Understanding Drug-Protein Interactions in *Escherichia coli* FabI and Various FabI Inhibitor Complexes

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Many ligands have been experimentally designed and tested for their activities as inhibitors against bacterial enoyl-ACP reductase (FabI), ENR. Here the binding energies of the reported ligands with the *E. coli* ENR-NAD⁺ were calculated, analyzed and compared, and their molecular dynamics (MD) simulation study was performed. IDN, ZAM and AYM ligands were calculated to have larger binding energies than TCL and IDN has the largest binding energy among the considered ligands (TCL, S54, E26, ZAM, AYM and IDN). The contribution of residues to the ligand binding energy is larger in *E. coli* ENR-NAD⁺-IDN than in *E. coli* ENR-NAD⁺-TCL, while the contribution of NAD⁺ is smaller for IDN than for TCL. The large-size ligands having considerable interactions with residues and NAD⁺ have many effective functional groups such as aromatic π rings, acidic hydroxyl groups, and polarizable amide carbonyl groups in common. The cation-π interactions have large binding energies, positively charged residues strongly interact with polarisable amide carbonyl group, and the acidic phenoxyl group has strong H-bond interactions. The residues which have strong interactions with the ligands in common are Y146, Y156, M159 and K163. This study of the reported inhibitor candidates is expected to assist the design of feasible ENR inhibitors.

**Key Words:** Enoyl-acyl carrier protein reductase, ENR inhibitor, ENR-NAD⁺-ligand interaction, Molecular dynamics

### Introduction

The *Escherichia coli* (E. coli) is one of the bacterial-disease-causing parasites. The enoyl-[acyl-carrier-protein] reductase (ENR), an enzyme involved in fatty acid biosynthesis, is known to be a good target for antibacterial drugs. The type II fatty acid synthetases (FAS II) are found in plants and other bacteria not in mammals. Plants and most prokaryotes perform the type II fatty acid synthesis, in contrast to eukaryotes which follow the type I fatty acid synthesis. Mammalian fatty acid synthase (FAS I) is different from FAS II. Fatty acid biosynthesis in bacteria is essential process to produce a number of lipid-containing components including the cell membrane. The enoyl reductase (ENR), also known as FabI, is one of the key components of the FAS II system. The ENR puts the last hand to the completion of the fatty acid chain elongation cycle by catalyzing the stereospecific reduction of the double bond between C2 and C3 positions of a growing fatty-acid chain. Hence, ENR becomes a important drug target for *E. coli* and other three critical infectious disease problems i.e., methicillin-resistant *Staphylococcus aureus* (MRSA),4 Tuberculosis (TB)5 and Malaria.6 The enoyl-acyl carrier protein reductase, ENR, inhibitors are known to be toxic compounds, and it was investigated on molecular basis study that a flipping loop (I192-S198) of *E. coli* ENR is involved in the active site with triclosan. In 2001 Heerding’s group reported that the 1,4-disubstituted imidazo[3,4-b]indoles are potential antibacterial agents.19 In 2005 Miller et al. discovered aminopyridine-based inhibitors of FabI,20 and in 2003 Seefeld et al. reported the test of indole napthryridiones as inhibitors against bacterial enoyl-ACP reductases FabI and FabK.21

It is very important to understand the functional groups and structures of good ligands and the important amino acid residues of the active site of ENR where the ligands would bind with different binding efficacies. Detailed understanding of the structure and energy relationship of these ENR inhibitors could help the design of new and improved ENR inhibitors. Therefore, we calculated, analyzed and compared the binding energies of the reported ligands with the *E. coli* ENR-NAD⁺, and performed their molecular dynamics (MD) simulation study. In particular we point out the important amino acid residues which are responsible for the improved binding efficacies with the inhibitors.

