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α-Amylases:structure And Function Relationship

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Abstract

In this review α-Amylases (α-1,4-glucan-4-glucanohydrolases, EC 3.2.1.1) are dealt with respect to their (a) types (b) assay method (c) structure (d) active site (e) post translational modifications (f) substrate and its digestion in vivo (starch) (g) inhibitors (h) future scope.

Keywords. α-Amylase, PPA, structure, mechanism, human amylases, glycosylation

Introduction

α-Amylases are a family of endo-amylases that catalyse the hydrolysis of α-D-(1,4) glycosidic linkages in starch and its components, i.e. amylose and amylopectin and glycogen releasing malto-oligosaccharides in the α-anomeric form[1-2]. The term 'α' relates to the anomeric configuration of the free sugar group released. α-Amylases are widely distributed in almost all the living organisms, i.e. mammals, plants and several microorganisms[3-5]. α-Amylases and related amylolytic enzymes are among the most important enzymes having great significance in several food industries. The range of technological applications of α-amylases are very wide [table 1]. α-Amylases are used for the liquefaction of cereal starch during the production of high fructose corn syrup and fuel ethanol. The paper and textile industry apply α-amylases in the sizing/desizing process. α-Amylases remove starch containing stains from clothes and dishes in household laundry and automatic dishwashing detergents. Besides these traditional applications, the spectrum of amylase usage has widened in many other fields, such as clinical medicine and analytical chemistry.

The Enzyme Commission (EC) classification of enzymes is based on the reaction catalysed. Each reaction is consequently assigned an EC number that uniquely identifies the reaction. For α-amylases this number is 3.2.1.1 and the reaction is described as the '*endohydrolysis of 1,4-α-glucosidic linkages in oligosaccharides and polysaccharides*'[6].

Types of starch degrading enzymes

Starch degrading enzymes can be classified into three main groups based on their mode of action: (1) endoamylases (2) exoamylases and (3) debranching enzymes.

(1) Endoamylases:

Endoamylases, also known as 'liquefying' enzymes cleave the α-1,4 glycosidic bonds in amylose, amylopectin and related polysaccharides such as glycogen but not the α-1,6 bonds in amylopectin. The products of hydrolysis are oligosaccharides of varying chain lengths, having α-configuration on the C_1 of the reducing glucose unit. Endoamylases hydrolyze the bonds located in the inner regions of the substrate resulting in rapid decrease of the viscosity as well as decrease in the iodine staining power of the gelatinized starch solution[7]. Activity is defined in terms of the amount of enzyme required to breakdown starch under a given set of conditions.

(2) Exoamylases:

Exoamylases, also known as 'saccharifying' enzymes act externally on the α-1,4 glycosidic bonds in amylose, amylopectin and glycogen substrate bonds from the non-reducing end and produce β-D-maltose by the inversion of the product. Gelatinized starch when subjected to exoamylases, results in a slow decrease in the viscosity and iodine staining power of starch in contrary to endo amylases. Cereal and bacterial β-amylases and fungal glucoamylases come under this category[8]

(3) Debranching enzymes:

The branch points containing α-1,6 glycosidic linkages present in starch and glycogen are resistant to attack by α- and β-amylases resulting in α/β limit dextrins, respectively. Pullulanase, first discovered in 1961 attracted interest because of its specific action on

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Table 1 Applications of alpha-amylase in various sectors of food industries

Industry	Purpose / Function
Baking	Bread making and to break down complex sugars such as starch (found in flour) into simple sugars, maltose production.
Brewing	For liquefaction, clarification and to supplement malt enzymes
Starch	Modification & conversion (ex. To dextrose or high fructose syrups)

pullulan, a linear D-glucose polymer with maltotriosyl units joined by α-1,6 bonds. Pullulanase is produced by mesophilic organisms such as *Klebisiella aerogenes* and *Aureobasidium pullulans* and are capable of specifically attacking α-1,6 linkages present in starch and glycogen[9]. Glucoamylase can also attack α-1,6 linkages but the reaction proceeds at relatively slow speed compared to pullulanase action.

