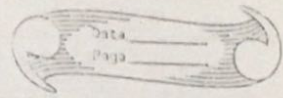


* Amylase *



→ Hydrolase class enzyme.

↓
Catalyze the hydrolysis of α -1,4 glycosidic linkage of polysaccharide.

→ Ca^{+2} metalloenzyme → Ca^{+2} ess. for activity.

→ does not attack on α , 1,6 linkage.

→ straight chain polyglycans
Amylase → maltose + glucose

→ glycogen/ Amylopectin
Amylase → maltose + glucose + limit dextrins

↓
Branch chain polyglycan

→ Various anion → chloride, bromide

↓
activators of enzyme (How?)

→ optimum pH for activity : 6.9 to 7.0

→ Amylase is very small

↓
So it can pass through glomeruli

↓
Only plasma enzyme found in urine

⊕ ↓ p-type + s-type (pancreatic is found in urine)
salivary

→ Isoenzymes :-

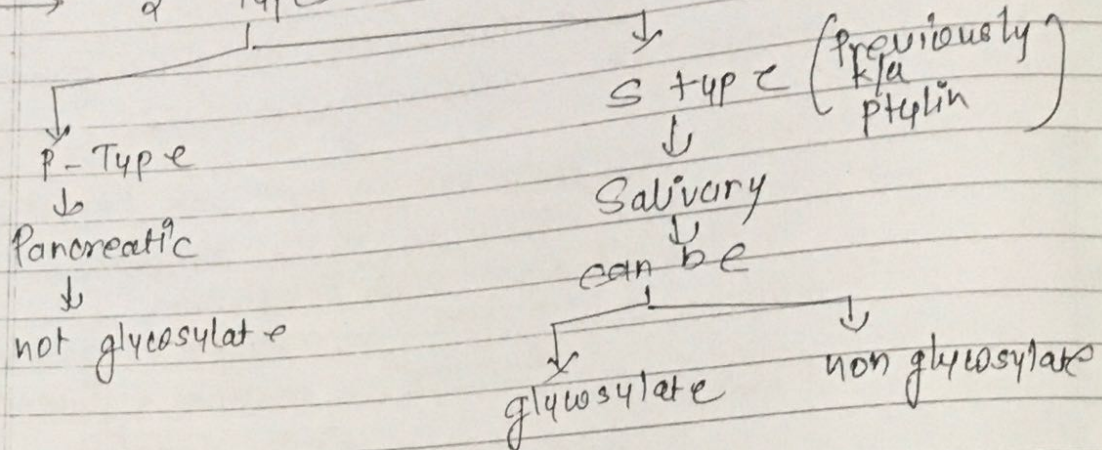
→ isoenzymes are product of 2 closely linked loci on chromosome 1.

→ Isoenzyme can also undergo posttranslational modification.

↳ like deamidation, glycosylation, deglycosylation.

→ Can be separated By Isoelectrophoresis/ electrophoresis

→ 2 Types



* Clinical Significance :-

→ ↑ in pancreatitis & Salivary gland inflammation

→ ① Acute pancreatitis :-

↓
rise in 5 to 8 hrs of onset of symptoms

↓
Activity returns to (N) by 3rd / 4th day

↓
4 to 6 fold elevation above Upper ref. limit

↓
Max. conc. attained in 12 to 72 hrs

→ magnitude of elevation of enzyme is not related to severity of pancreatic involvement.

→ greater the rise → greater the probability of Acute pancreatitis.

→ As \uparrow serum conc.

↓

It also leads to \uparrow urinary Amyl level.

→ Compared to serum, urinary level remains high for longer duration.

→ More accurate to measure P-Amyl than Total Amyl. $\$$

(2) Biliary tract d'se :-
such as cholecystitis

↓

\uparrow P-Amyl due to 2^o involvement of Pancreas

(3) Various Intra-abdominal events :-

↓

\uparrow of Amyl due to leakage of P-Amyl from intestine into peritoneal cavity & then into circulation.

(4) Renal Insufficiency :-

↓

\uparrow in proportional to extent of renal impairment

(5) Neoplastic d'se :-

like tumor of lung, Ovary (S-Type \uparrow)

⑥ Macroamylasemia :-

↓
Ordinary Amy complexes to IgG/IgA

↓
Forms macroamylase (mainly s-Amy)

↓
can not filter through kidney
as ↑ size

↓
So retained in plasma

⇒ ↓ P-Amylase level :-

↓
① Pancreatic Insufficiency
→ P-Amy highly specific for it.

* Methods :-

① Reference method :-

IFCC 4-NP-G7 (4-nitrophenyl maltoheptaosyl)
at 37°C :-

~~5-ethylidene~~ ethylidene-4-NP-G7 + 5H₂O $\xrightarrow{\alpha\text{-amylase}}$

2-ethylidene-G₅ + 2NP-G₂

2-ethylidene-G₄ + 2NP-G₃

2-ethylidene-G₃ + 2NP-G₄

2NP-G₂ + 2NP-G₃ + 10H₂O $\xrightarrow{\alpha\text{-glucosidase}}$
4 4-NP + 10G

as NP-G₄ is not acted upon by α -glucosidase

→ Formation of 4-NP- α_2 is 90%
4-NP α_3 is 31%.
4 NP α_4 is 60%.

→ $\alpha_6, \alpha_7, 4NP\alpha_6, 4NP\alpha_5$ are not produced in appreciable amount.

→ Free NP is detected by its absorbance at 405 nm.

→ α -glucosidase does not react to any oligo-~~glucose~~-saccharide containing more than 4 glucose molecules in the chain.

→ Poor stability of reconstituted assay mixture

↓
As mixture contains 4-NP-glycoside & α -glucosidase

↓
Because slow hydrolysis of 4NP-glycosides by α -glucosidase

↓
This effect has been reduced by covalently linking a "Blocking" group

↓
Blocking groups ~~are~~ ^{is} substance like ethylidene-protected substance (EPS)

↓
So α -glucosidase now can't act on ~~EPS~~ ^{EPS}-NP-glycoside.

↓
It also shows diff. & advantageous hydrolytic product, α

it produces ↓
4-NP- α_2 - 40%
4-NP- α_3 - 40%
(4-NP- α_4 - 20%) → not acted upon by β -glycosidase.

(d) CNP- α_3 method :-

- d-chloro - p-nitrophenol maltotriose method.
- direct assay.

* Sample type :-

→ use only serum / heparinized plasma

→ don't use EDTA, citrate buff

↓

as they chelate Ca^{2+}

↓

C is required for activity of amylase.

→ Very stable enzyme, even at room temp.

↓

retain its activity for 4 day for $37^{\circ}C$.

* Ref. Interval :-

31 to 107 U/L

→ S. P. Amy activity is not demonstrable in most children younger than 6 months.

↓

reach to adult conc. at 5 yrs of age ↓

reflects postnatal development of pancreas.

→ So In children → use lipase than Amylase.

* Methods for Amylase Isoenzyme :-

① Double monoclonal Ab Assay :-

↓
2. Immuno-inhibitory monoclonal Ab to
S-Amy is added

↓
S-Amy is inhibited But P-Amy
is not inhibited

↓
P-Amy activity is measured by
using EPS-4-NP- K_7 as a substrate.

Q.