### Computational Methods

The X-ray structures of *E. coli* ENR-NAD⁺-ligands were obtained from the reported Protein Databank (PDB ID for TCL ligand: 1C14, PDB ID for S54 ligand: 112Z, PDB ID for
E26 ligand: 1130,19 PDB ID for ZAM ligand: 1LX6,20 PDB ID for AYM ligand: 1LXC,20 and PDB ID for IDN ligand: 1MFP21). The names of ligands are given as followings: TCL; Triclosan, S54; 4-(2-Thienyl)-1-(4-methylbenzyl)-1H-imidazole, E26; 1,3,4,9-Tetrahydro-2-(hydroxybenzoyl)-9-[(4-hydroxyphenyl)methyl]-6-methoxy-2H-pyridazo[3,4-b]indole, ZAM; 3-[(Acetyl-methyl-amino)-methyl]-4-amino-N-methyl-N-(1-methyl-1H-indole-2-ylmethyl)-benzamide, AYM; 3-(6-Aminopyridin-3-yl)-N-methyl-N-[(1-methyl-1H-indole-2-yl)methyl]acylamide, and IDN; (E)-N-Methyl-N-(1-methyl-1H-indole-3-ylmethyl)-3-(7-oxo-5,6,7,8-tetrahydro-[1,8]naphthyridin-3-yl)-acrylamide. The structures of these ligands are shown in Figure 1. Tense X-ray structures of 1I30, 1LX6 and 1LXC complexes have been carried out using the Amber9 program.22 We also calculated the B-factor of the α-carbon atoms of the protein of each of the MD trajectories. Their average binding energy calculations were performed for last 1.5 ns MD simulations after convergence of total energy.

Results and Discussion

Figure 2. Interaction energies (IE) of the residues which have considerable interactions with ligands for (a) A configurations and (b) B configurations after the energy minimizations.
NAD$^+$-ligand interaction energies are $-18.56$ kcal/mol for TCL, $-16.62$ kcal/mol for S54, $-16.96$ kcal/mol for E26, $-34.36$ kcal/mol for ZAM, $-18.70$ kcal/mol AYM, and $-18.68$ kcal/mol for IDN. For B configurations the NAD$^+$-ligand interaction energies are $-18.72$ kcal/mol for TCL, $-26.86$ kcal/mol for S54, $-19.40$ kcal/mol for E26, $-35.14$ kcal/mol ZAM, $-16.78$ kcal/mol for AYM, and $-17.84$ kcal/mol for IDN. The main interactions of NAD$^+$-TCL in the 1C14 complex are the aromatic(cation)-aromatic [πircon] interaction$^{26,27}$ between positively charged nicotinamide ring and 5-monochloro-phenol (MCP) of neutral TCL and the H-bond interaction between the phenoxyl group of MCP and the ribose of NAD$^+$. The cation-π interaction is considerably strong and the acidic phenol hydroxy group also has strong H-bond interaction. For 1Z2 complexes the NAD$^+$-S54 interaction are attributed to the π-stacking interaction between the thiophene ring of S54 and the electron-poor nicotinamide ring of NAD$^+$. A total of $12$ H-bond interactions between one nitrogen atom of the imidazole ring of S54 and one ribose hydroxyl group of NAD$^+$. But in the B configuration S54 has another H-bond interaction between one hydrogen atom of the imidazole ring of S54 and one of the phosphate groups of NAD$^+$. S54 has larger BE in the B configuration than in the A configuration. In the E. coli ENR-NAD$^+$-E26 complexes, there are two interactions: the π-π stacking interaction between the aromatic ring of the 4-hydroxybenzamide (E26) and the nicotinamide ring of NAD$^+$ and the H-bond interaction between the amide oxygen of the 4-hydroxybenzamide and the ribose hydroxyl group of NAD$^+$. In 1LX6 complexes the central amide moiety of ZAM has the H-bond interaction between the oxygen atom of the ZAM amide and the ribose hydroxyl group of NAD$^+$ and the πircon interaction between the nitrogen atom of the central amide of ZAM and the nicotinamide ring of NAD$^+$. The carbonyl group of amide moiety is polarisable and has strong H-bond interaction. And the 4-aminogroup of ZAM has another H-bond interaction with one of the phosphate groups of NAD$^+$. So, among the concerned ligands the ZAM has the largest interaction energies ($-34.36$ kcal/mol for A configuration and $-35.14$ kcal/mol for B configuration) with NAD$^+$. In 1LXC complexes AYM has a central amide moiety which is engaged in the H-bond interaction between the oxygen atom of the AYM amide and the ribose hydroxyl group of NAD$^+$ and the πircon interaction between the nitrogen atom of the central amide of AYM and the nicotinamide ring of NAD$^+$. In 1MFP complexes the IDN ligand has similar interactions with NAD$^+$ to the AYM ligand. IDN has a central amide moiety which has contribution to the H-bond interaction between the oxygen atom of the IDN amide and the ribose hydroxyl group of NAD$^+$ and the πircon interaction between the nitrogen atom of the central amide of IDN and the nicotinamide ring of NAD$^+$. TCL has the main interactions of a strong H-bond interaction ($-5.22$ kcal/mol for A configuration and $-5.14$ kcal/mol for B) using the hydroxyl group with the phenolic hydroxy group of Y156, a H-π interaction using MCP hydrogen with the phenol π ring of Y146, a H-π interaction using π ring of 2,4-dichlorophenol (DCP) with the terminal thiomethoxy group of M159, and a H-π interaction using MCP π ring with hydrophobic side chain of I200. The acidic phenol hydroxy group is a good H donor as well as a good H acceptor in the H-bond interaction. S54 involves a strong H-bond interaction ($-8.22$ kcal/mol for A and $-7.76$ kcal/mol for B) using the unsubstituted imidazole.