The different starch hydrolysing enzymes are given in table 2

Assay methods for amylases

Amylase activity can be determined by a number of analytical methods. The quantitative procedures generally used involve the measurement of new reducing groups formed upon the amylolytic hydrolysis

Table 2 Starch hydrolyzing enzymes

Enzyme	EC number	Glycosidic bond specificity	Mode of action	End products
Phosphorylase (α-1,4-α-D-glucan orthophosphate α-D-glucosyl transferase)	2.4.1.1	α-(1-4)-glucosyl	Exo	Glucose 1-phosphate
Alpha-amylase (α-1,4-α-D-glucanglucanohydrolase)	3.2.1.1	α-(1-4)-glucosyl	endo	Linear and branched Oligosacchari-des
Beta-amylase (1,4-α-D-glucan maltohydrolase)	3.2.1.2	α-(1-4)-glucosyl	exo	Maltose and limit dextrins
Amyloglucosidase (glucoamylase exo-1,4-α-glucosidase)	3.2.1.3	α-(1-4)-glucosyl and α-(1-6)-glucosyl	exo/endo	Glucose
Isoamylase (glycogen 6-glucanohydrolase)	3.2.1.68	α-(1-6)-glucosyl	endo	Linear α-(1-4) glucan Chains
Pullulanase (limit dextrinase; amylopectin 6-glucanohydrolase)	3.2.1.41	α-(1-6)-glucosyl	endo	Linear α-(1-4) glucan

of starch. The amylase activity is represented as the micromoles of products formed or substrate transformed per minute under defined conditions[10]. The released reducing groups can be measured by colorimetric methods using the following reagents (a) alkaline copper[11] (b) alkaline ferricyanide[12] and (c) alkaline 3, 5, dinitrosalicylate (DNS)[13-14]. Among these methods, DNS method is the most extensively used because of its reliability and simplicity. Copper and ferricyanide procedures give equimolar reducing values for equimolar reducing ends of maltooligosaccharides[12]. The decrease in loss of the ferricyanide ion concentration is measured by the ferricyanide reagent method. The other method is the Neocuprine method[15] which is used for the estimation of the reducing sugar released during amylase activity assay.

The measurement of decrease in the blue color produced by starch when complexed with iodine solution can be semi-quantitatively determined[16]. This procedure reflects the endoclevage of starch and can be used routinely to assay α-amylases. In addition, the measurement of the decrease in the viscosity of the starch solution can also be taken as a measure of α-amylase activity[10]. Since this procedure measures only endoactivity, it can be extensively used to detect α-amylase.

Another method to assay α-amylase activity is the use of chromogenic substrates, which are useful for α-amylase assays of clinical samples. The dye is covalently linked to starch or one of its constituents (amylose or amylopectin) to give an insoluble azure derivative[17]. When these substrates are acted on by α-amylase, fragments containing the dye is solubilized, the remaining insoluble substrate is removed by centrifugation and the absorbance of the supernatant is taken as measure of amylase activity.

α/β- amylase activities can also be detected by the p-Nitrophenyl derivative of oligosaccharides[18-19]. The amount of p-nitrophenol released correlates with the amylase activity.

Structure of α-amylases

Primary structure

α-Amylases are small proteins with a molecular weight ranging from 20-55 kDa. They are monomeric, calcium-containing enzymes. Nakajima, et al.,[20] clearly pointed out the existence of four highly conserved regions, especially in the catalytic and substrate binding regions in eleven different α-amylases. The three active site residues Asp231, Glu261 and Asp328 (*Bacillus licheniformis* α-amylase numbering) were identified.

Secondary structure

X-ray studies of mammalian and bacterial α-amylases have shown that all α-amylases consist of three distinct domains i.e. domain 'A', 'B' and 'C'. A central $(\beta/\alpha)_8$ barrel (domain A) forms the core of the molecule with

Fig.1 Ribbon diagram of pancreatic α-amylase

three active site residues Asp231, Glu261 and Asp328. Domains 'B' and 'C' are located roughly at opposite sides of this barrel. The 'B' domain is firmly attached to the 'A' domain by disulphide bond. 'C' domain with a β-sheet structure is linked to the 'A' domain by a simple polypeptide chain and may carry a carbohydrate chain depending on the origin of the enzyme[21]. The ribbon diagram of pancreatic -amylase is presented in fig.1.

All known α-amylases contain a conserved calcium ion, which is located at the interface between domains 'A' and 'B'[22-25]. The calcium ion is bound very tightly as shown by the dissociation constants for PPA and hog pancreatic amylase, 44 nM and 0.0050 nM respectively[26]. The role of the conserved calcium ion is mainly structural since it is too far away from the active site to participate directly in catalysis [25,27,21].

