Examination of pancreas

The laboratory diagnostics of pancreas can be classified to:

- Examination of endocrine function
- Examination of exocrine function:
    - Demonstration of damage to acinar cells
    - Examination of secretion of pancreatic juice

The **endocrine part** of the pancreatic gland produces especially insulin, glucagon and somatostatin. Disorders of the endocrine pancreatic function are particularly relevant to diabetes mellitus (see that text for more information).

Examination of the **exocrine pancreatic function** is not very common. It is used especially in diagnostics of **chronic pancreatitis** and includes both **direct tests**, which measure activities of pancreatic enzymes (chymotrypsin, elastase) in stool, and **indirect tests**, based on administration of suitable substrates for the pancreatic enzymes and subsequent detection of their degradation products.

Biochemical examination of pancreas

A wide-spread injury to the pancreatic tissue is seen predominantly in **acute pancreatitis**. It is a life-threatening situation of medical emergency, in which the digestive enzymes of pancreatic juice become activated and digest the pancreatic tissue. The condition is typically triggered by overpressure in the common pancreatic and biliary ducts (cholelithiasis) and alcohol abuse.

The destruction of pancreatic acinar cells leads to spillage of their contents to the blood. High levels of pancreatic enzymes, especially **α-amylase** and **lipase** can then be found in the serum.

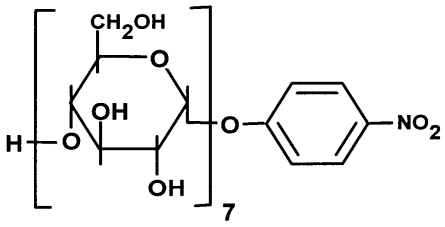
α-amylase

The α-amylase (α-1,4-glucan-4-glucanhydrolase, EC 3.2.1.1.) hydrolyses the α-1,4-glycosidic bonds in dietary starch and glycogen to smaller units such as dextrins, maltose and glucose. Its pH optimum is between 7.0 and 7.2. In the body it occurs in two isoforms: the ***salivary (S-amylase)*** and ***pancreatic (P-amylase) isoenzyme.*** Both forms differ in their sugar components and can be separated by electrophoresis, or by precipitation with a special lectin or antibody. The α-amylase is produced in the acinar pancreatic cells and accumulates in the zymogenic granules. It reaches the intestinal lumen in the pancreatic secretion (pancreatic juice) together with other digestive enzymes. Under physiological condition the enzyme molecule is not absorbed by the intestinal mucose and its serum level is low, corresponding to the activity of enzyme released to blood directly from the glandular cells or through the lymphatics. The relative molecular weight of α-amylase is about 55 kDa. It is removed from circulation by glomerular filtration in the kidney.

A macro-form of the enzyme (macroamylase) originates from binding of amylase to some proteins in the serum, especially immunoglobulins, circulating immunocomplexes or other glycoproteins. The macro-form has a much higher molecular weight (from 150,000 to 2,000,000) and therefore is not eliminated by glomerular filtration.

The laboratory estimation of α-amylase activity in serum and urine is one of the routine tests in clinical diagnostics.

The commonly used techniques for estimation of α-amylase are based on cleavage of chromogenic substrate. The early methods that used starch as natural substrate of α-amylase were difficult to standardize. Therefore, they were replaced with procedures employing synthetic substrates such as ***maltoheptaoside***, formed by a chain of seven glucosyl units. ***4-nitrophenol*** is attached to the ***reducing end*** of maltoheptaoside through α-glycosidic bond, and serves as a chromogen. In order to estimate separately the pancreatic isoenzyme of α-amylase, the salivary isoenzyme is first inhibited by a specific monoclonal antibody.



Maltoheptaoside with 4-nitrophenol attached

Reference values:

S-AMS total amylase in serum **0.30 – 1.67 µkat/l**
U-AMS total amylase in urine **< 7.67 µkat/l**

S-pAMS pancreatic amylase in serum **0.22 – 0.88 µkat/l**
U-pAMS pancreatic amylase in urine **< 5.83 µkat/l**

Macroamylase: physiologically undetectable

Evaluation of findings:

From a practical point of view, a significant finding is an increased activity of α-amylase in serum. It can be caused by:

- **increased release of amylase** from damaged cells of pancreas or salivary glands, or
- **decreased glomerular filtration**, when this small protein is excreted to urine less than usual

Hyperamylasemia due to **damage to pancreas or salivary glands** at normal renal function should be associated with an increased amylase in urine as well, albeit with a delay of several hours. If this is the case, it remains to be investigated whether the amylase comes from the pancreas, or rather from the salivary glands. Unless it can be figured out from the clinical symptoms, examination of isoenzymes provides the answer.

