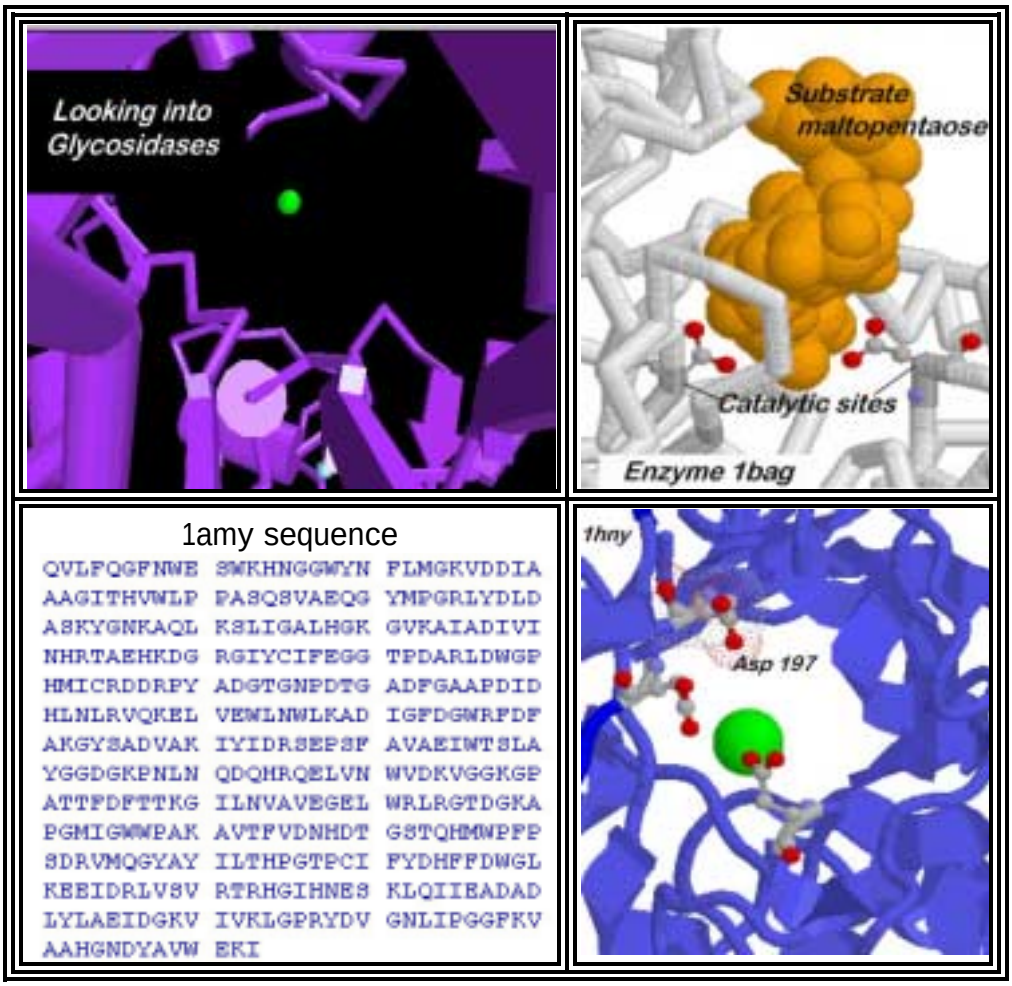

Ethel D. Stanley and Keith D. Stanley

Version 1.2 8/1/00

Looking into Glycosidases:
A Bioinformatics Resource for Biology Students

Information technology and escalating research in computational molecular biology are changing what it means to be biologically literate in the 21st century. Developing an appreciation for this wealth of molecular data and methodologies may seem a Herculean task. However, current issues such as antibiotic resistance, GM foods, evolution education, global demographics, environmental risks, and emerging diseases provide rationales for doing so.

Utilizing strategic molecular investigations, bioinformatics, and visualization tools in undergraduate biology is supported here by a number of scenarios for investigation. Several introductory molecular problem spaces are featured with appendices on the glycosidases, resources, internet tools, and selected literature. NOTE: None of these scenarios comes with a solution. We generated many supportable hypotheses while working on the problems and hope you will enjoy similar success!

The scenarios involve one or more proteins from the same family of enzymes. Utilizing a "shared" chemistry for the glycosidases narrows the problem space for investigation and may help learners gain familiarity with critical features of these proteins. Although the molecular structure of the enzymes and their properties are shared, the enzymes are introduced within unique biological situations. Students who are involved in different investigations can share their own research literature on protein folding, catalytic sites, enzymatic mechanisms, or sequence homology. This collaboration between peers is not unlike the sharing of problems, resolutions, and resources found in scientific research. In addition, students can utilize this glycosidase information throughout the semester as they engage in sequential investigations or independent research.

The enzyme family for the glycosidases (glycoside hydrolases, glycosyl hydrolases, E.C. number 3.2.1.x) includes enzymes such as the alpha-amylases that are routinely studied and has members that represent both diverse and ubiquitous biological functions. All of these enzymes hydrolyze glycosidic bonds, but some are also multifunctional. Sequence data for many of the glycosidases are well described in terms of their functional roles (active sites and protein folding) and a great deal of research can be found on evolutionary relationships between these enzymes in different taxa.

Strategies for molecular investigation, search skills for accessing molecular resources, and familiarity with online tools for doing bioinformatics and visualization are indispensable for the 21st Century biology student.

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Scenario 1.

Glycosidases and the modification of corn starch …

In the commercial production of maltodextrins and corn syrups, starch is hydrolyzed using an alpha-amylase either alone or combined with other enzymes.

- Maltodextrins are partially hydrolyzed starches used in foods to modify physical properties that contribute little or no sweetness or flavor. Alpha-amylase is used to make this product.

- Corn syrups are used primarily to add sweetness or enhance flavors in food products. High dextrose syrup is made by hydrolyzing starch first with alpha-amylase, then with glucoamylase (amyloglucosidase) which cleaves both alpha-1,4 bonds and alpha-1,6 bonds. To increase the rate of alpha-1,6 bond cleavage, a debranching enzyme such as pullulanase may also be added.

  High fructose corn syrup is made by converting dextrose to fructose using glucose isomerase (not a glycosidase) to create an equilibrium mixture of dextrose and fructose (42%fructose). Higher fructose concentrations can be prepared by separating fructose from dextrose using chromatographic methods and large-scale ion exchange columns. Pure crystalline fructose is made this way.