Tertiary structure

X-ray crystallography was carried out to describe the crystal structure of amylases from barley malt α-amylase isoforms Amy-2 (68 Å x 53a x 36Å), *Aspergillus oryzae* (Taka amylase 3.0 and 2.1 Å), *A. niger* (2.1 Å) and porcine pancreatic amylase (2.9 Å). The three-dimensional structures of porcine and human pancreatic α-amylases do not differ significantly at 2 Å resolution[28-30].

Active site of α-amylase

The active site of the α-amylase is located in a long cleft of about 3 nm between the carboxyl end of the 'A' domain and the 'B' domain. A model for the organization of active site and subsites, each capable of binding glucose has been proposed. The active site of different α-amylases are thereby made up of 511 subsites (A-K). The catalytic site is situated between subsites F and G. The reducing end of the α-glucose chain is located towards K subsite. Difference in the specificity of α-amylase, i.e. in the detailed way in which they hydrolyze a polysaccharide has been explained in terms of subsites at the active site. Each subsite interacts with one glucose unit of the substrate. The catalytic site of the enzyme is located between subsites F and G. Interaction of the enzyme with the primary hydroxyl of a glucose ring unmodified at C-6 is an important requirement for binding at that subsite.

α-Amylase catalytic mechanism

Catalytic mechanism of α-amylase was well characterized with respect to Taka amylase and found that active site has been localized in the cleft of the $(\beta/\alpha)_8$ barrel domain with Asp 206, Glu 230, Asp 297 residues playing the catalytic role whereas His 122 and His 296 might bind to glucosyl residues of substrate[21]. The active site is divided into two parts (a) the binding site made up of a number of subsites and (b) the catalytic site made up of 23 groups that are proton donors (electrophiles) and proton acceptors (nucleophiles). The number of subsites and their arrangement in conjunction with the catalytic groups determine the type of the products formed.

The retention of the configuration occurring during α-amylase action suggests a double displacement mechanism involving a covalent intermediate. It was proposed that transglycosylation which involves formation of both α-1,4 and α-1,6 bonds could be catalyzed by the same mechanism[28].

Human α-amylases

Human α-amylases are divided into two groups by the site of their biosynthesis. One group is the pancreatic α-amylase and the other is the salivary α-amylase. Human α-amylases from both salivary and pancreatic origin (HSA and HPA) have been extensively studied with respect to their three dimensional structure[29-31]. These groups are encoded by two loci Amy_1 (salivary) and Amy_2 (pancreatic)[32]. Their amino acid sequences have been deduced from the nucleotide sequences of cDNAs[33], and that of human salivary amylase was also determined from the cloned gene[34]. A 97% homology was observed with respect to the above two α-amylases.

Measuring α-amylase activity in serum, urine, saliva and other biological fluids is a useful diagnostic tool in evaluating diseases of the pancreas and salivary glands (e.g., acute pancreatitis, parotitis)[35,19].

Comparison of porcine pancreatic α-amylase amino acid (aa) sequences with other mammalian amylases

Structural comparisons were made between the PPA sequence and the established sequences of human[34], mouse[36] and rat[37] α-amylases. The highest homology of PPA amino acid (aa) sequence was found with that of human pancreatic alpha amylase sequence with a percentage identity of 87.1%. The mouse and rat α-amylases share slightly lower identity with the porcine sequence (85.5%). As already known α-amylases contain five conserved sequence regions[38], four located at or around the β- strands of the $(\beta/\alpha)_8$-barrel (domain A)[39] and a short one near the 'C'-terminus of domain 'B'[40]. The three-dimensional structures of porcine and human pancreatic α-amylases do not differ significantly at 2 Å resolution[29-30]. However, according to Brayer, et al.,[30] two main regions of conformational differences exist near the active site, these include residues 237-250 and 304-310. These above studies showed that the sequence of both porcine and human α-amylases are identical in the second region (aa 304-310). Together with the data obtained by X-ray analysis, the sequence conservation

of the 304-310 region among mammalian α-amylases favours a common role of this loop in the enzymatic mechanism of α-amylases[41].