### Table 1. Interaction energy contributions of important amino acid residues interacting with ligands calculated from the energy minimized complex structures

<table>
<thead>
<tr>
<th>residues</th>
<th>A configuration</th>
<th>B configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCL</td>
<td>S54</td>
</tr>
<tr>
<td>G93</td>
<td>-0.24</td>
<td>0.86</td>
</tr>
<tr>
<td>F94</td>
<td>-0.44</td>
<td>-1.22</td>
</tr>
<tr>
<td>G97</td>
<td>-0.16</td>
<td>0.66</td>
</tr>
<tr>
<td>D98</td>
<td>0.12</td>
<td>-1.12</td>
</tr>
<tr>
<td>L100</td>
<td>-1.66</td>
<td>-1.70</td>
</tr>
<tr>
<td>D101</td>
<td>-0.24</td>
<td>-0.44</td>
</tr>
<tr>
<td>Y146</td>
<td>-2.90</td>
<td>-1.98</td>
</tr>
<tr>
<td>M159</td>
<td>-2.64</td>
<td>-1.66</td>
</tr>
<tr>
<td>K163</td>
<td>0.48</td>
<td>-4.38</td>
</tr>
<tr>
<td>R171</td>
<td>-0.12</td>
<td>-0.70</td>
</tr>
<tr>
<td>L195</td>
<td>-0.02</td>
<td>-0.04</td>
</tr>
<tr>
<td>A196</td>
<td>-1.14</td>
<td>-0.46</td>
</tr>
<tr>
<td>A197</td>
<td>-0.82</td>
<td>-0.08</td>
</tr>
<tr>
<td>I200</td>
<td>-1.96</td>
<td>-1.16</td>
</tr>
<tr>
<td>D202</td>
<td>-0.26</td>
<td>0.18</td>
</tr>
<tr>
<td>F203</td>
<td>-0.22</td>
<td>-0.16</td>
</tr>
<tr>
<td>E211</td>
<td>-0.06</td>
<td>0.42</td>
</tr>
<tr>
<td>Total</td>
<td>-52.52</td>
<td>-55.35</td>
</tr>
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</table>
nitrigen with the phenolic hydroxyl group of Y156, a cation–π interaction using imidazole ring with terminal positively charged protonated amine of K163, and a H–π interaction using hydrogen atoms of thiophene ring with the phenolic π ring of Y146. The cation–π interaction has considerably large interaction energy (–15.94 kcal/mol for Bz-NH4+, –37.9 kcal/mol for Bz-Li+, –28.0 kcal/mol for Bz-Na+, –18.3 kcal/mol for Bz-K+). E26 makes a strong electrostatic interaction (–9.38 kcal/mol for A and –7.76 kcal/mol for B) using amide carbonyl group with terminal positively charged protonated amine of K163, a H-bond interaction (–8.14 kcal/mol for A and –8.32 kcal/mol for B) using amide carbonyl group with the phenolic hydroxyl group of Y156, an H–π interaction for B using aliphatic H atoms of I200. And also the TCL, S54 and IDN ligands have considerable H–π interactions with A196, A197 and I200 residues. ZAM, AYM and IDN have double interactions with Y156 using indole π ring and amide carbonyl group. Y156 has a phenol group. In summary large ligands with many functional groups and aromatic rings have large interaction energies with the ENR protein and NAD+ . The functional groups engaged in the interactions with the residues and NAD+ are large aromatic π rings, hydroxyl group, and polarizable amide carbonyl group. ZAM, AYM and IDN ligands have indole and amide moieties in common. AYM has one less carbonyl group than ZAM and IDN. IDN and ZAM ligands have similar functional groups to each other but their locations and structures are different. And it is shown in Table 1 that a flipping loop (I192-S198) is involved in the binding region. For six concerned ligands the residues Y146, Y156, M159 and K163 have strong interactions with the ligands in common. These four residues (Y146, Y156, M159 and K163), NAD+ coenzyme and ligands of 1C14, 1I2Z, 1I30, 1LX6, 1LXC and 1MFP of A configurations are presented in the superimposed form in Figure 3. In Figure 3 F94 and L100

