RECENT STATUS ON CARBOHYDRATE METABOLIZING ENZYME INHIBITORS IN REGULATION OF DIABETES: A MECHANISM BASED REVIEW

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The important therapeutic approach for treating type 2 diabetes mellitus is to decrease the post-prandial glucose levels which could be done by decreasing the absorption of glucose through the inhibition of the carbohydrates-hydrolyzing enzymes such as α-amylase and α-glucosidase present in the small intestinal brush border that are responsible for the breakdown of oligosaccharides and disaccharides into monosaccharide's and suitable for absorption. Inhibition of α-amylase generally considered as strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity. Among the marketed allopathic preparations carbohydrates-hydrolyzing enzymes Inhibitors like acarbose, voglibose etc delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise. Some of the plants are also considered as an important source of chemical constituent with potential for inhibition of α-amylase and can be used as therapeutic purposes. In this review our efforts have been devoted to explore the mechanism based carbohydrates-hydrolyzing enzymes Inhibitors for the regulation of diabetes.

Keywords: α- Glucosidase, α-amylase, Sucrase, Maltase, Diabetes

INTRODUCTION

Enzymes are biological catalysts which are important in digestion and for other biological reactions. Luminal digestion is mainly due to enzymes secreted by salivary glands, stomach and pancreas. Chemical degradation of food also occurs by hydrolytic enzymes present in the brush border of small intestine termed as membrane digestion.[1,2,3] The source and sites of various luminal and membrane bound digestive enzymes are illustrated in Fig. 1[5]

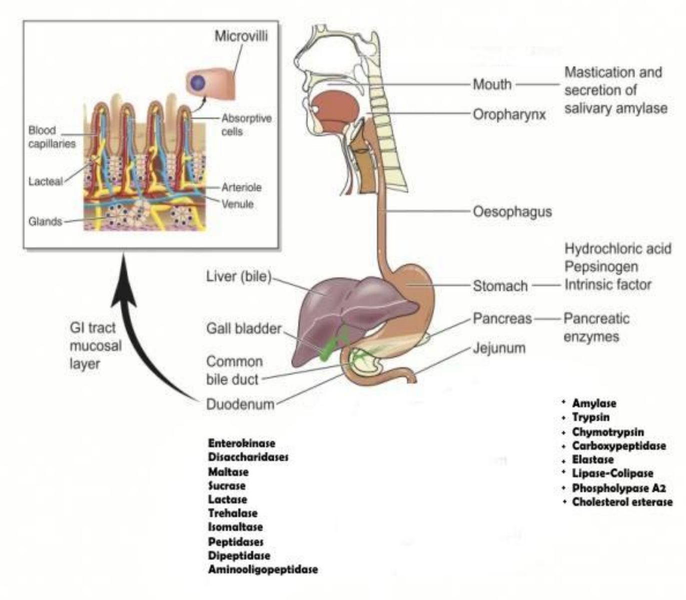

Fig. 1 Luminal and membrane bound digestive enzymes are illustrated

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METABOLISM OF CARBOHYDRATES

Distribution of glucose after a meal [5,6,]

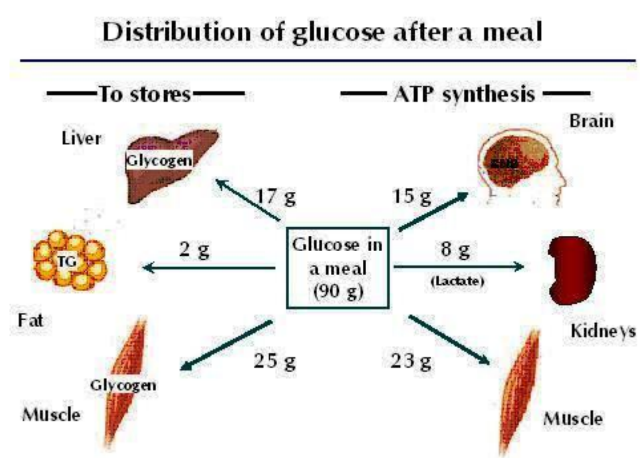

Fig. 2 Distribution of glucose after a meal

Carbohydrate digestion

Starch is composed of amylose, which is a linear alpha-1,4-linked glucose polymer, and highly branched amylopectin consisting of linear alpha-1,4-linked glucose chains with alpha-1,6-linked branch chains. Salivary and pancreatic alpha-amylases catalyze the endo-hydrolysis of alpha-1,4-glucosidic linkages releasing mainly maltose, maltotriose and related alpha-1,6-oligomers. Further digestion takes place in the small intestinal brush border by alpha-glucosidases, which hydrolyze the terminal alpha-1,4-linked glucose residues as the final step in the digestion of dietary carbohydrates to release glucose. The alpha-glucosidase activities, first described as maltases, are associated with maltase-glucoamylase and sucrase-isomaltase.