Pancreatic alpha amylase and its isoform

Porcine pancreatic α-amylase (PPA) was purified to homogeneity from tissue extracts[42] as two forms i.e. PPA-I and PPA-II[43]. They differ in their pI values of 7.5 (PPA-I) and 6.4 (PPA-II)[18]. The crystal structures of α-amylase from *Aspergillus oryzae* (TAA)[44] and α-amylase from porcine pancreas (PPA)[21] have revealed that the binding sites are similar in both the enzymes. Porcine pancreatic α-amylase contains 2 sulfhydryl groups[45,42].

The entire aa sequence of PPA-I was established[46-47], while only partial unordered and non-overlapping peptides have been determined for PPA-II[48]. However, the three dimensional structure of both PPA-I and PPA-II are known and were found to be identical at 2.1 Å resolution[29, 49]. Moreover, the three-dimensional structure of mixed PPA-I and PPA-II crystals has been reported at the same resolution[27].

According to the multiple attack hypothesis porcine pancreatic α-amylase (PPA), PPA (E) remains bound after the first endoglycosidic attack to the new reducing end of the glycoside half chain and switches to the exoglucosidase mode of action, releasing several maltose molecules before the ES complex is dissociated[50-51]. This highly unusual mechanism has not been properly elucidated yet nor have the functions of several structural features of PPA been explained[52]. The active site of PPA is a long deep polysaccharide binding cleft, crosses the C-terminal end of the β/α-barrel[27]. An additional surface carbohydrate-binding site has been detected in the C-terminal domain segment between β_9 and β_{10}[21]. Porcine pancreatic amylase has two metal ion binding sites, one selective for calcium, and the other to which zinc or copper may bind[53].

Post-translational modification of amylases

Glycosylation is the most important post-translational modification in newly synthesized proteins. Addition of oligosaccharides (glycans) to protein structures is a common occurrence in higher organisms[54-55]. The structural complexity of glycans, far greater than that of proteins and nucleic acids, allows them to encode information for specific molecular recognition and to determine protein folding, stability and pharmacokinetics. The interest in glycosylation is also due to the implication of carbohydrates in many pathological states, such as cancer, atherosclerosis and rheumatoid arthritis.

There are two main type of protein glycosylation: *N*-glycosylation, in which the glycan is attached to an Asn residue present in the tripeptide consensus sequon Asn-X-Ser/Thr (where X can be any amino acid except Pro), and *O*-glycosylation, in which the glycan is attached to a Ser or Thr residue. In rare cases other amino acid residues, e.g., cysteine or lysine, may also be glycosylated. N-linked glycans contain N-acetylglucosamine (GlcNAc) linked via an amide bond to asparagine residues. The complexity of N-linked oligosaccharide structures are reduced by the existence of their core region but enhanced through the variations in the antennal region. *N*-glycosylation is a co-translational event where prefabricated oligosaccharide units are transferred from the lipid carrier, i.e. dolichol diphosphate to *Asn* residues as soon as the growing polypeptide chain enters the lumen of the endoplasmic reticulam[56-57].

Complex oligosaccharides attached to an asparagine residue in glycoprotein, termed *N*-linked oligosaccharides, have a common pentasaccharide core structure and one or more antennae attached to each of the mannose residues at the nonreducing terminus. Glycosylation is observed in all eukaryotes and has also been found in prokaryotes[58]**.** Porcine pancreatic α-amylase is believed to be a glycoprotein[59]. In mammalian systems, these antennae consist either of mannose (Man) chains (termed "high-mannose" sugars) or chains containing N-acetyl-2-amino-2-deoxyglucose (GlcNAc) with or without galactose (Gal)[60]. The biological significance of the carbohydrate moiety of a glycoprotein has been a matter of much speculation. Carbohydrates in the form of asparagine-linked (*N*-linked) or serine/threonine-linked (*O*-linked) are major structural components of many eukaryotic proteins.

The diversity of oligosaccharides structure often results in heterogeneity in the mass and charge of glycoproteins. These glycosylation modifications are not merely decorations. The carbohydrate perform critical biological functions/ processes such as embryonic development, inter and intracellular activities[61], coordination of immune functions[62], cell division processes, and protein regulations and interactions.

To study the structure and function of a glycoprotein, it is often desirable to remove either all or only a selected class of oligosaccharides. This approach allows the assignment of specific biological functions to particular components of the glycoprotein. For the glycomic analysis, glycans are often released from protein back-bones. Asparagine (Asn)-linked glycans can be cleaved enzymatically by peptide *N*-glycosidase F (PNGase F, EC 3.5.1.52) and chemically by

hydrazinolysis.

