Food Dyes are Inhibitors of Human Protein Tyrosine Phosphatases (PTP1B) Molecular Docking Studies

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ABSTRACT
Protein Tyrosine Phosphatase 1B (PTP1B) is an enzyme that plays a critical role in down-regulating insulin signaling through dephosphorylation of the insulin receptor. Inhibitors of PTP1B showed increased insulin sensitivity and normalize plasma glucose level and thus are use full therapeutic agents for the treatment of diabetes. The aim of the current study is to identify PTP1B inhibitors by means of virtual screening with docking. Six food dyes molecules have been screened and based on energy MolDok scores and hydrogen bonding interactions, L1, L2 potential inhibitors were identified in cavity1 and 2 respectively.

Keywords
Diabetes, PTP1B, Food dyes, Interactions, Molecular Docking.

1. INTRODUCTION
The activities and functions of cellular proteins are often regulated by posttranslational modifications. Tyrosine phosphorylation is an example of those modifications and the reversible nature of the phosphorylation provides the basis for the communication between the signaling proteins inside cell [1-4]. The phosphorylation state of a protein is dynamically controlled by the action of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases); PTKs catalyze the tyrosine phosphorylation and PTPases catalyze the reverse reaction [5,6].

PTKases were discovered in 1980 [7] and later found to be extremely important for the regulation of cell transformation, growth, differentiation, apoptosis, and hormone actions. On the other hand, Protein tyrosine phosphatases (PTPs) constitute a large family of signaling enzymes. Deregulation of PTP activity can play a role in a number of diseases including diabetes, cancer, and dysfunction of the immune system. Fischer et al. purified and characterized the first PTPase, PTP-1B from the human placenta in 1988 [8,9]. PTP1B dephosphorylates the insulin receptor and causes resistance to insulin. Thus, it has been implicated in the development of type II diabetes [10]. Based on the structural similarity between it and leukocyte antigen CD45, they found that this antigen had PTPase activity [11]. Subsequently, more than 30 PTPases were cloned and reported [12]. PTPases form a large family of enzymes that can be divided into cytosolic types, such as SH2-domain-containing phosphotyrosine phosphatase (SHP2) and PTP-1B, and transmembrane types, such as CD45, receptor PTPase-a (RPTP-a), and leukocyte antigen related tyrosine phosphatase (LAR). In the other hand, Synthetic colorants are a very important class of food additives. They are widely used to compensate for the loss of natural colors of food and the way to improve the esthetic quality of a food product, which is destroyed during processing and storage, and to provide the desired colored appearance. The total world colorant production is estimated to be 80,00,000 tons per year [13]. However, some of these substances pose a potential risk to human health, especially if they are excessively consumed. For this reason, safety data, such as the acceptable daily intake, based on toxicological studies on experimental animals and human clinical studies, have been repeatedly determined and evaluated by Food and Agricultural Organization (FAO) and World Health Organization (WHO) [14]. They are divided into five major colorant classes: the azo compounds (such as amaranth and tartrazine…), the chinaphosphol derivatives of Quinoline Yellow, the triarylmethane group, xanthenes (such as erythrosine) and the indigo colorants [15].

While studying the enzymology of protein tyrosine phosphatases (PTPases), they observed that some of the dyes behaved as potent inhibitors of PTPases [16,17]. PTPases are a class of enzymes that hydrolyze the phosphate moiety from a phosphotyrosine residue of a protein thus regulating the cellular phosphorylation level of proteins in collaboration with protein tyrosine kinases [1,18]. Because PTPases play important roles as regulators of a diverse of signal transduction pathways, inhibition of PTPases by food colorants might disturb a certain signaling pathways resulting in adverse effects in human health. The dye attracted our attention because it is a competitive inhibitor of PTPases and also it contains free hydroxyl groups to introduce additional structural features without a significant change of the core structure. In present paper molecular modeling studies were performed to study the structural features of the inhibitor binding to PTP1B.

2. MATERIALS AND METHODS
X-ray crystallographic study has performed to explain the enzyme–inhibitor interactions with PTP1B and some food dyes. This work showed that PTP1B has two possible modes of inhibition depending on the structures of the ligands. Based on this result, molecular modeling was carried out to study the structural features of the interaction between PTP1B and food dyes.

2.1 Ligands Structures
Six food dyes was taken as ligands (Figure 1). These compounds were reported to bind with PTP1B and
subsequently disturb a certain signaling pathways resulting in adverse effects in human health [19]. Therefore those were also subjected to docking to examine their binding modes.

![Chemical structures](image)

**Fig1**: Chemical structure of the food dyes used. L1: Erythrosine B, L2: Phloxine B, L3: Allura Red AC, L4: Amaranth, L5: Tartrazine L6: Sunset Yellow FCF

The full geometrical optimization of the food dyes L1-6 (Figure 1) in the gas phase were carried out at the level of semi-empirical AM1 method [20], as well as density functional theory (DFT) [21] using a gradient technique [22,23] and 6-31G* [24,25] basis set. The DFT calculations were carried out with the B3LYP functional, in which Becke’s nonlocal exchange [26,27] and the Lee-Yang-Parr correlation functional [28] semi-empirical and DFT calculations were performed using GAUSSIAN 03 for Windows program package [29]. All calculations were done on a Pentium IV PC computer.

