A Review on Structure Based Drug Design of Protein Tyrosine Phosphatase 1B Inhibitors for Target for obesity and Type 2 Diabetes Mellitus

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ABSTRACT

Type 2 diabetes is characterized by tissue resistance to the action of insulin combined with a relative deficiency in insulin secretion. Obesity and diabetes are due to the resistance of hormones insulin and leptin. Protein Tyrosine Phosphatase 1B (PTP1B) is involved in the negative regulation of insulin signaling. In this article, we are reviewing PTP1B as a potential target for type 2 diabetes due to controlled Tyrosine phosphorylation.

Key words: PTP1B inhibitors, Structure-based drug design.

INTRODUCTION

Type 2 diabetes is the most common diabetes accounting for 80-90% of the diabetic population [1]. It occurs due to genetic and environmental factors. Overeating coupled with under activity leading to obesity is associated with the development of Non-insulin dependent diabetes mellitus. Obesity acts as a diabetogenic factor in genetically predisposed individuals by increasing the resistance to the action of insulin. This is due to a decrease in insulin receptors on the insulin responsive cells. The patients of Non-insulin dependent may have either normal or even increased insulin levels. It is suggested that overeating causes increased insulin production but decreased synthesis of insulin receptors. This is based on the fact that weight reduction by diet control alone is often sufficient to correct Non-insulin dependent diabetes [2-4].

Tyrosine phosphorylation is controlled by protein tyrosine kinases (PTKs) and phosphatases (PTPs). Protein protein phosphatases (PTPases) form a large, structurally diverse family of enzymes expressed in all eukaryotes. Recent reports indicate that there are about 60 PTPase genes encoded within the human genome, including transmembrane, receptor-like, and nonreceptor-like enzymes. Six PTPases have been found to have mutations that contribute to inherited human diseases, such as familial stroke, hypercholesterolemia, coronary artery disease, Alzheimer’s disease, and diabetes. Each PTPase is composed of at least one conserved domain characterized by a unique 11-residue sequence motif (IVHCXAXXXST/G) containing a cysteine and an arginine known to be essential for catalytic activity. Their catalytic mechanism involves a nucleophilic attack by the conserved cysteine on a phosphotyrosine substrate; resulting in a covalent phosphocysteine intermediate that is subsequently hydrolyzed by an activated water molecule. PTP1B was the first PTPase to be isolated in homogenous form. Since then a number of biological and enzyme kinetic studies on PTP1B have suggested that PTP1B is an important regulator of the insulin-signaling pathway. In particular, PTP1B seems to regulate negatively insulin signaling by dephosphorylating the phosphotyrosine residues of the tissue insulin receptor kinase. Recent studies on PTP1B knock mice showed increased insulin sensitivity and obesity resistance. Thus PTP1B is an attractive target for the treatment of type 2 diabetes and obesity, and selective PTP1B inhibitors could be a significant therapeutic utility [5-10].

Role of PTP1B in diabetes and obesity

Insulin plays a key role in the regulation of carbohydrate; lipid and protein metabolism. Insulin exerts anabolic and antitrophic influences on the body metabolism.

Metabolic insulin signal transduction occurs through activation of the insulin receptor, including autophosphorylation of tyrosine (Tyr) residues in the insulin-receptor activation loop. This leads to recruitment of insulin receptor substrate (IRS) proteins, followed by activation of phosphatidylinositol-3-kinase (PI3K) and downstream protein kinase B (PKB also known as AKT), and activation and subsequent translocation of the glucose transporter GLUT4. This process is negatively regulated by Protein Tyrosine Phosphatases (PTPs), and is a general mechanism for down regulation receptor tyrosine kinase (RTK) activity. Several PTPases, including receptor protein tyrosine phosphatase-a (rPTP-a), leukocyte antigen-related tyrosine phosphatase (LAR), SH2-domain containing phosphotyrosine phosphatase (SHP2) and protein tyrosine phosphatase 1B (PTP1B), have been implicated in modulating insulin signal transduction. PTP1B seems to be a key regulator of insulin-receptor activity that acts at the insulin receptor and a downstream signaling components, such as IRS1. Reduced insulin sensitivity in omental fat has been thought to contribute to overall insulin resistance and PTP1B expression and activity is elevated in this tissue.