The enzymatic methods are more preferably employed, since it yields intact oligosaccharides regardless of size or structure of the substrate/carbohydrate moiety and a slightly modified protein in which Asn residues at the site of de-N-glycosylation are converted to aspartate (Asp).Chemical deglycosylation by hydrazinolysis causes chemical modification including N-deacetylation of sialic acids and N-acetyl-D-hexosamines such as GlcNAc and GalNAc residues, as well as extensive cleavage of polypeptide backbones[63-64].

The reasons for deglycosylation experiments are manifold: i) for simplifying amino acid sequence determination of glycoproteins ii) to remove heterogeneity in glycoproteins for X-ray crystallographic analysis iii) to remove carbohydrate epitopes from antigens iv) to enhance or reduce blood clearance rates of glycoprotein therapeutics v) to investigate the role of carbohydrates in enzyme activity and solubility vi) to investigate ligand binding vii) for quality control of glycoprotein as pharmaceuticals.

Even though all animal α-amylases include glycosylation sequons (Asn-Xaa-Thr/Ser) in their sequences, amylases purified from natural sources are not quantitatively glycosylated. These glycosylation sequons suggest that the protein is a potential target of the glycosylation machinery. There are one or two of these sequons per amylase depending on the species and they are always located at the C-terminus of the protein in the C-domain. The two rat glycosylation sequons are located at positions 410 (Asn^{410}-Gly-Ser-Asn) and 459 (Asn^{459}-Cys-Thr- Gly)[37]. According to studies on the influence of the Xaa residues in the glycosylation sequon on the efficiency of glycosylation, the glycine and the asparagine of the rat Asn^{410} sequon favour N-glycosylation[65]. In the case of α-amylases, any function of glycosylation on the enzyme activity of the mature protein is quite unlikely since the non-glycosylated form of amylase has been proven to be fully active. However, it is remarkable that all glycosylation sequons of animal α-amylases are located in the C-domain, a domain of unknown function. One of the roles proposed for this domain is to help stabilize the $(\beta/\alpha)_8$ barrel of the A-domain[66].

The N-glycosylation site of PPA is located between the amino acid residues from position 235 to 293 and for taka-amylase it is located between the amino acid residues 234 to 290. These regions are located on the enzyme surface close to their active-site cleft in their three-dimensional structures. Such a carbohydrate