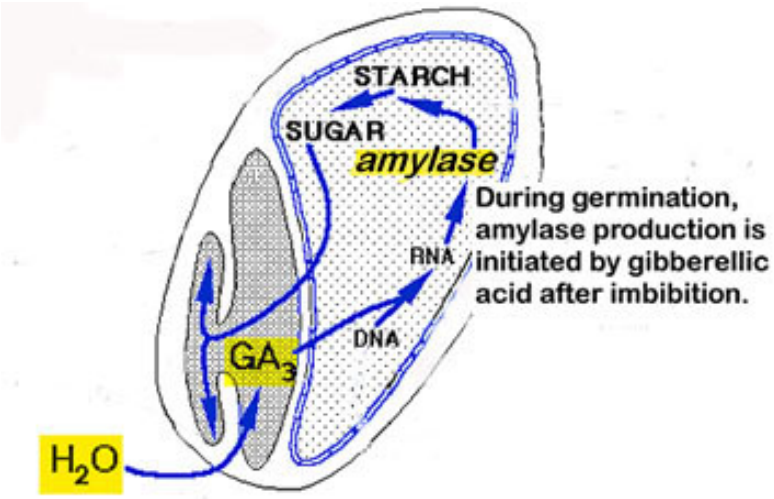
  - *List the organisms that are likely sources for each of the industrial enzymes above. Are all of these bacteria?*
  - *Choose a food product containing high-fructose corn syrup other than a can of pop. Create a poster showing the role of glycosidases in production of the product.*

See also: http://home3.inet.tele.dk/starch/ industrial starch processing

Scenario 2.

Amylases in the brewery

http://koning.ecsu.ctstateu.edu/Plant_Physiology/Seedgerm.html



Adding barley seeds to water is an important step in beer-making. The seeds germinate and maltose (a disaccharide) is eventually produced from starch by beta-amylase that is abundant in barley. The success of "malting" directly affects the resulting alcohol yield.

You are asked to increase productivity in a beer brewery.

Choose one of the following roles and related activity:

- *plant molecular biologist: sketch out your research plan to increase maltose production via barley plant genetics*

  http://www.css.orst.edu/barley/nabgmp/97/97sum.htm
  The North American Barley Genome Mapping Project

- *marketing team member: describe an advertising scheme you might use to dissuade criticism of GM(Genetically Modified) food*
- *environmental engineer: describe three environmental factors that impact the success of processing the beer,*
- *consumer advocate: provide a list of potential health and safety concerns*

List your resources for the work above.

Scenario 3.

Alpha-amylase inhibitors, weight loss, and beans

Any calories that are absorbed and are not used by the body for energy are stored as glycogen and body fat. A gram of starch, when digested and absorbed, provides 4 calories.

When trying to lose weight, dieters limit the amount of starch in their diet. The usual amounts of starchy foods, such as potatoes, bread, beans, corn and pasta, are reduced.

Starch provides from 500 to 700 calories per day in the average American adult diet. Individuals may consume as much as 1,500 or more calories per day from starch contained in their foods. However, starch is a large molecule that cannot be absorbed if it is not first broken down. Undigested starch will pass on through the digestive tract.

An over-the-counter product is Phase'oLean Starch Blocker.
http://www.uhs4u.com/lifeplus/phaseole.htm

Each tablet contains a minimum of 25,000 AIU's (alpha-amylase inhibiting units) consisting of unique plant extracts, including phaseotein from legumes. These extracts are said to inhibit the absorption of up to 100 grams of starch by blocking the enzyme alpha-amylase.

- *Would you take alpha-amylase inhibitor (AIU) tablets in order to lose weight? Explain.*
- *Do bean plants make the alpha-amylase inhibitor (AIU) phaseotein in order to lose weight? Provide an alternative explanation.*

Scenario 4.

Are plants really passive? Explore defense proteins in higher plants

See http://dmd.nihs.go.jp/latex/defense-e.html

Pathogen attacks, wounding, application of chemicals, air pollution, ultraviolet rays, and harsh growing conditions all may trigger defense responses in higher plants.

Proteins accumulated in seeds and fruits may provide defense against microbial pathogens and invertebrate pests as well as their storage function. These defense mechanisms are relatively conserved. Most plants either produce or accumulate similar proteins under certain situations. Proteins known to act defensively have been classified into several families based on sequence similarities, serologic or immunologic relationships, and enzymatic properties. Defense-related proteins are intensively studied by agricultural researchers.

Plant breeders see defense-related protein genes as a tool for the genetic modification of crops. Although these proteins act against microbial pathogens and invertebrate pests, they may also act as latex allergens.

- *Should we just test for latex allergenicity in GM (Genetically Modified) fruits and vegetables? Explain.*
- *Since human use of pollen in food is rare (e.g. saffron), should we be concerned about latex allergens in pollen?*

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[9] Hanninen, A.R., Mikkola, J., Kalkkinen, N., Turjanmaa, K., Ylitalo, L., Reunala, T. and Palosuo, T.: Increased allergen production in turnip (*Brassica rapa*) by treatments activating defense mechanisms., *J. Allergy Clin. Immunol.*, 104, 194-201 (1999).
[10] Salcedo, G., Diaz-Perales, A., Sanchez-Monge, R.: Fruit allergy: plant defence proteins as novel potential panallergens., *Clin. Exp. Allergy.*, 29, 1158-1160 (1999).

Web sites:

Plant Defense-Related Proteins as Latex Allergens
Latex-Allergic People Cross-React to Many Plants
Latex Allergens
Latex Allergy Links - What's New!
Latex Allergy Links - US Government
Latex Allergy Links - Health Canada
Internet Symposium on Food Allergens - Links
Dermatology Links - Allergy/Latex Allergy (HAD)
Japanese Society of Latex Allergy (Japanese)
Latex Allergy Forum (Japanese)

Scenario 5.

Enzyme replacement therapy: Should you try increasing your own levels of alpha-amylase?

There are a number of over-the-counter products that contain enzymes that aid in the digestion of proteins, starches, fats and dairy foods. For example, Lactaid® contains the enzyme lactase for helping the digestion of dairy products.

