

Review

Amylases and their applications

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Amylases are widely distributed and are one of the most studied enzymes. These enzymes have wide scale application ranging from textile to effluent treatment.

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INTRODUCTION

Amylases are starch degrading enzymes. They are widely distributed in microbial, plant and animal kingdoms. They degrade starch and related polymers to yield products characteristic of individual amyolytic enzymes. Initially the term amylase was used originally to designate enzymes capable of hydrolyzing α -1,4-glucosidic bonds of amylose, amylopectin, glycogen and their degradation products (Bernfeld, 1955; Fisher and Stein, 1960; Myrback and Neumuller, 1950). They act by hydrolyzing bonds between adjacent glucose units, yielding products characteristic of the particular enzyme involved.

In recent years a number of new enzymes associated with degradation of starch and related polysaccharides structures have been detected and studied (Boyer and Ingle, 1972; Buonocore et al., 1976; Griffin and Fogarty, 1973; Fogarty and Griffin, 1975).

1. The enzymes of actual or potential commercial importance of microbial origin that split α -1,4 or α -1,4 and/or α -1,6 bonds in these structures, may be divided in the following six classes (Fogarty and Kelly, 1979):
2. Enzymes that hydrolyze α -1,4 bonds and bypass α -1,6 linkages e.g. α -amylase (endoacting amylases).
3. Enzymes that hydrolyze α -1,4 and cannot bypass α -1,6 linkages e.g. β -amylase (exoacting amylases producing maltose as a major end product).
4. Enzymes that hydrolyze α -1,4 and α -1,6 linkages e.g. amyloglucosidase (glucoamylase) and exoacting amylase.
5. Enzymes that hydrolyze only α -1,6 linkages e.g. pullulanase and other debranching enzymes.

6. Enzymes that hydrolyze preferentially α -1,4 linkages in short chain oligosaccharides produced by the action of other enzymes on amylose and amylopectin e.g. α -glucosidases.
7. Enzymes that hydrolyze starch to a series of nonreducing cyclic D-glucosyl polymers called cyclodextrins or Sachardinger dextrans e.g. *Bacillus macerans* amylase (cyclodextrin producing enzyme).

STARCH

Before describing the action pattern and properties of amyolytic enzymes, it is essential to discuss the features of the natural substrate, starch. Starch is a major reserve carbohydrate of all higher plants. In some cases it accounts for as high as 70% of the undried plant material. It occurs in the form of water insoluble granules. The size and shape of the granules are often characteristic of the plant species from which they are extracted. When heated in water the hydrogen bonds holding the granules together begin to weaken and this permits them to swell and gelatinize. Ultimately they form paste or dispersion, depending on the concentration of polysaccharide. Starches are produced commercially from the seeds of plants, such as corn, wheat, sorghum or rice; from the tubers and roots of plants such as cassava, potato, arrowroot and the pith of sago palm. The major commercial source of starch is corn from which it is extracted by a wet milling process (Berkhout, 1976).

Starch is a heterogeneous polysaccharide composed of two high molecular weight entities called amylose and amylopectin. These two polymers have different structures and physical properties (Table 1). Starch may

Table 1. Comparison of amylose and amylopectin.

Properties	Amylose	Amylopectin
Basic Structure	Essentially linear	branched
Stability in aqueous solution	retrogrades	stable
Degree of polymerization	C. 10^3	C. $10^4 - 10^5$
Average chain length	C. 10^3	C. 20-25
β amylase hydrolysis	87%	54%
β amylase and debranching nzyme hydrolysis	98%	79%
Iodine complex max.	650 nm	550 nm

