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 monosaccharides 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]

METABOLISM OF CARBOHYDRATES
Distribution of glucose after a meal[5,6,]

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.

**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.\[12,13,14\]

**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 phosphofructokinase and pyruvate kinase.
- Pyruvate dehydrogenase complex is activated via dephosphorylation of enzyme molecules which lead to increased production of acetyl-CoA from pyruvate.

Disorders of carbohydrate uptake may cause severe health problems such as diabetes and obesity. 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. Long term complications of diabetes mellitus include retinopathy, nephropathy, neuropathy, microangiopathy and increased risk of cardiovascular disease. Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia. This can be achieved by the inhibition of carbohydrate hydrolysing 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.

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 to decrease the post-prandial glucose levels. 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 monosaccharides 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.

**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 200μl of 0.02 M sodium phosphate buffer (Ph- 6.9), 20μl of enzyme

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

200μl 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 of 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

\[
\text{Inhibition} = \frac{\text{Abs}_{540(\text{control})} - \text{Abs}_{540(\text{drug sample})}}{\text{Abs}_{540(\text{control})}} \times 100
\]

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

Incubated for 10 minutes room temperature

Addition of 200μl of starch in all test tubes

The reaction was terminated

The addition of 400μl DNS reagent

Heated on boiling water bath for 5 min

Cooled and diluted with 15 ml of distilled water

Absorbance was measured at 540 nm

\[
\text{Inhibition} = \frac{\text{Abs}_{540(\text{control})} - \text{Abs}_{540(\text{drug sample})}}{\text{Abs}_{540(\text{control})}} \times 100
\]

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:**

Enzyme solution was prepared by dissolving 0.5 mg α-glucosidase 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) as (DMSO) (sample blank)

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

Na2CO3 (100 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

\[
\text{Absorbance (blank)} - \text{Absorbance (test/standard)} \times 100
\]

\[
\% \text{alpha-glucosidase inhibition} = \frac{\text{Absorbance (blank)}}{\text{Absorbance (blank)}}
\]

**METHOD – B:**

200 μl of α-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°C to allow enzymatic action as well as drug action

Heating at 100°C 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 α-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:**

**DETERMINATION OF YEAST ALPHA-GLUCOSIDASE INHIBITOR ACTIVITY:**

P-Nitrophenyl-α-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-α-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 buffer 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**

Mixture containing 200 μl of 0.02 M of (Ph-6.9) sodium phosphate buffer 20 μl of enzyme solution and different concentration (20-100 μg/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 400 μl of 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**

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°C for 3 minutes.

After thoroughly mixing the Sample and blank tubes were incubated at 37°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 provided 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.

REFERENCES

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