- *Do you have any concerns about enzyme replacement therapy? Explain.*

Another commercial product, Digestol®, is advertised as an all-purpose digestive aid. http://www.kramerlabs.com/digesto.html

The product contains the following enzymes:

Enzyme	For	Amount
Papain	Protein	50mg
Bromelain	Protein	50mg
Lactase	Dairy	35mg
Amylase	Starch	25mg
Lipase	Fats	25mg

- *Support or reject claims made by the manufacturer on the efficacy of this diet aid. Provide evidence and identify your sources.*

Scenario 6.

Micro-ecology of San Francisco Sourdough

http://www.landfield.com/faqs/food/sourdough/faq/section-21.html

The yeast *Candida milleri sp. Nov.* and the dominant lactobacillus *Lactobacillus sanfrancisco sp. nov*. occur in a ratio of 1:100 in sourdough. (Sugihara) Maltose is released from starch through the action of amylase enzymes. Though most strains of yeast can metabolize maltose*, Candida milleri* cannot. As a result, maltose is available to the lactobacilli which have an absolute requirement for this sugar. Lactobacilli cannot utilize other sugars present in dough.

The yeast is able to utilize the other sugars present in dough, so the two organisms do not compete for a carbon source. In addition, the lactobacilli release glucose into the media while assimilating maltose. The yeast use glucose to boost their reproduction.

Lactobacilli secrete an antibiotic cycloheximide which "sterilizes" the dough since it kills many organisms. *Candida milleri* is resistant to cycloheximide. *Candida milleri* is also moderately tolerant to the acetic acid that the lactobacilli produce. Dead yeast cells provide a number of amino acids and fatty acids needed by the lactobacilli.

- *Make a diagram showing the relationships between Candida milleri and Lactobacillus sanfrancisco.*
- *What is the role of alpha-amylase in sourdough production?*
- *How does the L. sanfrancisco alpha-amylase differ from your own salivary amylase? Structural and functional differences?*

Scenario 7.

Allergic to your breakfast cereal?
Is the alpha-amylase inhibitor in wheat the culprit?

http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?uid=9042052&form=6&db=m&Dopt=b

James JM, Sixbey JP, Helm RM, Bannon GA, Burks AW. 1997. Wheat alpha-amylase inhibitor: a second route of allergic sensitization. *Journal of Allergy Clinical Immunology.* 99(2): 239-44

Using serum samples collected from children with a known wheat allergy and one adult with baker's asthma, a wheat protein was identified which bound IgE. Control serum samples were collected from wheat-tolerant patients. No IgE binding to this wheat protein was demonstrated in any of the control subjects.

Samples representing the 15 kd wheat protein (isoelective point, 5.85) were selected and the N-terminal peptide sequence of this protein (residues 1 to 20) matched to a wheat alpha-amylase inhibitor.

- *Could you use this sequence to search for similar alpha-amylase inhibitors in other grains? Explain.*

- *Develop a poster for this research that would be appropriate for public education about wheat allergy at a children's health center.*

Scenario 8.

So what can I learn about biology from alpha-glucosidases?

Alpha-glucosidases are starch degradation enzymes that can hydrolyze various glycosidic bonds found in starch, maltose and even glycoproteins. These enzymes are found in a wide variety of organisms such as plants, fungi and mammals, but not in prokaryotes.

- *Why don't prokaryotes have alpha-glucosidases?*
- *Are plants, fungi and mammals more closely related to each other than to prokaryotes?*

Arabidopsis thaliana, is a well-known plant with a short life cycle and small genome(first plant genome to be completely sequenced). Working with *Arabidopsis thaliana*, Monroe (1998) identified 3 different forms of the enzyme located in the endoplasmic reticulum, the apoplast (outside the plasma membrane) and the chloroplast.

- *Why are alpha-glucosidases found in subcellular structures where starch is not found?*
- *Does the molecular structure vary for alpha-glucosidases found in different parts of the cell?*
- *Is there a relationship between molecular structure and physiological function of various forms of alpha-glucosidase?*

Other studies have reported that acidic alpha-glucosidase could prevent or delay infection by fungal conidia.

- *Suggest how the acidic alpha-glucosidase enzyme could act as a fungicide.*

Scenario 9.

Genetic disease and the human alpha-glucosidase gene

Glucose is a major source of energy for the body. It is stored in the form of glycogen in both the liver and muscles and later released with the help of enzymes. Persons affected by glycogen storage disease (GSD) have an inherited defect in one of the enzymes responsible for forming or releasing glycogen as it is needed by the body during exercise and/or between meals. There are eleven types of GSD known at this time.

Read the following brochure written by a mother whose son inherited an infantile form of Pompe's Disease which reduces glycogen storage function to less than 2% of normal. This is an autosomal recessive disorder that is always fatal.

POMPE'S DISEASE: A Guide for Families

http://www.agsd.org.uk/

- *Construct a family pedigree to use to explain the genetic basis of this disorder.*
- *Choose two known alpha-glucosidase mutations and explain why the enzyme doesn't function normally.*

Resources

Clinical Genetics Site:

http://www.eur.nl/FGG/CH1/pompe/

NiceZyme View of ENZYME: EC 3.2.1.3

http://www.expasy.ch/cgi-bin/nicezyme.pl?3.2.1.3

GSD II Database: A register of mutations in Human acid alpha-glucosidase

http://www.eur.nl/FGG/CH1/pompe/mutation.htm

Note: Names used for this disease:

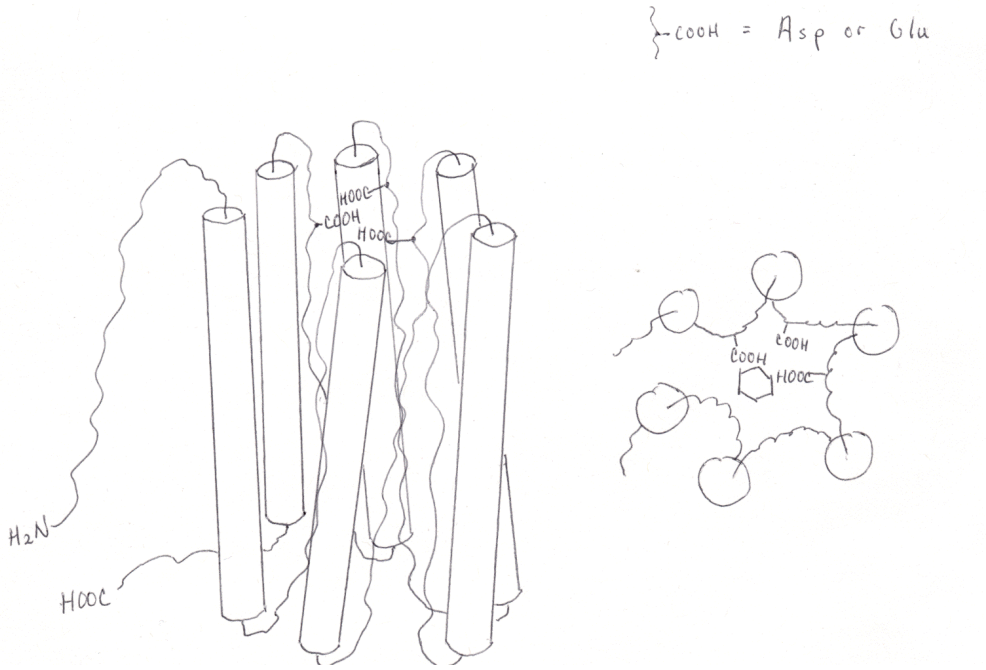
Glycogen Storage Disease Type II (GSD II)
Acid Maltase Deficiency
Pompe Disease
Lysosomal alpha-glucosidase Deficiency

Appendix A: Getting Started with Glycosidases

What does a glycosidase look like?
How does a glycosidase break down starch?
Conservation of active sites
Notes on a bioinformatics approach in industry including the use of sequence data to look for similar structure and function in other proteins.

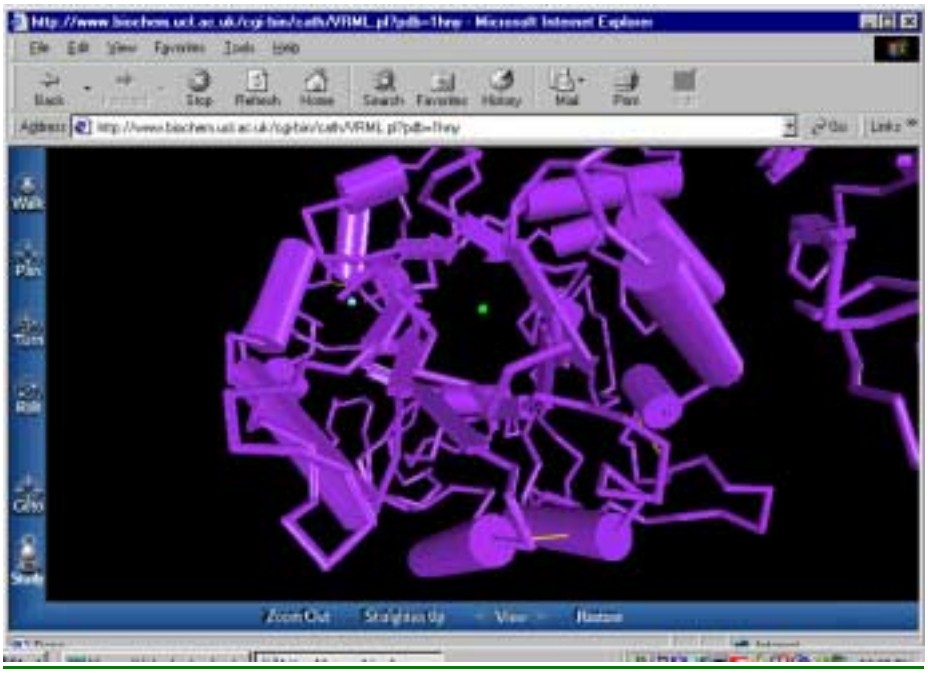
What does a glycosidase look like?

The alpha-amylases contain eight alpha-helices and eight beta-strands in beta alpha/beta alpha order. Only six of each are shown in the following illustration for simplicity.



Beta-alpha barrel structure

The alpha-helices provide rigidity to the catalytic sites and substrate binding sites which are contained within the beta-strands.



PDB file: View down the substrate binding site of the amylase 1hny

See also: Alpha/Beta Topologies
http://www.cryst.bbk.ac.uk/PPS95/course/8_folds/alph_bet_wnd.html#barrels

How does a glycosidase break down starch?

If we look at the alpha-amylase enzyme, we can find both the catalytic sites and the substrate binding site. The amino acid sequence of alpha-amylases may vary, but there are specific aspartic acid and glutamic acid units found in the beta-strand region of alpha-amylases responsible for the catalysis of glycosidic bond cleavage. Other amino acid units such as histidine shown in step 1. of the following starch hydrolysis are necessary for enzyme activity involved in establishing conformation and binding of the substrate.

Steps in enzymatic hydrolysis of starch.

1. Acid catalyzed nucleophilic displacement. One aspartic acid acts as the nucleophile, while the other aspartic acid is the acid catalyst. Note that His 296 and His 122 both form hydrogen bonds to the substrate to hold it in place.