First Crystal Structures of PTP1B

The first crystal structure of the catalytic domain of PTP1B was published in 1994. A 10-stranded mixed b-sheets that adopts a highly twisted conformation forms the core of the protein. The sodium tungstate was used to form a heavy-metal derivative in the structure determination since it was found to bind tightly to PTP1B. It presumably acts as a phosphate mimic, and thus the structure of tungstate complexed to PTP1B provides the first details of the interactions between PTP1B and its active site ligands. The catalytic site is located at the base of a shallow cleft. The phosphate recognition site is created from a loop that is located at the N-terminus of an a-helix. This site consists of the 11-residue sequence motif which is typical for PTPases and contains the catalytically active cysteine and arginine. There are H-bonds between the tungstate and the backbone amides of Ser216, Ala217, Gly218, Ile219, Gly220, Arg221 and the side chains of the conserved Arg221, suggesting that these H-bond functions are recognizing the phosphate in the phosphorylated substrate peptide. The position of the conserved cysteine within the phosphate binding site is consistent with its role as a nucleophile in the catalytic reaction.

Structural Biology of PTP1B

PTP1B was the first PTP1B enzyme to be purified homogeneity from human placental tissue. The native protein consists of 435 amino acid residues, of which amino acid 30-278 comprise the catalytic domain. The 35 carboxy-terminal amino acid residues are rich in proline, and are involved in targeting the enzyme to the cytoplasmic face of the endoplasmic reticulum. The main structural features are the catalytic loop containing the catalytic residue cys 215, the WPD (tryptophan, proline, aspartic acid) loop and the secondary Aryl-Phosphate-binding site. Features involved in phosphatases selectivity are the YRD (tyrosine, arginine, aspartic acid) motif ad the gateway residue, glycine (Gly).

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Active Site:
The active site of PTP1B contains a common structural motif of PTPs. The base of the catalytic site is defined by the 214-221 PTP signature motif, a loop of eight amino acid residues that forms a rigid, cradle-like structure. This region is known as the YRB motif. By forming a salt bridge interaction with the substrate phosphate, in this transformed conformation, Cys 215 is in a position to undergo a nucleophilic attack on the substrate phosphate. All molecules that inhibit PTP1B binding to the open conformation are typically weaker inhibitors than molecules that induce the closed conformation.

Secondary aryl-phosphate binding site:
This site is catalytically inactive and provides weaker binding interactions compared with the primary site owing to its more open exposure to solvent.

Selectivity at the molecular level:
Targeting interactions between Arg 47 and Asp 48, which are close to the active site has allowed selectivity to be achieved over most other PTPs. Arg 47 and Asp 48 form a charged region at the top of the binding pocket of PTP 1B. This region is known as the YRB motif. By forming a salt bridge between ASP 48 and an inhibitor that contains basic nitrogen in that region, selectivity can be achieved over 6 other PTPs. The reported inhibitors are difluoromethylene phosphonates, 2-carbomethoxybenzoic acids and lipophilic compounds.

CONCLUSION:
Structure-based drug design relies on knowledge of three-dimensional structure of the biological target obtained through X-ray crystallography/NMR spectroscopy. The drug is mostly an organic small molecule which activates or inhibits the function of biomolecules such as protein in turn results in therapeutic benefit to the patient. In most basic sense drug design involves design of small molecule that is complementary in shape and charge to the bimolecular target to which they interact and therefore bind to it. PTP1B is a new therapeutic option to patients in the treatment of type 2 diabetes. Several structural features of PTP1B in and adjacent to the active site do offer the possibility of developing selective inhibitors of this phosphatase.

Structure-based drug design can successfully contribute to the discovery process at different stages. It can be used at a very early stage at which no leads are available. PTP1B is a novel target for diabetes. Protein Tyrosine Phosphatase 1B (PTP1B) is increasing importance in the pathophysiology of insulin resistance in diabetes mellitus but also a drug target for the management of insulin resistant states such as obesity and type 2 diabetes mellitus. The crystal structure of PTP1B with the inhibitors reveals that in addition to the phosphotyrosine binding site (catalytic site) (residues Cys 215-Arg 221) there is a second binding site (site 2) (Arg 24 and Arg 254). Inhibitors that bind to both the sites are found to be highly potent with activities in the nanomolar range. Recently, a third binding site (site 3) (residues Tyr 46-Asp 48) was also found to contribute to the potency and selectivity of inhibitors. All the inhibitors that have been developed so far are either non-peptidic or peptidomimetic in nature. Detailed literature survey and physicochemical properties of PTP 1B inhibitors have to be studied. The protein structure and interaction of amino acids with PTP1B are represented in Fig 1 & Fig 2.

REFERENCES

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