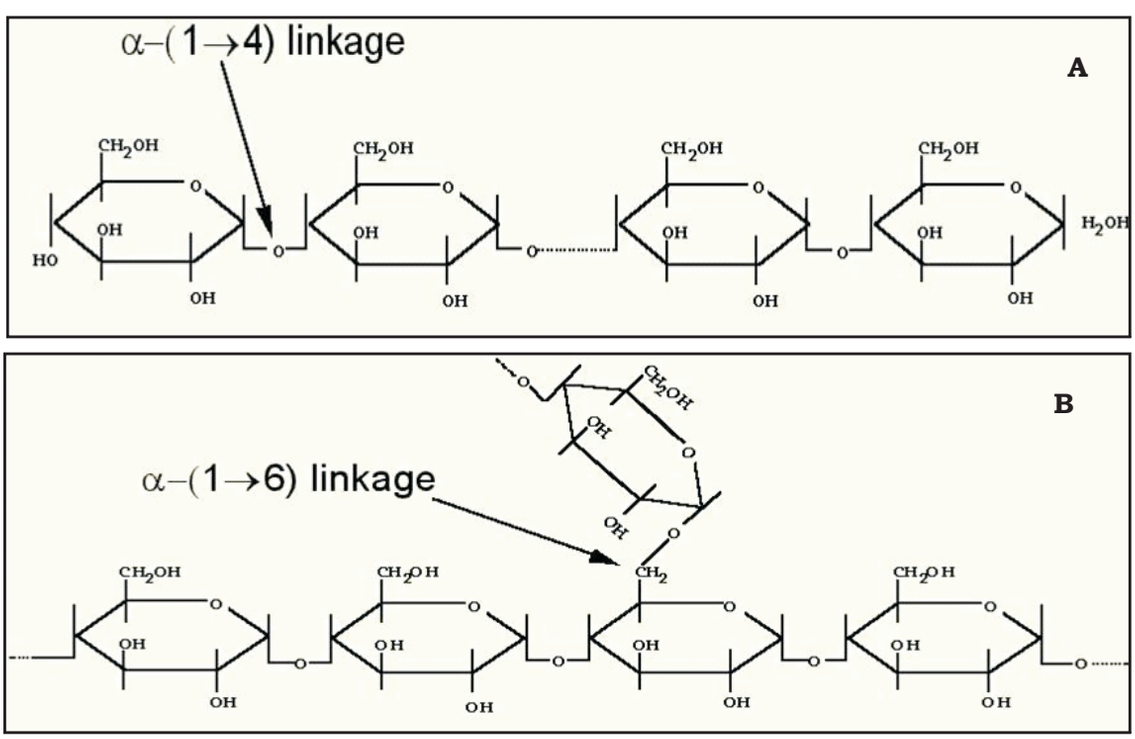

Fig 2 : Structure of (A) Amylose and (B) Amylopectin

chain might interact with the substrate entering into the active-site cleft. The presence of the carbohydrate chain near the active-site cleft might improve its efficiency, because α-amylase is known to hydrolyze the soluble starch several times by single attack (multiple attack)[67-68].

Effects of carbohydrate chains on thermostability were reported for glucoamylase[69]. These results suggest that the presence of the carbohydrate chain is important for stabilization of the three-dimensional structure. It is important to consider the position of the N-linked carbohydrate chain on the enzyme to elucidate the effects of the carbohydrate chain on the enzyme kinetics.

Structural identification of these oligosaccharides requires determination of the constituent monosaccharide, their sequence, the branching pattern and the hydroxyl groups involved in the linkage of one residue with another. Composition and linkage are best determined by gas chromatograph and MS following hydrolysis, whereas sequence and branching information can be deduced from fragment ions produced by fast atom bombardment (FAB)[60].

Starch the substrate

Starch is one of the major digestible carbohydrates in the human diet and contributes a substantial amount of calories. Starch granules are composed of two types of α-glucans, amylose and amylopectin which represent approximately 98-99% of the weight. Amylose, a linear polymer of α-D-glucopyranosyl units linked by the α-1,4 bonds and amylopectin is a branched polymer of α-D-glucopyranosyl units linked by α-1,4 and α-1,6 linkages (4.0-5.5% branching) [fig. 4][70-71]. Amylopectin has a cluster-like organization and forms crystalline regions developed from double helices of linear

Table 3 Characteristics of native starch granules from common sources

Starch	Size (μm)	Shape	Distribution
Barley	2-5 (B-granules) 15-25	Spherical Lenticular	Bimodal
Maize (waxy and normal)	2-30	Spherical/polyhedral	Unimodal
Amylomaize	2-30	Irregular	Unimodal
Millet	4-12	Polyhedral	Unimodal
Oat	3-10 (single) 80 (compound)	Polyhedral	Unimodal
Pea	5-10	Rentiform	Unimodal
Potato	5-100	Lenticular	Unimodal
Rice	3-8 (single) 150 (compound)	Polyhedral	Unimodal
Rye	5-10 (B-granules) 10-40 (A-granules)	Spherical Lenticular	Bimodal
Sorghum	5-20	Spherical	Unimodal
Tapioca	5-35	Spherical/ Lenticular	Unimodal
Triticale	1-30	Spherical	Unimodal
Wheat	2-10 (B-granules) 15-35 Lenticular (A-granules)	Spherical	Bimodal

branched chains. This results in the formation of amorphous and crystalline lamellae arranged in an overall semicrystalline structure.

Starch is acted upon by the salivary and pancreatic α-amylases and the brush border enzymes to produce glucose which is utilized by the human system. Undigested or resistant starch is fermented in the large intestine producing short chain fatty acids. The susceptibility of starch granules to degradation by α-amylase depends on their botanical origin and the α-amylase source[72-73]. An important parameter correlated to the extent of amylolysis is starch crystalline type (A or B)[74]. Starch granules from various botanical sources have one of the three X-ray diffraction patterns. Most cereal starches, normal maize, rice, wheat and oats exhibit the type A pattern, alternatively called the cereal type which are more readily hydrolyzed by α-amylase. Potato, lily, canna and tulip starches show B-type diffraction pattern called the tuber type. Some of the rhizome and bean starches exhibit the C-type pattern which is a mixture of A and B types in varying proportions.

