, etc. Production of complete or partial hydrolysates of protein by enzymatic methods is another example of an industrial use of proteinases. This is the process used in the liquefaction of fish proteins to make products with good flavors (Belitz and Grosch, 1987).

One of the concerns in the enzymatic hydrolysis of proteins is to avoid the release of bitter-tasting peptides and/or amino acids. Their occurrence in the majority of proteins treated (an exception is collagen) can not be ignored, especially when the extent of hydrolysis yields peptide fragments of less than 6 kdal. However, plastein reaction enables peptide fragments of hydrolysate to join enzymatically through peptide bonds at higher pH than the hydrolysis itself (Belitz and Grosch, 1987). Plastein reaction can be used to make several specialty products including surfactants, emulsifiers, whipped topping, phenylalanine-free high protein products and nutritionally enriched food products (Chobert *et al.*, 1992). A schematic diagram of plastein reaction is shown in Fig. 1. The reaction rate is affected by, among other things, the nature of the amino acid residues. Hydrophobic amino acid residues are preferably linked together (Arai *et al.*, 1978). Incorporation of amino acid esters into protein is affected by the alkyl chain length of the ester. Short-chain alkyl esters have a low rate of incorporation, while the long-chain alkyl esters have a higher rate of incorporation. This is especially important for the incorporation of amino acids with a short side chain, such as alanine.

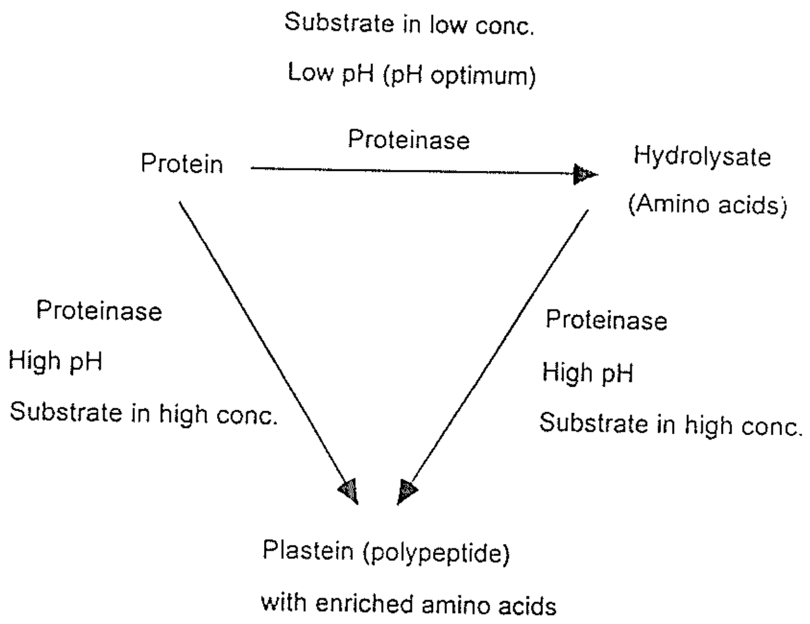

Fig. 1. An outline for single- and two-step plastein reactions.

The plastein reaction can help to improve the biological value of a protein (Aso *et al.*, 1974). Enrichment of a protein with selected amino acids can be achieved with the corresponding amino acid esters or, equally well, by using suitable partial hydrolysates of another protein (Yamashita *et al.*, 1971). The plastein reaction also makes it possible to improve the solubility of a protein, as for example, by increasing the content of glutamic acid (Yamashita *et al.*, 1975). Proteins with an increased content of glutamic acid show an interesting sensory effect: partial hydrolysis of modified plastein does not result in a bitter taste, rather it generates a pronounced "meat broth" flavor. Elimination of the bitter taste from a protein hydrolysate is also possible without incorporation of hydrophobic amino acids: bitter-tasting peptides, such as Leu-Phe, which are released by partial hydrolysis of protein, react preferentially in the subsequent plastein reaction and are incorporated into higher molecular weight peptides with a neutral taste.

Undesired amino acids in certain products can also be removed by enzymatic processes through plastein reaction (Yamashita *et al.*, 1979). For example, a phenylalanine-free diet which can be satisfied by mixing amino acids, is recommended for certain metabolic defects. However, the use of a phenylalanine-free higher molecular weight peptide is more advantageous from sensory and osmotic aspects.

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ISSN-0854-9230