be separated into its two components by addition of a polar solvent, e.g. n-butanol, to a dispersion of starch. The insoluble amylose complex can then be separated from soluble amylopectin fraction. Amylose is composed of linear chains of α -1,4 linked D-glucose residues. Hence it is extensively degraded by α -amylase. Some amylose is not totally degraded to maltose by this enzyme. Amylose has a degree of polymerization of several thousands of glucose units (Banks and Greenwood, 1975). Because of the molecular shape and structure of amylose, it is not stable in aqueous solution and retrogrades (precipitates spontaneously). This is because linear chains align themselves by hydrogen bonding and thus forms aggregates. This process is irreversible. Retrograded amylose will only dissolve in alkaline solution. Amylose has considerable viscosity in alkaline solutions due to its molecular shape. Amylose forms complex with iodine to form intense blue color and this forms the basis of a method for quantitative determination of amylose.

Amylopectin may account for 75 to 85% of most starches. It has molecular weight in excess on $10^7 - 10^8$ and has a branched structure composed of chains of about 20 – 25 α -1,4 linked D-glucose residues. Amylopectin which is branched by α -1,6 linkages may contain 4 to 5% α -1,6-D-glucosidic bonds. In aqueous solutions, amylopectins are relatively stable due to branched molecules and are not able to form compact aggregates. There is no apparent relationship between the limiting viscosity number and the degree of polymerization. Due to the nature of branched structure, the iodine binding power is reduced. The branched components of starch is amylopectin which has different types of chains referred to as A, B and C chains (Fogarty, 1983)

The hydrolysis of starch may be carried out using either acid or enzyme as catalyst. Enzyme hydrolysis has several advantages: it is more specific, therefore fewer byproducts are formed, and hence yields are higher. Conditions for enzyme hydrolysis are milder therefore refining stages to remove ash and color is minimized. The enzymatic hydrolysis of starch has been practiced

on an industrial scale for many years and is gradually replacing the traditional acid hydrolysis process (Underkofler et al., 1965; Barfoed, 1976).

APPLICATIONS OF AMYLASES

The history of the industrial production of enzymes dates back to the time when Dr. Jhokichi Takamine began the production of digestive enzyme preparation by wheat bran koji culture of *Aspergillus oryzae* in 1894. Industrial production of dextrose powder and dextrose crystals from starch using α -amylase and glucoamylase began in 1959. Since then, amylases are being used for various purposes. Conversion of starch into sugar, syrups and dextrans forms the major part of the starch processing industry (Marshall, 1975). The hydrolysates are used as carbon sources in fermentation as well as sources of sweetness in a range of manufactured food products and beverages. Hydrolysis of starch to products containing glucose, maltose, etc. is brought about by controlled degradation (Norman, 1978; Barfoed, 1976; Hurst, 1975; Slott and Madser, 1975). Some of the applications of amylase are:

Liquefaction

Liquefaction is a process of dispersion of insoluble starch granules in aqueous solution followed by partial hydrolysis using thermostable amylases. In industrial processes, the starch suspension for liquefaction is generally in excess of 35% (w/v). Therefore the viscosity is extremely high following gelatinization. Thermostable α -amylase is used as a thinning agent, which brings about reduction in viscosity and partial hydrolysis of starch. Retrogradation of starch is thus avoided during subsequent cooling.

The traditional thinning agent used in starch technology was acid (hydrochloric or oxalic acids, pH 2 and 140 – 150°C for 5 min). The introduction of thermostable α -amylases has meant milder processing conditions. The formation of byproducts is reduced and refining and

recovery costs are lowered (Greenshields and Macgrillivray, 1972; Birch and Schallenger, 1973).