Porcine pancretic α-amylase degrades rice or wheat starches (A-type) over 6 times faster than banana starch and over 20 times faster than potato starch (B-type)[75]. The final rates after extensive hydrolysis of the banana starch by common α-amylases from plants or animals are in the range of 5 to 20% [76]. The lower susceptibility of potato starch has been attributed to a higher granular size than in normal cereal starches and to a greater crystalline structure[77]. Studying reference crystalline materials is a way of understanding the differences observed in the susceptibility of native starch granules towards amylolysis[78].

The processed i.e. cooked starch is easily digested in the human body as its crystalline structure is destroyed during cooking. Digestion of starch is effected by hydrolysing enzymes in a complex process which depends on many factors; these include the botanical origin of starch, whether the starch is amorphous or crystalline, the source of enzymes, substrate to enzyme concentration, temperature and time as well as the presence of other substances in the multicomponent matrix in which starch occurs naturally, *e.g.* cereal grains [table 3]. Native starch is digested slowly compared to processed (gelatinised) starch whose crystallinity has been lost and where the accessibility of substrate to enzymes is greater and not restricted by α-glucan associations such as double helices (especially in crystallites) or amylose-lipid complexes (cereal starches). The restriction of starch digestion in the human digestive system due to forms which are resistant to hydrolysis has led to the concept of dietary 'resistant-starch'[79].

Starch digestion *in vivo*

Animals and humans produce a range of digestive enzymes which hydrolyse starch. Starch digestion and absorption essentially consists of three phases: the intraluminal phase, the brush borded phase and the phase of glucose absorption. The digestion of starch is initiated in the oral cavity by salivary α-amylase secreted from the parotid glands. Chewing disintegrates the food, thus increasing the surface area to volume ratio in the solid phase and hence enzyme accessibility. The pancreas plays a major role in starch digestion where starch in the duodenum is acted upon by pancreatic α-amylase and sucrase iso-maltase complex to hydrolyse α-(1-4) bonds and thus produce glucose, oligosaccharides and dextrins.

Glucose can be absorbed from the small intestine (especially terminal end of the duodenum and jejunum) whereas maltose and malto-dextrins generated from starch hydrolysis cannot be absorbed. Absorptive epithelial cells which line the intestinal villi produce a number of other 'brush border' enzymes that allow digestion and subsequent absorption of disaccharides[80-81].

The α-limit dextrins and small linear oligomers that are not absorbable into the bloodstream are converted to glucose in the human small intestine by the combined action of mucosal maltase-glucoamylase (MGAM, E.C. 3.2.1.20 and 3.2.1.3), and sucrase-isomaltase (SIM, E.C. 3.2.1.48 and 3.2.1.10). These enzymes are not released into the lumen of the small intestine but are bound to the membrane of microvilli. Consequently, some carbohydrate hydrolysis occurs at the surface of epithelial cells in the small intestine rather than the lumen of the gut. Brush border enzymes include sucrase which converts sucrose to glucose and fructose, and lactase converting lactose to glucose and galactose, but these are not relevant to starch metabolism. However, maltase (converting maltose generated from α-amylase activity to glucose) and isomaltase (α-dextrinase) which hydrolyses α-(1-6) bonds of isomaltose and α-dextrins continue the digestive process and thus convert the available starch to glucose.

Amylase inhibitors

α-Amylase inhibitors are diverse as they can be synthetic, microbial and of plant origin. α-Amylase and α-glucosidase have been targeted as potential avenues for modulation of postprandial hyperglycemia through mild inhibition of the enzymatic breakdown of complex carbohydrates to decrease meal-derived glucose absorption. Hyperglycemia is a condition characterized by an abnormal excess of sugar in the blood. Elevated postprandial blood glucose levels are widely

recognized as one of the earliest disease markers in the prediction of subsequent microvascular and macrovascular complications that can progress to full symptomatic type 2 diabetes (T2DM)[82-83]. Majority of available synthetic antidiabetic drugs target the dual metabolic defects that characterize T2DM, impaired insulin secretion and insulin resistance, while some of these drugs (e.g. metformin) can have negative side effects at high doses[84-85, 83]. Thus, a major goal of antidiabetic research is the discovery of anti-hyperglycemic agents that are safe and that lack any negative side effects. Dietary α-glucosidase and α-amylase inhibitors that act in the gut by inhibiting the enzymatic breakdown of starch/soluble carbohydrates have been identified as a potentially natural and safe approach for controlling hyperglycemia through modulation (i.e. decrease) of meal-derived glucose absorption.