In addition to alpha-1,4-glucosidic activity, sucrase-isomaltase displays specific activities against the alpha-1,2 linkages of sucrose and alpha-1,6 linkages of is maltose.[7,8]

Digestion in Mouth

Digestion of carbohydrates starts at the mouth, where they come in contact with saliva during mastication. Saliva contains a carbohydrate splitting enzyme called salivary amylase (ptyalin).[9,10]

Action of ptyalin (salivary amylase)

It is α - amylase, requires Cl- ion for activation and optimum pH 6-7. The enzyme hydrolyzes α-(1,4) glycosidic linkage at random, from molecules like starch, glycogen and dextrins, producing smaller molecules maltose, glucose and disaccharides maltotriose. Ptyalin action stops in stomach when pH falls to 3.0[10,11]

α ***–Amylase***

Starch or glycogen ⟶ Glucose, Maltose, Maltotriose

Digestion in Stomach

No carbohydrate splitting enzymes are available in gastric juice. HCl may hydrolyze some dietary sucrose to equal amounts of glucose and fructose.

Digestion in Duodenum

Food reaches the duodenum from stomach where it meets the pancreatic juice. Pancreatic juice contains a carbohydrate-splitting enzyme pancreatic amylase.

Action of pancreatic Amylase

It is also an α - amylase, optimum pH 7.1. Like ptyalin it also requires Cl- for activity. The enzyme hydrolyzes α-(1,4) glycosidic linkage situated well inside polysaccharide molecule. Other criteria and end products of action are similar of ptyalin.[10,11]

Digestion in Small Intestine

Action of Intestinal Juice

Pancreatic amylase

It hydrolyzes terminal α-(1-4), glycosidic linkage in polysaccharides and Oligosaccharide molecules liberating free glucose molecules.

Lactase

It is a β- glycosidase, its pH range is 5.4 to 6.0. Lactose is hydrolyzed to glucose and galactose.

$$\textit{Lactose} \xrightarrow{\textit{lactase}} \textit{Glucose + Galactose}$$

Maltase

The enzyme hydrolyzes the α -(1,4) glycosidic linkage between glucose units in maltose molecule liberating two glucose molecules. Its pH range is 5.8 to 6.2. [11]

$$\textit{Maltose} \xrightarrow{\textit{Maltase}} \textit{Glucose + Glucose}$$

Sucrase

PH ranges 5.0 to 7.0. It hydrolyzes sucrose molecule to form glucose and fructose.[11]

$$\textit{Sucrose} \xrightarrow{\textit{Sucrase}} \textit{Glucose + fructose}$$

INSULIN

Insulin is a protein hormone secreted by β-cells of Islets of Langerhans of pancreas.[12]

Chemistry

- Insulin 51 amino acids in an insulin molecule.
- They are two chain Polypeptide .
  - Chain A-has-21 amino acids,
  - Chain B-had-30 amino acids.
- Both chains are connected by Disulphide Bridge.
- Half life of insulin - 4-6 minutes.[12,13,14]

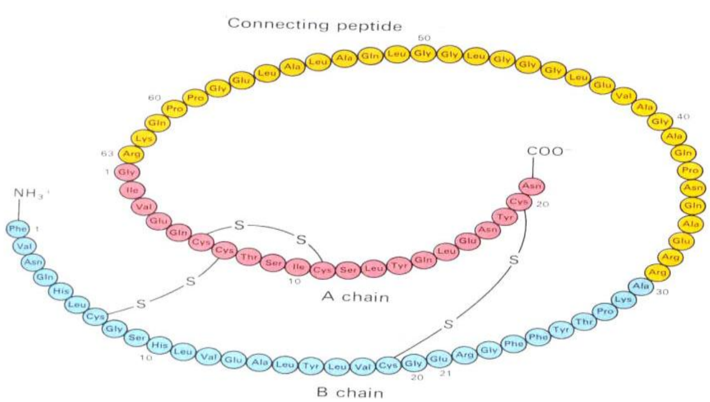

Fig. 3 Structure of Insulin

C peptide=31-65, A chain=66-86, B chain=1-30

Metabolic Role of Insulin

Carbohydrate metabolism: Insulin produces lowering of blood glucose and increases glycogen stores. This is achieved at several metabolic stages.