In the enzymatic process the hydrolytic action is terminated when the average degree of polymerization is about 10-12. Two distinct types of thermostable α -amylases are commercially available and used extensively in starch processing technology. The amylase of *Bacillus amyloliquefaciens* was the first liquefying α -amylase used on a large scale. Later, a more heat stable enzyme from *Bacillus licheniformis* was introduced commercially (Madsen et al., 1973). Liquefaction can be done by two methods:

Single stage enzyme liquefaction: In 1973, Novo Industri A/S Copenhagen developed and patented the process. In this process, starch slurry containing 30 – 40% dry solids is prepared in the feed tank. The pH is adjusted to about 6 – 6.5 with sodium hydroxide. Calcium salts may be added if the level of free calcium ions is below 50 ppm. The liquefying enzyme is then added. The slurry is then pumped continuously through a jet cooker where the temperature is raised to 105°C by direct injection of live steam. Tremendous shearing forces are exerted on the slurry as it is pumped through the jet cooker. So in addition to the viscosity reduction action of the enzyme, some mechanical thinning also occurs. The slurry is maintained at this high temperature in the pressurized holding cell for about 5 min, after which it is discharged via a spring loaded release valve into a reaction, where enzyme action is allowed to continue for about 2 hours at 95°C. After this treatment the liquefied starch will have dextrose equivalent (DE) of 10 – 20 depending on the amount of enzyme used. DE is defined as reducing sugars expressed as dextrose and calculated as a percentage of dry substance. This process is simple energy consumption is relatively low because the maximum operating temperature is only 105°C as compared to 140 – 150°C normally used.

Acid enzyme liquefaction: This is another process which takes advantage of the thermostability of *B. licheniformis* amylase. The enzyme is added after the starch has been cooked and cooled to 100 – 95°C. A starch slurry containing 30 – 40% dry solids is cooked at a high temperature for about 5 min. A jet cooker is used so that sufficient mechanical thinning, due to shearing takes place. The pH may be in the range 2 – 5, but if it is too low, byproduct formation will be significant. If it is too high there will be no thinning effect from the acid and there will be an increased color formation. After cooking, the slurry is flash cooled to about 100°C and the pH is set to 6 to 6.5 before the addition of enzyme. By this process the enzyme consumption is slightly reduced. The filtration properties are also improved because better fat/protein separation is achieved. There is an increase in steam consumption and hence fuel costs due to high temperature cooking.

Liquefaction is the first and most important step in starch processing. The purpose is to provide a partially

hydrolyzed starch suspension of relatively low viscosity which is free from by products, stable to retro gradation and suitable for further processing i.e saccharification. If the liquefaction process does not go well, problems like poor filtration and turbidity of the processed solution occurs. The most important factor for ideal liquefaction of starch is that the starch slurry which contains suitable amount of α -amylase is treated at 105 to 107°C as quickly and uniformly as possible (Hattori, 1984). Thermostable amylase are not sufficiently heat stable to be used during liquefaction process, but they can be used as saccharifying enzymes. The most widely used enzymes in this group are the maltogenic enzymes.

Manufacturing of maltose

Maltose is a naturally occurring disaccharide. Its chemical structure has 4-O- α -D-glucopyranosyl-D-glucopyranose. It is the main component of maltosugar syrup (Sugimoto, 1977). Maltose is widely used as sweetener and also as intravenous sugar supplement. It is used in food industries because of low tendency to be crystallized and is relatively nonhygroscopic.

Corn, potato, sweet potato, and cassava starches are used for maltose manufacture. The concentration of starch slurry is adjusted to be 10 – 20% for production of medical grade maltose and 20 – 40% for food grade. Thermostable α -amylase from *B. licheniformis* and *B. amyloliquefaciens* are used.

Manufacture of high fructose containing syrups

High fructose containing syrups (HFCS) 42 F (Fructose content = 42%) is prepared by enzymic isomerization of glucose with glucose isomerase. The starch is first converted to glucose by enzymic liquefaction and saccharification.

Manufacture of oligosaccharides mixture

Oligosaccharides mixture (maltooligomer mix) is obtained by digestion of corn starch with α -amylase, β -amylase and pullulanase. Maltooligomer mix is a new commercial product. Its composition is usually as follows: glucose, 2.2%; maltose, 37.5%; maltotriose, 46.4%; and maltotetraose and larger maltooligosaccharides, 14%.