Table 1. Shows energies of each ligands used DFT/ B3LYP (6-31(G*))

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Energies (u.a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>HF=-0.0074</td>
</tr>
<tr>
<td>L2</td>
<td>HF=-0.1020</td>
</tr>
<tr>
<td>L3</td>
<td>HF=-0.3203</td>
</tr>
<tr>
<td>L4</td>
<td>HF=-0.4099</td>
</tr>
<tr>
<td>L5</td>
<td>HF=-0.2881</td>
</tr>
<tr>
<td>L6</td>
<td>HF=-0.2635</td>
</tr>
</tbody>
</table>
As shown in Table 2, broad spectrum of IC50 values was observed in food dyes (L1–L6) [19].

**Table 2. Inhibition of PTP1B by various synthetic dyes. Compounds L1–L6**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50(µM)x</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>L2</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>L3</td>
<td>33 ± 6</td>
</tr>
<tr>
<td>L4</td>
<td>34 ±7</td>
</tr>
<tr>
<td>L5</td>
<td>&gt;190</td>
</tr>
<tr>
<td>L6</td>
<td>&gt;220</td>
</tr>
</tbody>
</table>

xIC50 values were usually derived from duplicates or more of experiments using a range of inhibitor concentrations. The numbers indicate mean value ± standard deviation

### 2.2 Enzyme Structure

The X-ray crystal structures of PTP1B bound with 6-[(difluoro-phosphono-methyl)-naphthalene-2-carboxylic acid (1BZJ) [30], were downloaded from RCSB Database (www.rcsb.org/pdb) [31].

### 2.3 The Simulation Details

The structure of the protein was corrected for missing atoms or unknown units using Molegro Virtual Docker (MVD2011) [32-34] program, graphical-automatic software (http://molegro.com/mvd-product.php). 1BZJ has a monomer structure. So to simplify the simulation water was removed.

To locate the appropriate binding orientations and conformations of ligands on PTP1B, docking was performed by using Molegro Virtual Docker (MVD) software package. The identification of ligand binding modes is done by iteratively evaluating a number of candidate solutions (ligand conformations) and estimating the energy of their interactions with the macromolecule. MVD performs flexible ligand docking, so the optimal geometry of the ligand will be determined during the docking. The MolDock [35] scoring function (MolDock Score) used by MVD is derived from the PLP scoring functions originally proposed by Gehlhaar et al. and later extended by Yang et al. [36]. The MolDock scoring function further improves these scoring functions with a new hydrogen bonding term and new charge schemes.

### 2.4 The Docking

To obtain better potential binding sites in the PTP1B (PDB ID: 1BZJ), a maximum of five cavities was detected using default parameters. The volume of cavity 2 (48.128 Å3) was found to be highest than the other cavities, also we found that the reference ligand of 1BZJ is fixed in cavity 1 (41.472 Å3). Out of the detected cavities, cavity 1 and 2 was selected for further studies (figure 2). The chosen cavity was further refined using side chain minimization by selection of an add- visible option set at a maximum of 10,000 steps per residue and at a maximum of 10,000 global steps. The grid resolution was 0.30 Å; the max iterations were 1,500; the max population size was 50 and the energy threshold was 100.

**Fig2:** Graphical interface with the cavities (cavity 1 and cavity 2 indicated by ellipses) identified by MolDock

### 3. RESULTS AND DISCUSSION

#### 3.1 Study of Ligand-Substrate Interaction

X-ray crystallographic study has previously performed to explain the enzyme–inhibitor interactions with PTP1B and benzofuran and benzothiophene biphenyls [37]. This work showed that PTP1B has two possible modes of binding at the active site depending on the structures of the ligands. Based on this result, molecular modeling was carried out to study the structural features of the interaction between PTP1B and six food dyes. Docking of these six food dyes were performed using MVD program. It was found that the residues in the site1(cavity1) as follows: Arg221, Ser216, Asp181, Phe182, Ala217, Val49, Asp48, Tyr46, Lys120, Gln262, Asp48, Ile219, Gly220, Cys215, Arg47, Glu262 and Lys116 are highly conserved within the active site. Also for the residues in the site2(cavity2) as follows: Lys248, Ala77, Glu252, Glu75, Leu234, Met75, Thr230, Val249, Glu76, Met74, Asp245, Phe256, Lys73, Val244, Gly78, Lys255, Leu251, Ser243, Arg238 are highly conserved within the active site. In this study, site1 and site2 are chosen as the binding site to dock with the food dyes diversity set molecules.
3.2 Virtual Screening