- There is increased uptake of glucose, galactose by various tissues like muscles, adipose, mammary glands etc .It is due to increased translocation of glucose transporters from Golgi to plasma membrane.
- Insulin induces the synthesis of glucokinase which phosphorylates and decreases the intracellular glucose in liver.
- Insulin enhances glycolysis by inducing the synthesis of phosphofructokinse and pyruvate kinase.
- Pyruvate dehydrogenase complex is activated via dephosphorylation of enzyme molecules which lead to increased production of acetyl- CoA from pyruvate.

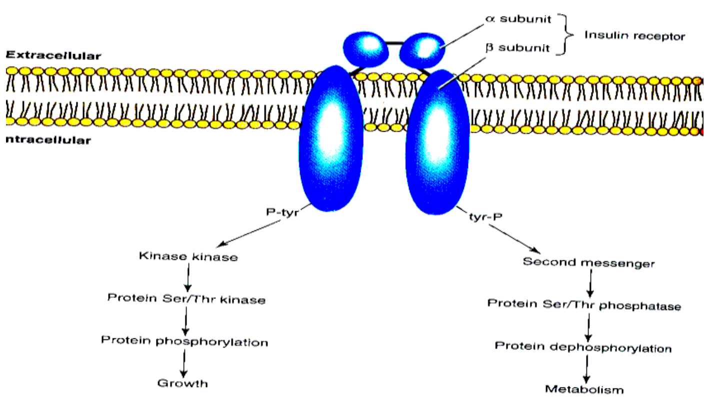

Fig. 4 Paradoxycal action of insulin

- Insulin stimulates protein phosphatase-1 which dephosphorylates and activates key enzyme glycogen synthase. This leads to increased synthesis of glycogen.
- Insulin reduces gluconeognesis by repressing at gene level, PEP (Phosphoenol pyruvate) carboxykinase, and it inhibits F-1, 6 bisphosphatase via F- 2, 6 bis phosphatase inhibition.
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- Insulin decreases glycogenolysis by dephosphorylating glycogen phosphorylase (inactivate) and also repressing glucose - 6phosphatase.
- It stimulates HMPshunt by inducing the enzymes glucose-6 phosphate dehydrogenase, 6-phosphogluconate dehydrogenase. [13,14,15]

Disorders of carbohydrate uptake may cause severe health problems such as diabetes and obesity[17]. Diabetes mellitus (DM) is a metabolic disorder resulting from deficiency in insulin secretion, insulin action, or both, promoting disturbance of carbohydrate, fat and protein metabolism[18]. Long term complications of diabetes mellitus include retinopathy, nephropathy, neuropathy, microangiopathy and increased risk of cardiovascular disease[19,20]. Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase. Alpha glucosidase and alpha amylase are the important enzymes involved in the digestion of carbohydrates.

Alpha amylase is involved in the breakdown of long chain carbohydrates and alpha glucosidase breaks down starch and disaccharides to glucose. They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and alpha glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes.[16 ,22,23]

The drugs commonly used in the treatment of diabetes such as, sulfonylureas, biguanide, glucosidase inhibitors, aldose reductase inhibitor, thiazolidinediones, carbamoylmethyl benzoic acid, insulin-like growth factor. They are used for treating type 2 diabetes mellitus is to decrease the post-prandial glucose evels [23,24]. This could be done by retarding the absorption of glucose through the inhibition of the carbohydrates-hydrolysing enzymes, alpha- glucosidase and alpha-amylase, present in the small intestinal brush border that are responsible for the breakdown of oligosaccharides and disaccharides into monosaccharide's suitable for absorption. Inhibitors of these enzymes, like acarbose, delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [16, 20,25].