Figure 3. (a) Stick structures and (b) ribbon and stick structures of the main binding sites of ligands in the ENR-NAD+–ligand complexes. Six ligands: TCL (green), S54 (yellow), E26 (magenta), ZAM (cyan), AYM (pink) and IDN (grey).
are included. The positions of these six residues and the NAD⁺
coenzyme are little changed. Especially, in the cases of E26,
ZAM, AYM and IDN the distances between the nitrogen atom of
the protonated amine of K163 and the oxygen atoms of amide
carbonyl groups of ligands are even very long as much as 4.56 Å
− 6.13 Å but the lysine163 (K163) has strong electrostatic inter-
actions with the amide carbonyl groups of these ligands.

The experimental IC₅₀ values of these ligands against E. coli
FabI are listed in Table 2. Smaller the value of the IC₅₀, stronger
the solvent effect for the ligand bindings in Table 1, TCL has
71.4%, 51.4%, 49.1% and 46.0%, respectively. The decrease
of the interaction energies of TCL is relatively low in com-
aparison with other ligands. TCL has smaller number of polar
functional groups than any of the other ligands. But the inter-
action energy decrease of small-size E26 ligand is relatively
large.31,32 From the MD simulations the total interaction energies
of TCL, S54, E26, ZAM, AYM and IDN were not changed in general, but the conformational change of
the solvent effect for the ligand bindings in Table 1, TCL has

**Table 2. Experimental IC₅₀ values of ligands against E. coli. FabI**

<table>
<thead>
<tr>
<th>Residues</th>
<th>TCL</th>
<th>S54</th>
<th>E26</th>
<th>ZAM</th>
<th>AYM</th>
<th>IDN</th>
</tr>
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<tbody>
<tr>
<td>IC₅₀ (μM)</td>
<td>0.43</td>
<td>13.7</td>
<td>&gt;4.2</td>
<td>0.52</td>
<td>0.37</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Table 3. Interaction energy contributions of important amino acid residues interacting with ligands TCL, S54, and IDN**

<table>
<thead>
<tr>
<th>Residues</th>
<th>TCL</th>
<th>S54</th>
<th>IDN</th>
</tr>
</thead>
<tbody>
<tr>
<td>IE (kcal/mol)</td>
<td>1.66</td>
<td>2.84</td>
<td>1.08</td>
</tr>
</tbody>
</table>