The potential of phenolic-optimized aqueous extracts of selected foods in a typical American and Asian diets for anti-amylase and anti-glucosidase activities (antidiabetic potential) have been investigated[86]. Tannins are natural polyphenolic compounds of high molecular weight, which form insoluble complexes with proteins. The hydroxyl groups of these molecules are partially or totally esterified with phenolic compounds, such as gallic acid (gallotannins) or ellagic acid (ellagitannins). The tannins have general protein complexing properties, which can cause variable enzyme inhibition. Inhibition of salivary α-amylase by tea polyphenols was also documented[87]. Recently, soft fruit extracts (rich in ellagitannins and anthocyanins) were found to be effective α-amylase inhibitors[88]. The exact structure of active tea polyphenols and kinetic properties of tea and fruit extracts have not yet been investigated. It is a well known fact that the inclusion of polyphenols in the diet can lead to perturbation of mineral absorption from the intestinal canal, decrease the bodyweight gain, retard growth, and inhibit the digestive enzymes[89-90].

Hydroxycinnamic acids, especially ferulic acid, are the most important phenolic compounds to form cross-links in plant cell wall. Ferulic acid and *p*-coumaric acid are ester-linked to arabinoxylans in cereals and other grasses and ferulic acid is linked to pectins in some dicots[91]. Similarly, sinapic acid is thought to be bound to polysaccharides via ester-linkages[92]. These polysaccharides can potentially be developed into useful therapies for treatment of some diseases like diabetes, obesity, hyperlipoproteinemia, cancer and HIV[93]. The search for glucosidase inhibitors has yielded a number of chemically distinct inhibitors from microbes and plants[94-96]. Among them acarbose, voglibose and miglitol have been in clinical use. Most of them were isolated from microbial origins and so microorganisms are an important source for screening α-glucosidase and α-amylase inhibitors.

Acarbose is a powerful pseudotetrasaccharidic inhibitor of amylase and α-glucosidase[97-98]. The half-chair conformation of the cyclitol unit mimics the distortion expected in the transition state prior to hydrolysis. The enzyme activity is lost upon binding to the active site[49]. Acarbose may also bind to a secondary carbohydrate binding site to make another abortive complex[43].

The structure of PPA complexed with acarbose, a pseudotetrasaccharide[49] was determined by Fourier difference analysis and the interactions occurring at the active site were identified[99]. An additional surface carbohydrate binding site has been detected in the A_7A_8 region facing the C-terminal domain segment between β9 and β10 in the 4,4'-dithio-α-maltotrioside-PPA complex[21,100]. Two additional carbohydrate-binding sites have also been observed in the maltopentaose-PPA complex[101]. PPA is much more sensitive to subtilisin attack in the inhibitor-enzyme complex, which suggests that the conformation of PPA changes when complexed. A change in the three-dimensional structure has in fact been detected by Fourier difference analysis[49].

The modulation of postprandial hyperglycemia can be brought about by mild inhibition of the enzymatic breakdown of complex carbohydrates to decrease meal-derived glucose absorption. The significance of food-grade, plant-based amylase inhibitors for modulation of carbohydrate breakdown and control of glycemic index of foods in the context of preventing hyperglycemia and diabetes mellitus complications is hypothesized. PPA is a glycoprotein with a carbohydrate moiety anchored to the protein / enzyme molecule. Studying the differences in the glycosylated and deglycosylated forms of the glycoprotein (PPA, PPA-I and PPA-II) are very useful to monitor the effect of the bound carbohydrate on temperature stability and changes in spectral and circular dichroism (CD) spectra

Future perspectives

Future studies can be contemplated in (a) screening of the plant originated bioactive molecules such as oligosaccharides derived from dietary fibres, and polyphenolic compounds for their potential to inhibit α-amylases (b) studying the molecular mechanism by which these inhibitors inhibit α-amylases especially pancreatic α-amylases and brushborder α-glucosidase. These will facilitate in developing tailor made low glycemic foods which are the need of hour with ever increasing diabetic population all over the globe.

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