IN-VITRO MODELS USED IN DIABETIC RESEARCH:

Inhibition Of Carbohydrate Digesting Enzymes Are:

- ✓ Alpha-amylase
- ✓ Alpha-glucosidase
- ✓ Sucrase
- ✓ Maltase

ALPHA -AMYLASE ENZYME METHOD

METHOD – A:[27,28]

Mixture prepare containing 200ul of 0.02 M sodium phosphate buffer (Ph- 6.9), 20µl of enzyme

↓

Test sample prepare with five different concentration (20-100µg/ml)

↓

200ul of 1% (w/v) starch solution prepare

↓

Incubated for 10 min. at room temperature

↓

Addition 200µl of starch solution in all test tubes

↓

The reaction was terminated with the addition of 400µl -3, 5 Dinitro salicylic acid (DNS) reagent

↓

Boling water bath for 5 min, cooled and diluted with 15 ml of distilled water

↓

Absorbance between control sample (without extract) and test sample measured at 540nm.

The inhibition is calculated according to the formula

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (drug sample)}}{\text{Abs 540 (control)}} \times 100$$

METHOD – B:[27]

EXTRACTION OF WHEAT ALPHA AMYLASE

500g of malted whole wheat flour was added slowly with stirring to 1 litre of 0.2% calcium acetate solution at room temperature and continuously stirred for 2 hours on a stirrer. The suspension was stored at 2°C to 3°C prior to heat treatment. Since beta-amylase interferes with the enzymatic determination of alpha-amylase it was inactivated by heating the extract at 70°C for 15 minutes. Alpha-amylase is resistant to inactivation by this treatment at pH between 6.5 and 8.0. the pH of the extract was first adjusted to 6.6 was cold 4% ammonium hydroxide. Heat treatment was carried out at 85°C to 90°C and other at 72°C to 74°C using a water bath with continuous stirring. The extract was then cooled to 2°C to 3°C until use.

DETERMINATION OF WHEAT ALPHA-AMYLASE INHIBITOR ACTIVITY:

200µl of 0.02 M sodium phosphate buffer, 20µl of enzyme and the plant extracts in concentration range 20-100µg/ml

$\downarrow$ Incubated for 10 minutes room temperature

Addition of 200µl of starch in all test tubes

$\downarrow$ The reaction was terminated

The addition of 400µl DNS reagent

$\downarrow$ Heated on boiling water bath for 5 min

Cooled and diluted with 15 ml of distilled water

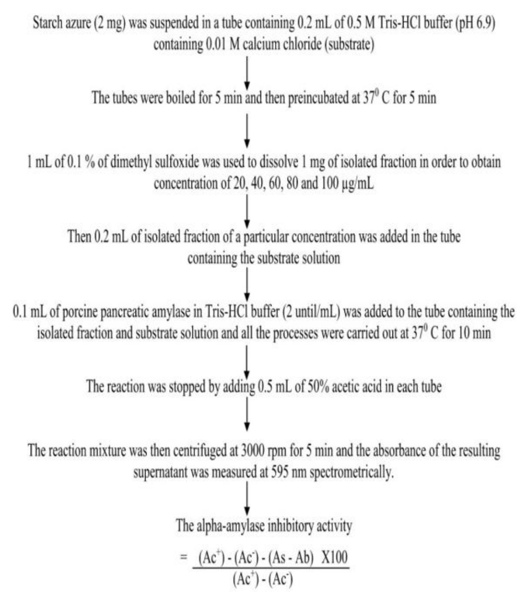
$\downarrow$ Absorbance was measured at 540 nm

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (drug sample)}}{\text{Abs 540 (control)}} \times 100$$

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-amylase inhibitor. All tests were performed in triplicate.

METHOD – C:[31,35]

PORCINE PANCREATIC ALPHA-AMYLASE INHIBITION



Where Ac^+ is absorbance of 100% enzyme activity (only solvent with enzyme), $A\ c^-$ is absorbance of 0% enzyme activity (only solvent without enzyme), As is absorbance of test sample (with enzyme), and Ab is absorbance of blank (a test sample without enzyme), respectively.

ALPHA-GLUCOSIDASE ENZYME METHOD

METHOD – A:[31]

Enzyme solution was prepared by dissolving 0.5 mg α-glycosidase in 10 ml phosphate buffer (ph 7.0)

↓ Containing

20 mg bovine serum albumin

↓ Further diluted just before use

1:10 with phosphate buffer

↓

Sample solutions were prepared by dissolving 4 mg sample in 400 µl dimethyl sulfoxide (DMSO) and (DMSO) (sample blank)

↓

Five concentrations: 50, 100, 150, 200, and 250 µg/ml were prepared

5 µl each of the sample solutions and DMSO (sample blank)

↓ Added

P-nitrophenyl-α-D –glucopyranoside with phosphate buffer (pH 7.0)