**Figure 4. Average interaction energies (IE) of the residues which have considerable interactions with ligands (TCL, S54, and IDN) in A configurations for last 1.5 ns in the 3ns MD simulations.**

IDN-F203 H-π interaction into π-π stacking interaction, in which the interaction energy increased from −1.76 kcal/mol to
−3.10 kcal/mol, was observed. For π-π stacking interaction is maximal
when two π-rings are displaced by forming a half overlapped sandwich.39,40 The π-π stacking interaction is known to
be compatible to the H-π interaction according to their substitu-
ts.41,42 From the MD simulations the total interaction energies
of ligands decreased by 5.8% for TCL, 9.7% for S54 and 14.9%
for IDN case. And the NAD⁺ -ligand interaction energies de-
creased by 7.5% for TCL, 29.0% for S54 and 41.3% for IDN
case. This means that the decrease of total ligand interaction
energies stems from the decrease of NAD⁺ -receptor interaction
energies. But the IDN ligand has the largest total interaction
energy (−80.51 kcal/mol) even after the MD simulations. These
total interaction energy of IDN is larger than the total interaction
energy of TCL. The total interaction energy (−50.00 kcal/mol) of S54 is compatible to that (−49.49 kcal/mol) of TCL. But the free energy change (−45.50 kcal/mol) with the solvent effect of only IDN is larger than that (−34.88 kcal/mol) of TCL. This trend agrees with the experimental results as shown in Table 2.

Figure 5 shows the B-factors from the MD simulations of E. coli ENR-NAD^+ -ligands. In the E. coli ENR-NAD^+ -TCL complex the 1I92-S198 loop was known to be flexible for the docking of TCL ligand. As shown in Figure 3 the B-factors of the residues of this loop and the residues in its vicinity have generally large values. The residues around L100-A115 and E150 make helical structures, which have relatively large B-factors but they are located aloof from the ligands. The residues Y156, K163, Y146 and M159 which have strong interactions with the ligands in common were gauged to show relatively small B-factors.

Conclusions

In the experiments actually IDN ligand is known to have considerably good inhibitory activities against FabI and FabK in comparison with TCL. And ZAM and AYM were proposed to show reasonable inhibitory activities against E. coli FabI. Through the energy minimizations and molecular dynamics simulations it is understood that ZAM and AYM ligands have large interaction energies with ENR-NAD^+ complex of E. coli FabI which are compatible to the interaction energy of TCL ligand and IDN has larger interaction energy than TCL, ZAM and AYM. This result agrees well with the experimental inhibitory activities against E. coli FabI. The experimental IC_{50} values against E. coli FabI of TCL, ZAM, AYM and IDN are 0.43, 0.52, 0.37 and 0.07 μM. For TCL ligand the ratio of NAD^+ -ligand interaction energy to the total interaction energy of ligand is 36.4% on average, but for IDN ligand the average ratio is 19.7%. The contribution of residues to the ligand binding is larger in E. coli ENR-NAD^+ -IDN than in E. coli ENR-NAD^+ -TCL. The large-size ligands with many functional groups and aromatic rings are calculated to have large interaction energies with the ENR protein and NAD^+ coenzyme. The functional groups of ligands which have considerably strong interactions with residues and NAD^+ are aromatic π rings, acidic hydroxyl groups, and polarizable amide carbonyl groups. IDN, ZAM and AYM ligands have indole and amide moieties in common. For the six handled receptors the residues Y156, K163, Y146 and M159 strongly interact with the ligands in common. The side chain of Y145 and Y156 is phenol group, M159 thiomethoxy group and K163 positively charged protonated amine. In general, the cation-π interactions including π_cation-π interaction have stronger interactions and also cations have strong interactions with polarisable carbonyl group. And the acidic phenolic hydroxyl group has strong H-bond interactions. IDN and ZAM ligands have similar functional groups to each other but their locations and structures are different. We hope that this information would be useful in the design of ENR inhibitors.

References


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