Maltooligomer mix powder obtained by spray drying is highly hygroscopic. Therefore it serves as a moisture regulator of the food with which it is mixed. Maltooligomer mix tastes less sweet than sucrose. Its solution shows a lower viscosity than corn syrup because of its low content of glucose. Maltooligomer mix is mainly used as a substitute for sucrose and other saccharides. It is also used for preventing crystallization of sucrose in foods

and keeping a certain level of hardness of the texture during storage.

Manufacture of maltotetraose syrup

Maltotetraose syrup (G4 syrup) is produced by subjecting starch to the action of maltotetraose forming amylase (G4 amylase). The sweetness of the syrup is as low as 20% of sucrose. Therefore a partial replacement of sucrose with G4 syrup reduces the sweetness of foods without affecting their inherent taste and flavor. It has high moisture retention power which serves to prevent retrogradation of starch ingredient and retains suitable moisture in foods. G4 syrup shows less Millard reaction as it has less glucose and maltose content. It has higher viscosity than sucrose thus improving the food texture. G4 syrups depresses the freezing point of water more moderately than sucrose or high fructose syrup. Therefore, G4 syrup can be used to control the freezing points of frozen foods. G4 syrup imparts gloss and can be used in industry such as a paper sizer. Commercial thermostable α -amylase of *B. licheniformis* or *B. subtilis* is used to make G4 syrups.

Production of anomalously linked oligosaccharides mixture (Alo mixture)

“Alo mixture” is a mixture of isomaltose, panose, isomaltotriose and branched oligosaccharide composed of 4 and 5 glucose residues. The ‘Alo mixture’ has properties that are favorable to food industry. It is mildly sweet, has low viscosity, high moisture retaining capacity and low water activity convenient in controlling microbial contamination.

For the manufacturing of “Alo mixture”, starch is dextrinized using thermostable bacterial α -amylase. The degree of hydrolysis (DE) of starch is kept between 6 to 10. The simultaneous reaction of saccharification and transglucosidation of dextrin is done by using soybean β -amylase and *Aspergillus niger* transglucosidase. The reaction mixture is finally purified and concentrated to 25% moisture.

Manufacturing of high molecular weight branched dextrans

Branched dextrans of high molecular weight are prepared by hydrolysis of corn starch with α -amylase. The extent of starch degradation to be carried out depends on the type of starch and the physical properties desired. The branched dextrans are obtained as powder after chromatography and spray drying. These are used as extender and a glozing agent for production of powdery foods and rice cakes, respectively.

Removal of starch sizer from textile (desizing)

In textile weaving, starch paste is applied for warping. This gives strength to the textile at weaving. It also prevents the loss of string by friction, cutting and generation of static electricity on the string by giving softness to the surface of string due to laid down warp. After weaving the cloth, the starch is removed and the cloth goes to scouring and dyeing. The starch on cloth is usually removed by application of α -amylase.

Direct fermentation of starch to ethanol

The amylolytic activity rate (Abouzied and Reddy, 1986) and amount of starch utilization and ethanol yields increase in several fold in cocultures (Van Lenen and Smith, 1968). Moulds amylases are used in alcohol production and brewing industries. The advantages of such system are uniform enzyme action in mashes, increase rate of saccharification, alcohol yield and yeast growth (Van Lenen and Smith, 1968).

Treatment of starch processing waste water (SPW)

Starch is also present in waste produced from food processing plants. Starch waste causes pollution problems. Biotechnological treatment of food processing waste water can produce valuable products such as microbial biomass protein and also purifies the effluent (Bergman et al., 1988; Friendrich et al., 1987; Jamuna and Radhakrishna, 1989; Kingspohn et al., 1993).

Other applications

Amylases, especially alkaline amylases are used in detergents. To some extent amylases are also used as digestive aids (Beazell, 1942) to supplement the diastatic activity of flour and to improve digestibility of some of the animal feed ingredients.

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