The six ligands molecules having minimum energy were screened out as the possible inhibitors for PTPases (Table 3). In cavity1: All selected molecules were having minimum energy MolDock score as follows: Ligand L₁ is having minimum energy MolDock score -145.584 Kcal/Mol. Ligand L₂ is having -138.408 Kcal/Mol. Ligand L₃ is having -103.515 and Ligand L₄ is having energy MolDock score -137.544 Kcal/Mol. Ligand L₅ is having energy MolDock score -147.036. Ligand L₆ is having energy MolDock score – 113.958. With the help of the Ligplot+ study we have selected Ligand L₁ as the possible inhibitor lead molecule, as it has minimum energy MolDock score and one of the highest number of interactions with the active site residue, it has 07 hydrophobic and 09 hydrogen interactions. Figure 3 represent the binding modes of the different docking poses of the ligands were investigated on Ligplot+ program [38], according to the energy MolDock score in cavity1.

Also in cavity 2 : All selected molecules were having energy MolDock score as follows: Ligand L₄ is having minimum energy MolDock score -141.283 Kcal/Mol. Ligand L₂ is having -106.417 Kcal/Mol. Ligand L₃ is having -104.632 and Ligand L₄ is having energy MolDock score -101.029 Kcal/Mol. Ligand L₅ is having energy MolDock score -115.254. Ligand L₆ is having energy MolDock score – 95.455. Also with the help of the Ligplot study we have selected Ligand L₁ as the possible inhibitor lead molecule, as it has minimum energy MolDock score and one of the highest number of interactions with the active site residue, it has 08 hydrophobic and 1 hydrogen interactions. Figure 4 represent interactions of the six ligands drawn by ligplot+ according to the energy MolDock score in cavity2.

Table 3(a,b) shows all hydrogen bonds and hydrophobic interactions exist between six ligands (L₁-₆) and active site(Cavity1 and Cavity2) respectively.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Hydrogen bond (≤3.6Å)</th>
<th>Hydrophobic interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>L₁</td>
<td>Arg221</td>
<td>Ser216, Asp181, Phe182, Ala217, Val49, Asp48, Tyr46, Lys120, Ile219, Val49, Ala217, Phe182, Lys120, Asp181, Phe182, Tyr46.</td>
</tr>
</tbody>
</table>
Table 4(b): List of amino acid residues involved in ligands-protein interaction between L₁-₆ and cavity2 as predicted by Ligplot+

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Hydrogen bond (≤3.6Å)</th>
<th>Hydrophobic interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>L₁</td>
<td>Lys248</td>
<td>Ala77, Glu252, Glu75, Leu234, Met75, Thr230, Val249, Glu76</td>
</tr>
<tr>
<td>L₂</td>
<td>Glu75, Ala77, Lys248</td>
<td>Glu252, Met74, Val249, Glu76</td>
</tr>
<tr>
<td>L₃</td>
<td>Glu76, Asp245</td>
<td>Phe256, Lys73, Met74, Glu75, Ala77, Thr230, Leu234, Lys248, Val244, Val249, Glu252</td>
</tr>
<tr>
<td>L₄</td>
<td>Glu76, Glu75</td>
<td>Val249, Leu234, Met74, Glu78, Lys248, Glu252</td>
</tr>
<tr>
<td>L₅</td>
<td>Lys255, Ala77, Glu75</td>
<td>Lys248, Glu252, Glu76, Leu251, Phe256, Lys73, Met74</td>
</tr>
<tr>
<td>L₆</td>
<td>Glu252, Asp245, Ser243, Arg238</td>
<td>Glu75, Glu76, Met74, Ala77, Leu234, Val249, Lys248, Val244</td>
</tr>
</tbody>
</table>


According to the results obtained by molecular docking, result of cavity 2 has agreement with experimental study [19] and detailed binding pattern of an inhibitor L1 exhibiting the second lowest IC₅₀ is shown in Table 2. L1 extended deep into the active site pocket, making several hydrogen bonds and hydrophobic interactions with key residues of the catalytic site. One carboxyl group of L1 forms one hydrogen bond with Lys248 and Ala77, Glu252, Glu75, Leu234, Met75,
Thr230, Val249, Glu76 forms hydrophobic interactions with \( L_4 \) (Table 4(b)).

4. CONCLUSION
The molecular docking analysis resulted in the identification of potential ligands (\( L_{1,4} \), \( L_4 \)) molecules. Hence, in present study, it can be concluded that molecules \( L_4 \) and \( L_4 \) in cavity 1 and 2 respectively have the potential to inhibit the activity of PTP1B and can might cause harmful effects in human health. \( L_4 \) and \( L_4 \) does not only show interactions with identified active residues that are important for catalytic activity of PTP1B but the free energy of binding also ensures that \( L_4 \) and \( L_4 \) shows a very strong binding with PTP1B at polymerase active site.

5. ACKNOWLEDGMENTS
Our thanks to the experts who have contributed towards development of the template.

6. REFERENCES


