

# Antidepressant specificity of serotonin transporter suggested by three LeuT–SSRI structures

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Sertraline and fluoxetine are selective serotonin re-uptake inhibitors (SSRIs) that are widely prescribed to treat depression. They exert their effects by inhibiting the presynaptic plasma membrane serotonin transporter (SERT). All SSRIs possess halogen atoms at specific positions, which are key determinants for the drugs' specificity for SERT. For the SERT protein, however, the structural basis of its specificity for SSRIs is poorly understood. Here we report the crystal structures of LeuT, a bacterial SERT homolog, in complex with sertraline, R-fluoxetine or S-fluoxetine. The SSRI halogens all bind to exactly the same pocket within LeuT. Mutation at this halogen-binding pocket (HBP) in SERT markedly reduces the transporter's affinity for SSRIs but not for tricyclic antidepressants. Conversely, when the only nonconserved HBP residue in both norepinephrine and dopamine transporters is mutated into that found in SERT, their affinities for all the three SSRIs increase uniformly. Thus, the specificity of SERT for SSRIs is dependent largely on interaction of the drug halogens with the protein's HBP.

SSRIs bind directly to the serotonin-transporter protein and inhibit neurotransmitter recycling, making them effective drugs for the treatment of depressive disorders<sup>1,2</sup>. SSRIs, however, are rather promiscuous in that they also bind to the homologous norepinephrine and dopamine transporters (NET and DAT, respectively), although with much lower affinity than to their principal target, SERT<sup>3,4</sup>. The selectivity of SSRIs for SERT is intriguing. Merely one or two different functional group substitutions are sufficient to convert an SSRI into a norepinephrine-reuptake inhibitor (NRI) with higher affinity to NET<sup>5–7</sup>. It is recognized that both the position and type of substitution on an aromatic moiety of the SSRI molecule are important for the higher specificity to SERT<sup>8,9</sup>. In particular, halogen substitutions on this ring are found to be largely responsible for SSRIs' specificity to SERT<sup>5,6,10</sup>. On the protein side, however, the transporter–SSRI interactions that define the specificity of SERT for these drugs have not yet been described, which hinders the development of more specific antidepressants<sup>11</sup>.

The human SERT, NET and DAT proteins are all members of the neurotransmitter:sodium symporter (NSS) family<sup>12–14</sup>. The same protein family also contains members from bacterial cells, and such proteins often function as amino acid transporters<sup>15</sup>. One family member is the leucine transporter LeuT from *Aquifex aeolicus*. LeuT shares 20–25% identity in primary sequence with the human neurotransmitter transporters, and the crystal structure of LeuT<sup>16</sup> and its transport mechanism have proven to be good model systems for the study of mammalian NSS proteins<sup>17–20</sup>. To understand the structural basis of the serotonin transporter's specificity for SSRIs, we carried out crystallographic studies of the bacterial leucine transporter LeuT in

complex with three different SSRIs. This was followed by mutagenesis and pharmacological studies of the human SERT, NET and DAT proteins at the equivalent drug-binding site.

## RESULTS

We first showed that three SSRIs—sertraline, R-fluoxetine and S-fluoxetine—all bind to LeuT (Fig. 1a) and that they also inhibit substrate transport by the protein reconstituted into proteoliposomes (Fig. 1b). We then cocrystallized LeuT with either sertraline, R-fluoxetine or S-fluoxetine (Prozac contains equal amounts of the R- and S- enantiomers, both pharmacologically active), along with substrate leucine and sodium ions, and determined these complex structures at a resolution of 2.15 Å, 2.35 Å and 2.45 Å, respectively (Table 1 and Supplementary Fig. 1a online). In all these crystals the overall structure of the protein–substrate complex is similar to that of the drug-free form<sup>16</sup>. However, in all three complexes, we observed a strong electron-density peak in the vestibule between the tip of the extracellular loop EL4 and the extracellular gate (Fig. 1c and Supplementary Fig. 1a), which is formed by residues Arg30, Asp404, Tyr108 and Phe253. The density was assigned to sertraline, R-fluoxetine and S-fluoxetine, respectively (Figs. 1d–f). This drug binding location is similar to the tricyclic antidepressant (TCA)-binding site in LeuT<sup>21,22</sup>, and no secondary SSRI-binding site was found in the protein.

## Sertraline-binding site in LeuT

Although the position of binding to the protein is similar to that observed for TCAs, how the SSRIs bind to the protein is markedly

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