↓

Incubated condition at 37°C for 15 min. store

↓

After 15 min. reaction was then stopped

↓ Addition

Na2 CO3 (1000 µl) solution

↓

P-nitrophenol released

↓ Measured

Absorbance of sample against a sample blank

↓ Using

400 nm UV visible spectrophotometer

↓

The inhibition activity were calculated according to the formula

↓

$$(\%)\ \text{Inhibition} = \frac{EC-(ET-EC)}{EC} \times 100$$

METHOD – B:[32,36]

200 µl of alpha-glucosidase enzyme solution was pre-incubated with different concentration of test and standard drug solution for 5 min

↓ Adding

200 µl of 37 mM sucrose to all the tubes

↓

All tubes were incubated for 30 min 37^0C to allow enzymatic action as well as drug action

↓ Heating at 100^0C for 10 min

The liberated glucose was determined by glucose oxidase-peroxidase (GOD-POD) method at 546 nm and by calculating with relative blank controls

↓

The alpha-glucosidase inhibitory activity of the test drug was calculated as follow;

↓

$$\%\ \text{alpha-glucosidase inhibition} = \frac{\text{Absorbance (blank)} - \text{Absorbance (test/standard)} \times 100}{\text{Absorbance (blank)}}$$

METHOD –C:[27]

DETERMINATION OF YEAST ALPHA-GLUCOSIDASE INHIBITOR ACTIVITY:

P-Nitrophenyl-alpha-D-glucopyranoside, Acarbose, Baker's Yeast alpha glucosidase were purchased from Sigma (USA)

↓

The yeast alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 as

↓ Used

The enzyme extract P-Nitrophenyl-alpha-D-glucoside was used as the substrate

↓

Plant extract were used in the concentration ranging from 20-100 µg/ml

↓

Different concentration of plant extract were mixed with 320 µl of 100 mM phosphate

↓ Read at 410 nm

The control samples were prepared without any plant extract

↓

The % inhibition was calculated according to the formula

$$\text{Inhibition (\%)} = \frac{\text{Abs 410 (control)} - \text{Abs 410 (extract)} \times 100}{\text{Abs 410 (control)}}$$

SUCRASE ENZYME METHOD[34]

Mixture containing 200µl of 0.02 M of (Ph-6.9) sodium phosphate buffer 20 µl of enzyme solution and different concentration (20-100µg/ml) test sample prepare

↓

Incubated for 10 min. at room temperature

↓

Addition of 200 µL of starch in all test tubes

↓

The enzyme reaction started by addition 100 µl of starch solution in all test tubes

↓ After 30 min.

The reaction was terminated with the addition of 400ul -3,5 Dinitro salicylic acid (DNS) reagent

↓

Treated the mixture placed in a boiling water bath for 5 min, cool and diluted with 15 ml of distilled water.

↓

The absorbance were measured at 540nm control sample (without sample) and test sample (with sample)

↓

The inhibition activity were calculated according to the formula

↓

$$\text{Inhibition (\%)} = \frac{\text{Abs (control)} - \text{Abs(drug sample)}}{\text{Abs (control)}} \times 100$$

MALTASE ENZYME METHOD[35]

0.5 ml of 25 mM maltose in 0.1 M potassium phosphate buffer (PH 7)

↓ Mixed

0.1 ml of the MCG at different concentrations (1.25-10mg\ml in DMSO).

↓

Crude rat intestinal alpha glucosidase solution equivalent to 0.5 mg of protein

↓ Added

After incubation at 37^0 C for 3 minutes.

↓ After thoroughly mixing the

Sample and blank tubes were incubated at 37^0 C for 15 minutes and then action was stopped by adding 200µL of 2 M Tris-HCL buffer (pH 6.9).

↓

The amount of liberated glucose was determined by the glucose oxidase method using a commercial reagent kit by Merck Ltd. Simultaneously, a control test with only DMSO was carried out.

↓

$$\%\ \text{Rat intestinal maltase inhibitory activity} = \frac{\text{(OD Control- OD Sample)}}{\text{OD Control}} \times 100$$

CONCLUSION

The present review has provides information of various In-vitro studies used in antidiabetic assessment which can establish a mechanism for the antidiabetic activity of drug. In conclusion, more research is required for developing a potential and valuable anti diabetic therapies using alpha amylase alpha glucosidase inhibitors of plant origin and intensive studies of the mechanism of action of the known drug have provide further validation of several new molecular drug targets.

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