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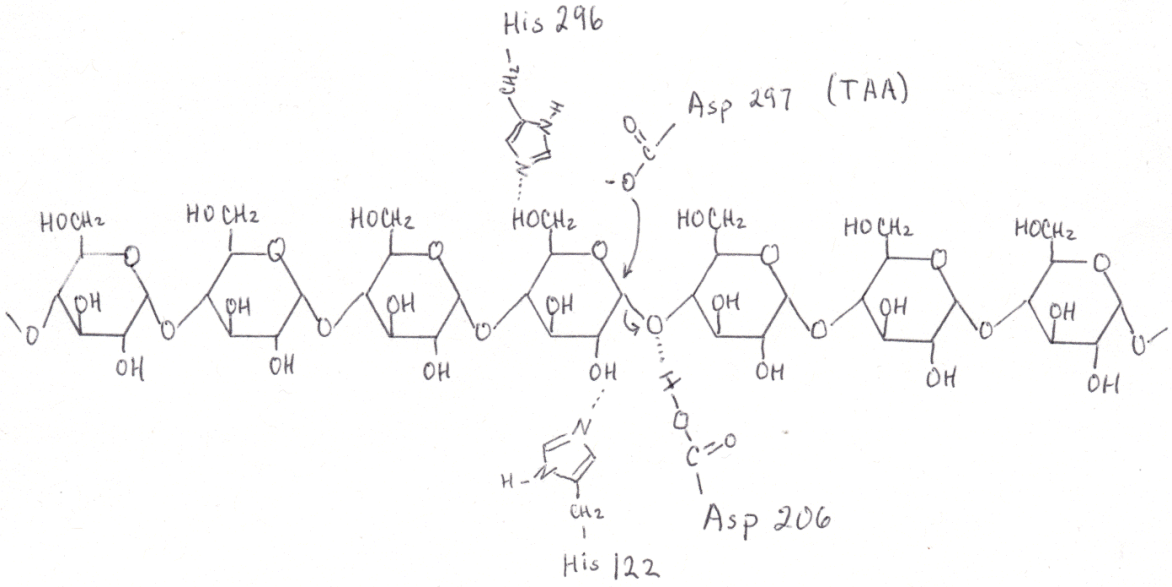
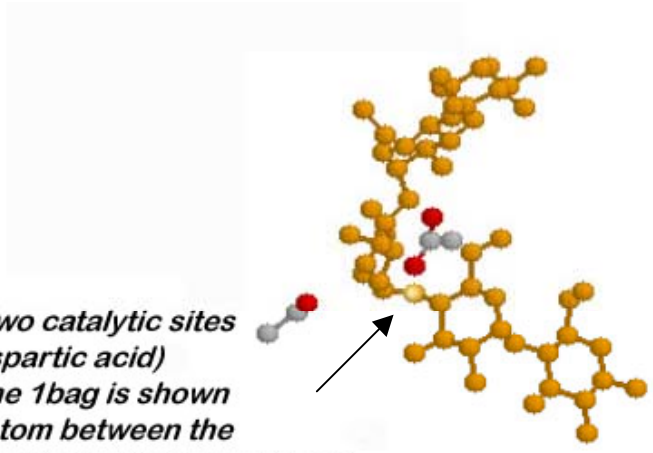
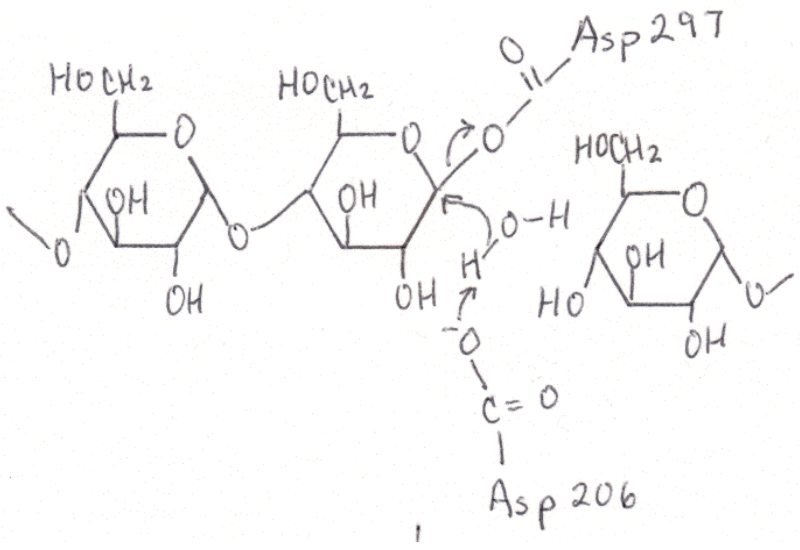


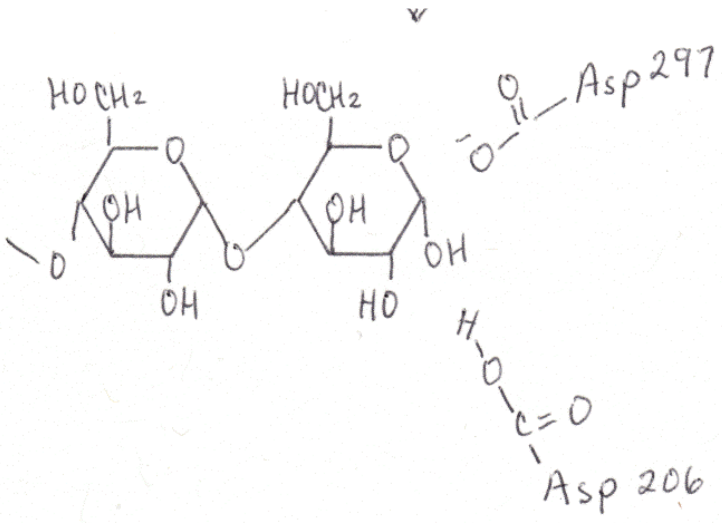


One of the two catalytic sites (both are aspartic acid) in the enzyme 1bag is shown near the O atom between the rings in the substrate maltopentaose. The rest of the enzyme is hidden.

2. Acid catalyzed hydrolysis of the link between the substrate polysaccharide and the enzyme (a carbohydrate protein ester link).

