* 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
→ Amylose (→ straight chain polyglycans) —Amylase→ maltose + glucose
→ glycogen/Amylopectin —Amylase→ maltose + glucose + limit dextrins
(Amylopectin → Branch chain polyglycan)
→ Various anion → chloride, bromide
↓
activators of enzyme (CHOW?)
→ optimum pH for activity : 6.9 to 7.0

→ Amylase is very small
↓
So it can pass through glomerulo
↓
Only plasma enzyme found in urine
(↓ P-type & Pancreatic is found in urine; S-type Salivary)

→ Isoenzymes :—

→ isoenzymes are product of 2 closely linked loci on chromosome 1.
→ Isoenzyme ~~is due to~~ Can also undergo post translation modification
↳ like deamida", glycosyla", deglycosylation.

→ Can be seperated By Isoelectrophoresis/ electrophoresis

→ 2 Types

- P-Type → Pancreatic → not glycosylate
- S type (previously k/a ptylin) → Salivary → can be → glycosylate / non glycosylate

* Clinical Significance :-

→ ↑ in pancreatitis & Salivary gland inflammation.

→ ① Acute pancreatitis :-

↓

rise in 5 to 8 hrs of onset of symptoms

↓

activity returns to ⓦ 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 dise → greater the probability of Acute pancrealitic.

→ As ↑ serum Conc.
↓
it also leads to ↑ urinary Amyl level.

→ Compared to serum, urinary level remains high for longer duration.
→ More accurate to measure P-Amy than Total Amyl.

② Biliary tract d'se :-
such as choleqystitis
↓
↑ of Amy due to 2° involvement of Pancreas

③ Various Intra-abdominal events :-
↓
↑ of Amy due to leakage of P-Amy from intestine into peritoneal cavity & then into circulation.

④ Renal Insufficiency :-
↓
↑ in proportional to extent of Renal impairement

⑤ Neoplastic d'se :-
like tumor of lung, Ovary (S-Type ↑)

⑥ Macro Amylasemia :—

↓

Ordinary Amy complexes ∝ IgG / IgA

↓

forms macro Amylase (mainly S-Amy)

↓

∴ can not filtes through kidney as ↑ insize

↓

So retained in plasma

⟹ ↓ P-Amylase level :—

↓

① Pancreatic Insufficiency.

→ S-Amy highly specific for it.

✱

✱ Methods :—

① Reference method :—

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

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

2-ethylidene-G5 + 2 NP-G2

2-ethylidene-G4 + 2NP-G3

2 ethylidene-G3 + 2NP-G4

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

4 4-NP + 10 G

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

→ formation of 4-NP-G_2 is 9%
4-NP G_3 is 31%.
4 NP G_4 is 60%.

→ G_6, G_1, 4NP-G_6, 4NP G_5 are not produced in appreciable amount.

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

→ α-glucosidase does not react ~~c~~ any oligo-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 4 NP-glycosides By α-glycosidase

↓

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

↓

Blocking groups ~~are~~ substance like ethylidene-protected substance (EPS)

↓

so α-glucosidase now can't act on EPS-NP-glycoside.

↓

it also shows diff. & advantageous hydrolytic product, *

it produces ↓
4-NP-G_2 – 40%.
4-NP-G_3 – 40%.
4-NP-G_4 – 20% → not acted upon by β-glycosidase.

ⓓ CNP-G_3 method :–

→ 4-chloro-p-nitrophenol maltotriose method.
→ direct assay.

* Sample type :—

→ use only serum / heparinized plasma

→ don't use EDTA, nitrate bulb

↓

as they chelate Ca^{+2}

↓

Ca^{+2} is required for activity of amylase.

→ Very stable enzyme, even at room temp.

↓

retain its activity for 4 day for 37°C.

* Ref. Interval :—

31 to 107 U/L

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

↓

reach to adult conc. at 5 yrs of age

↓

reflects postnatal development of pancrease.

→ so In children → use lipase than Amylase.

* Methods for Amylase Isoenzyme :-

① Double monoclonal Ab Assay :-

↓

2 Immunoinhibitory 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-G7 as a substrate.

②.