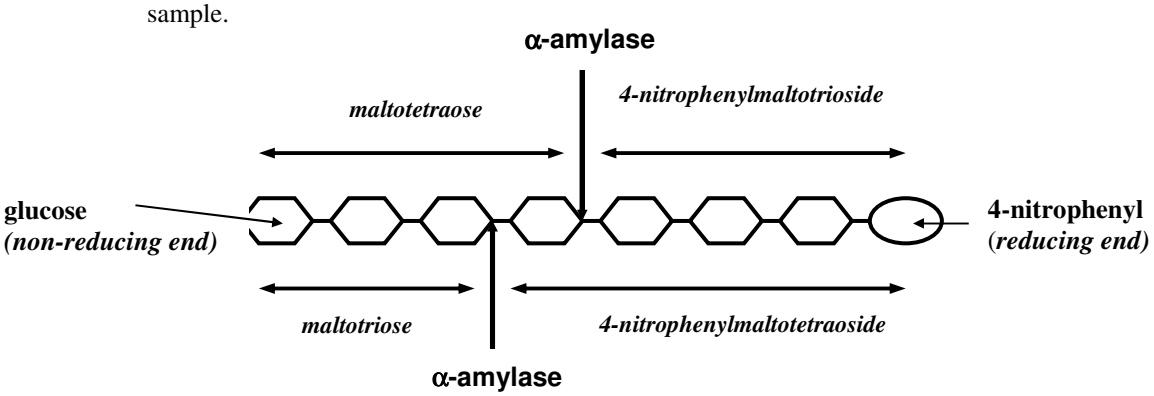
A **decreased glomerular filtration of amylase** is most often due to **renal insufficiency**. In this case hyperamylasemia is accompanied with a low concentration and activity of amylase in urine. Another, much rarer cause of a low renal clearance of amylase is **macroamylasemia**.

Cleavage of maltoheptaoside with α-amylase

Following reactions take place:

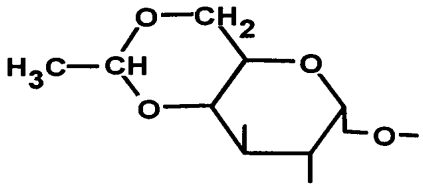
1) α-amylase cleaves internal bonds in the chromogenic substrate producing shorter oligosaccharides, such as 4-nitrophenylmaltotetraoside and maltotriose or 4-nitrophenylmaltotrioside and maltotetraose.

2) The subsequent cleavage of α-amylase hydrolysis products is performed by α-glucosidase, a 'helper' enzyme added to the reaction mixture. α-glucosidase hydrolyses all glycosidic bonds from non-reducing ends releasing molecules of free glucose and finally the yellow 4-nitrophenol, whose absorbance is measured. In the kinetic measurement the increase in 4-nitrophenol concentration per unit of time is directly proportional to the catalytic concentration of α-amylase in the analyzed sample.



Blocking the non-reducing end of synthetic substrate with ethylidene:

In order to prevent uncontrolled premature action of α-glucosidase, the substrate has its non-reducing end 'blocked' with ethylidene, which connects fourth and sixth carbons of the terminal glucose. This modification renders the non-reducing end of the substrate inaccessible to α-glucosidase. Only after the hydrolysis by α-amylase the resulting 4-nitrophenyl-containing fragments have their non-reducing ends unprotected and can be cleaved by α-glucosidase.



Lipase

Lipase (triacylglycerol acylhydrolase, EC 3.1.1.3.) is a newer and more specific marker of pancreatic injury than amylase. It is a glycoprotein consisting of 420 – 449 amino acyl residues; the relative molecular weight is 46,000 – 56,000 for pancreatic lipase and 32,000 – 39,000 for serum lipase.

Lipase is a hydrolytic enzyme that cleaves triacylglycerols with fatty acids longer than 12 carbons. In the presence of bile acids it cleaves fat to monoacylglycerols and diacylglycerols. The fatty acids in positions *sn*-1 and *sn*-2 are cleaved preferentially. Like the α-amylase, the lipase is produced by the glandular cells of the pancreas and secreted to the intestinal lumen with pancreatic juice. Concentration gradient of lipase between the pancreatic tissue and serum is about 20,000:1.

Estimation of lipase activity involves various approaches:

- Enzymatic cleavage of natural substrate
- Enzymatic liberation of chromogenic and fluorogenic substrates
- Immunological methods (ELISA, latex-agglutination)

Nephelometric and turbidimetric procedures based on cleavage of the natural substrate triacylglycerol are used most often. Majority of the kits for lipase estimation contains the co-lipase as well. The turbidimetric estimation of lipase activity is based on clearing of the oil emulsion by action of the lipolytic activity. However, the process can be affected also by other serum components, e.g. by a clearing factor known as "pseudolipase." It consists most often from circulating immunocomplexes of the IgM type. A procedure for turbidimetric estimation of pancreatic lipase in the presence of pseudolipase has been developed in which the pseudolipase is inactivated by addition of β-mercaptoethanol, which leads to dissociation of the IgM complexes.

There are also new chromogenic tests for lipase activity that are based on an enzymatic cascade of lipase cleaving 1,2-diacylglycerol, glycerol kinase, glycerol 3-phosphate oxidase and peroxidase with a chromogenic product. Another, completely new approach to the pancreatic lipase estimation is based on a change of solution conductivity due to release of fatty acids from the substrate triolein; it is detected by an acoustic sensor and the measured variable is a frequency response.

The **increase in serum concentration of pancreatic lipase** is a more specific marker of acute pancreatitis than α-amylase. Its level remains elevated about two weeks following the acute episode. It is not substantially increased in renal diseases.

According to:

Kocna, P.: Miniencyklopedie laboratorních metod v gastroenterologii
http://www1.lf1.cuni.cz/~kocna/glab/glency1.htm

Wikiskripta: Biochemická vyšetření pankreatu
http://www.wikiskripta.eu/index.php/Biochemick%C3%A1_vy%C5%A1et%C5%99en%C3%AD_pankreatu