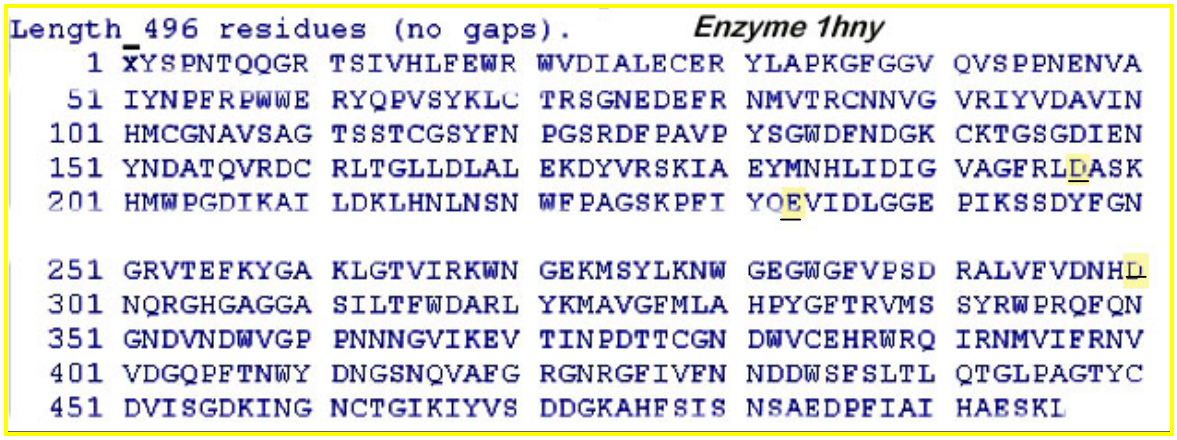


3. The end products are the two fragments of the substrate polysaccharide and the freed enzyme.

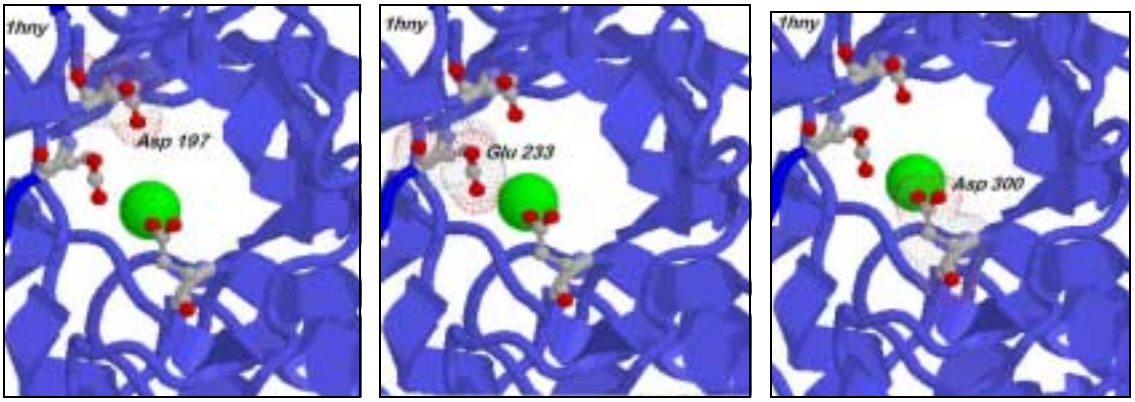


Conservation of active sites and catalytic sites

Not only alpha-amylases, but also beta-amylases and starch debranching enzymes such as the pullulanases and isoamylases also contain the beta/alpha barrel domain including the same catalytic amino acids. The mechanisms differ, but the relatedness of these enzymes is clear. Amino acid sequences of the beta-strands are well conserved within this family of enzymes. This provides a rationale for using the glycosidases to investigate the evolutionary relationships between organisms.



Highlighted amino acid sequences **D** (Aspartic acid 197), **E** (Glutamic acid 233) and **D** (Aspartic acid 300) are the catalytic sites.



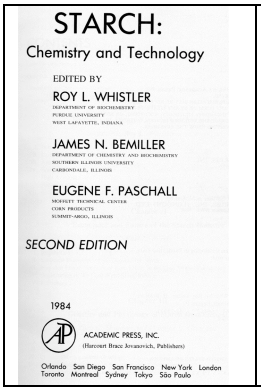
Catalytic sites Aspartic acid 197, Glutamic acid 233, and Aspartic acid 300 in the alpha-amylase 1hny. Note the green Chloride ion near the sites.

Notes on a bioinformatics approach in industry including the use of sequence data to look for similar structure and function in other proteins…

To an industrial organic chemist specializing in starch modification for food products, bioinformatics is a routine part of the work. Major steps in this process include:

1. Literature review

   Find out what structural information such as molecular weight, active sites, 3-D structure, and substrate interaction site (where the enzyme attacks the substrate) is available for a specific starch hydrolyzing enzyme. References available on site probably include "industry standards such as:



Starch: Chemistry and Technology. Whistler, BeMiller & Paschall, Eds. 1984. San Diego: Academic Press.

Starch: Properties and Potential, Galliard, Ed. 1987. New York: John Wiley & Sons.

See also: http://home3.inet.tele.dk/starch/
International Starch Institute

Additional literature searches such as NERAC professional searches are also routine.

2. Using the research literature above, correlate the amino acid sequence with active sites and 3D structure

   - establish location of the amino acids that control both the catalytic sites and the substrate binding sites

3. Compare sequence data and test for homology in order to:

    - determine if there are other enzymes with similar sequences
    - look for new sources of an enzyme with similar activity
    - determine sequences that are responsible for desired physical properties such as temperature stability, pH stability, and metal ion requirements (some need Ca ions).

4. Develop a genetic engineering strategy for generating "economically viable" enzymes. If an enzyme of interest is in a "bug" that is difficult to culture in sufficient quantities, investigate the potential of cloning by inserting nucleic acid sequence (DNA) for the enzyme into more easily cultured "bug" already in production.

Appendix B: Tools for molecular investigation and the visualization of enzymes

Looking for enzymes… it's so E.C.
Visualization: See more with pdb files
Protein Explorer: Seeing is believing!
Biology Workbench provides super fast multiple access and saves your sessions with data sets

It's so EC…

Enzyme functions are classified by **E.C.** numbers:

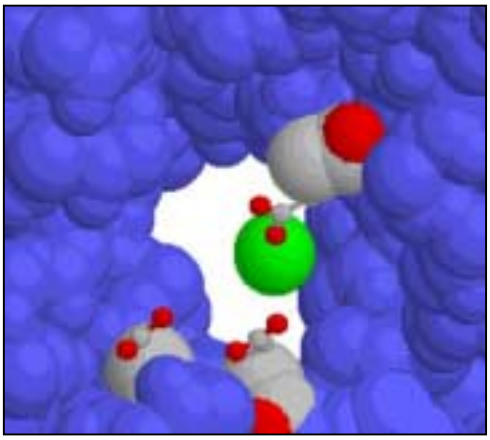
- E.C.1. *Oxidoreductases.* [**1013** PDB entries]
- E.C.2. *Transferases.* [**1370** PDB entries]
- E.C.3. *Hydrolases.* [**3035** PDB entries]
- E.C.4. *Lyases.* [**400** PDB entries]
- E.C.5. *Isomerases.* [**299** PDB entries]
- E.C.6. *Ligases.* [**140** PDB entries]

(Enzyme Data Bank, v.25.0. July 1999)

Solving the problem of synonyms: Besides providing essential information on enzyme classification, E.C. numbers are very useful for doing searches when variations of enzyme names are encountered. Listed below are the E.C. numbers of several starch hydrolyzing enzymes further characterized by their mode of action:

- Alpha-amylases (EC number 3.2.1.1) hydrolyze starch by cleaving alpha 1,4 linkages randomly within the chain (endo mechanism)
- Beta-amylases (EC number 3.2.1.2) hydrolyze starch by cleaving alpha 1,4 linkages producing maltose units from the non-reducing end (exo mechanism)
- Amyloglucosidases (EC number 3.2.1.33) hydrolyze starch by cleaving glucose units from the non-reducing end (exo)
- Pullulanases (EC number 3.2.1.41) and isoamylases (EC number 3.2.1.68) are debranching enzymes that hydrolyze starch by cleaving alpha 1,6 linkages (specific so not referred to as either endo or exo)

Visualization: See more with pdb files



View down the substrate binding site of the amylase 1hny

Structural data files for many of the glycosidases are readily available from the Protein Data Bank. All pdb files have unique 4 character names that include numbers and letters. The Protein Data Bank Education page provides a good introduction to the international repository for 3-D molecular structure data. It is found at: http://www.rcsb.org/pdb/education.html

"Our vision is for the PDB to enable scientists worldwide to gain a greater understanding of structure-function relationships in biological systems," Helen Berman, Rutgers, is principal investigator for the PDB project.

http://www.biochem.ucl.ac.uk/bsm/pdbsum/viewers.html
PDB Viewers, RasMol program and VRML browser

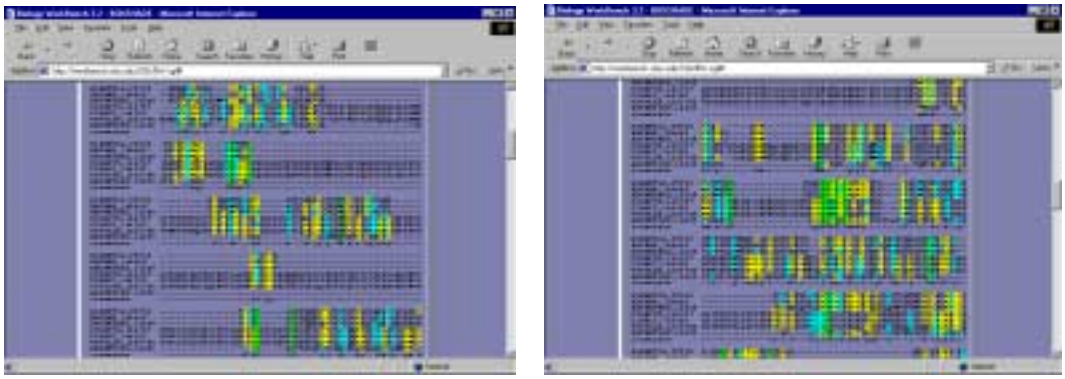
http://www.biochem.ucl.ac.uk/bsm/pdbsum/index.html
PDBsum - Summaries and structural analyses of PDB data files

Protein Explorer

http://www.umass.edu/microbio/chime/explorer/
"Protein Explorer can make visual exploration of protein structure much more accessible to novices, occasional users, or nonspecialists, as well as making it much more convenient than RasMol, even for experts."

You can use this viewer by directly entering the pdb file name or by setting up a web page of pdb file name links that you are interested in. This viewer can also be used off line with downloaded pdb files.

- A web resource helpful for getting started with the glycosidases: http://bioquest.org/amylase)



Investigating possible sequence homology using Boxshade

Biology Workbench http://workbench.sdsc.edu/

The Biology WorkBench is a web-based resource containing a suite of bioinformatics tools for analyzing and visualizing molecular data. It was developed at the National Center for Supercomputing Applications (NCSA), now undergoing continued development at the San Diego Supercomputer Center. Any computer with access to the Internet can use the Biology WorkBench to search large public domain databases (like Genebank); compare molecular sequences (for example building multiple sequence alignments); visualize and manipulate molecular structures (such as viewing protein secondary structures); and generate phylogenetic hypotheses (for instance building phylogenetic trees).

The Biology WorkBench is innovative because it integrates access to many tools within a simple graphical user interface. As a web-based resource, the Biology Workbench overcomes platform incompatibility issues and concerns about local computing power. Biology students and instructors can use the tools of bioinformatics to investigate a wide range of biological concepts. Building biological meaning from molecular sequence data requires access to rich data sources, powerful analysis tools and concrete biological questions that will drive investigations.

The Biology WorkBench Investigation Portal introduces multiple resources and tutorials for students and instructors at:
http://glycine.ncsa.uiuc.edu/educwb/index2.html

Here are three problems that you can use Biology Workbench to investigate:

Problem 1.

Since the amino acid sequences of the beta-strands of the beta alpha barrel are well conserved within the glycosidases, a rationale for using the glycosidases to investigate the evolutionary relationships between organisms is supported.

- Use one or more glycosidases to probe evolutionary relationships between major phyla of your choice.

Problem 2:

WITH OR WITHOUT INTRONS: THE AMYLASE ALTERNATIVE (1996)

http://www.cnrs.fr/Cnrspresse/en34a1.html

Did introns, the non-coding sequences in DNA, appear early or late over the course of evolution? Three researchers intrigued by this question studied the case of Drosophila genes for the enzyme amylase. Marie-Louise Cariou and Jean-Luc Da Lage, respectively Director and Researcher at the CNRS "Populations, Genetics and Evolution" Laboratory in Gif-sur-Yvette, working with Maurice Wegnez, Director of the "Development and Morphogenesis" Laboratory in Orsay (CNRS-University of Paris 11), discovered that various species of this fruit fly -- and even a single fly within the same species -- present amylase genes both with and without introns.

- *Use the intron sequence to probe mammalian alpha-amylases.*
- *Propose a methodology for investigating another known intron sequence using Biology Workbench.*

Problem 3: What are your chances of finding an alpha-amylase inhibitor in a haystack…

James JM, Sixbey JP, Helm RM, Bannon GA, Burks AW. 1997. Wheat alpha-amylase inhibitor: a second route of allergic sensitization. *Journal of Allergy Clinical Immunology.* 99(2): 239-44

Using serum samples collected from children with a known wheat allergy and one adult with baker's asthma, a wheat protein was identified which bound IgE. Control serum samples were collected from wheat-tolerant patients. No IgE binding to this wheat protein was demonstrated in any of the control subjects.

Samples representing the 15 kd wheat protein (isoelective point, 5.85) were selected and the N-terminal peptide sequence of this protein (residues 1 to 20) matched to a wheat alpha-amylase inhibitor.

- *Use this sequence to search for similar alpha-amylase inhibitors in other grains?*
- *If you were able to locate similar proteins, make a case for or against nucleic acid sequence homology.*

Appendix C: Selected web sites with information on starch hydrolyzing enzymes (glycosidases)

http://expasy.cbr.nrc.ca/prosite/

Prosite database of protein families and domains provides specific information on enzymes.

http://afmb.cnrs-mrs.fr/~pedro/CAZY/ghf.html

Glycoside Hydrolase Family Server

http://savba.savba.sk/sav/inst/ue/stefan/alamy/alamy.htm

ALAMY Alpha Amylase Database

http://www.biochem.ucl.ac.uk/bsm/pdbsum/1bag/main.html

PDB code: 1bag *Alpha-amylase from bacillus subtilis complexed with maltopentaose*

http://xtal1.sdsc.edu/pdbmirror/pdb25sp10/abstracts/Hasson.html

Understanding sequence relationships in enzymes families through comparison of active-site structures

http://www.csb.ki.se/users/xray/joyce.html

Structural and Thermodynamic Study of Molecular Adaptations in Thermostable Proteins

Hyperthermophiles are microorganisms that have their optimal temperature for growth above 80 °C and many of them thrive optimally even above the normal boiling point of water. In order to be able to survive and reproduce efficiently under these extreme conditions, hyperthermophiles must have developed mechanisms in order to stabilize their macromolecules from thermal inactivation and denaturation. In particular, enzymes and other proteins on the one hand should be flexible in order to perform their dedicated function, while on the other hand they should be sufficiently rigid in order to prevent thermal unfolding.

http://afmb.cnrs-mrs.fr/~pedro/CAZY/ghf.html

Glycoside Hydrolase Family Server

http://www.protomap-old.cs.huji.ac.il/Amino/Prosite/ByFamily/BETA_AMYLASE_1

Beta-amylase (EC 3.2.1.2) [1,2] is an enzyme that hydrolyzes 1,4-alpha-glucosidic linkages in starch-type polysaccharide substrates so as to remove successive maltose units from the non-reducing ends of the chains. Beta- amylase is present in certain bacteria as well as in plants. Three highly conserved sequence regions are found in all known beta-amylases.

http://www.worthington-biochem.com/manual/A/AA.html

[[Alpha]]-Amylase acts upon large linear polymers at internal bonds. The hydrolytic products have [[alpha]]-configuration. The activity is present in all living organisms, however the enzymes vary remarkably even from tissue to tissue within a single species.

http://www.biochemj.org/bj/331/0929/bj3310929.htm

Biochem. J. (1998) 331, 929–935 (Printed in Great Britain)

Protein heterogeneity of spinach pullulanase shows coexistence of interconvertible isomeric forms of the monomeric enzyme. Anette HENKER*, Ilka SCHINDLER*, Andreas RENZ† and Erwin BECK*[1]Purified pullulanase (EC 3.2.1.41) from spinach (*Spinacia oleracea* L.) chloroplasts separated into at least seven individual enzymically active proteins.

http://home3.inet.tele.dk/starch/

Industrial starch processing

http://www.biotech.iastate.edu/news_releases/Dec_19_96.html

Pruning Branches of Starch Molecules Could Stimulate New Growth in Corn Profits. Cheaper ethanol production and new starches for food and industrial uses could result from research at Iowa State University to modify the branches of starch molecules in corn.

http://www.public.iastate.edu/~pedro/glase/stack92-82.html

Abstracts of Papers and Patents (1982-1992) Keywords - "Glucoamylase" and "Amyloglucosidase" 1209 Entries at Stack-Serpukhov, Russia

http://www.agron.missouri.edu/cgi-bin/sybgw_mdb/mdb3/GeneProduct/167598

Gene Product alpha-dextrin endo-1,6-alpha-glucosidase

Synonyms

limit-dextrinase

pullulanase

starch debranching enzyme

amylopectin 6-glucanohydrolase

http://water-cooler.com/WC/patentviewer/patent-4657865.html

United States Patent 4,657,865 Takasaki April 14, 1987

Pullulanase-like enzyme, method for preparation thereof, and method for saccharification of starch therewith

http://www.munksgaard.dk/plantarum/abs/pp101310.html

Pullulanase in mung bean cotyledons.

http://www.worthington-biochem.com/manual/A/AA.html

Amylase, Alpha

http://xtal1.sdsc.edu/pdbmirror/pdb25sp10/abstracts/Hasson.html

Understanding sequence relationships in enzymes families through comparison of active-site structures

http://xtal1.sdsc.edu/pdbmirror/pdb25sp10/abstracts/Wexler.html

Use of bioinformatics for analysis of an outer membrane protein (HMP-1) isolated from *Bacterodes fragilis*, the anaerobe most commonly involved in clinical infections

http://www.ifas.ufl.edu/~jmfc/Starch.htm

STARCH SYNTHESIS IN MAIZE

http://www.jic.bbsrc.ac.uk/staff/alison-smith/amylopec.htm

The synthesis of amylopectin

http://home3.inet.tele.dk/starch/
International Starch Institute

http://www.cryst.bbk.ac.uk/PPS95/course/10_interactions/crapp.html
Glycosidases

http://www.biochem.osakafu-u.ac.jp/EC/ab-e.htm
University of Osaka Department of Applied Biochemistry

http://link.springer.de/link/service/journals/00253/bibs/4042001/40420051.htm
Applied Microbiology and Biotechnology Volume 42 Issue 1 (1994) pp. 51-56. General characteristics of thermostable amylopullulanases and amylases from the alkalophilic *Bacillus sp.*

http://csm.jmu.edu/biology/monroejd/localize.html
Localization of apoplastic alpha-glucosidase activity in crucifers.

http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?uid=9042052&form=6&db=m&Dopt=b
1: J Allergy Clin Immunol 1997 Feb; 99(2): 239-44Wheat alpha-amylase inhibitor: a second route of allergic sensitization.

http://www.talkorigins.org/faqs/behe/publish.html
The evolutionary history of the amylase multigene family in *Drosophila pseudoobscura*

http://www.css.orst.edu/barley/nabgmp/97/97sum.htm
The North American Barley Genome Mapping Project

Appendix D: Journal Articles

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