

Molecular basis for substrate and inhibitor interactions with the glycine transporter, GlyT2

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Two subtypes of glycine transporters, GlyT1 and GlyT2, work to regulate extracellular glycine concentrations. While both glycine and sarcosine are GlyT1 substrates, GlyT2 is exquisitely selective for glycine. No other amino acid can be transported or modulate glycine transport by GlyT2. The primary substrate binding site, S1, lies towards the centre of GlyT2 and residues lining this site are proposed to contribute to substrate specificity (Yamashita *et al.*, 2005). More recently, a second substrate binding site (S2) has been proposed to lie above S1, with substrate binding to S2 triggering the release of substrate from the S1 (Shi *et al.*, 2008). We have investigated the molecular basis for substrate selectivity of GlyT2 and explored the possible existence of S2 in this transporter. Site-directed mutagenesis was employed to mutate residues within the predicted S1 and S2 sites of GlyT2. Transporters were expressed in *Xenopus laevis* oocytes and activity was monitored using two-electrode voltage clamp. Mutations within the GlyT2 S1 site changed substrate selectivity. For example, W482F changes GlyT2 from being highly selective for glycine to a promiscuous transporter that allows transport of alanine, valine, leucine, cysteine, serine, tyrosine and phenylalanine. Mutations within the proposed S2 site did not alter substrate selectivity of GlyT2. The GlyT2 inhibitor, ALX1393, causes a reduction in maximal rate of glycine transport and also increases the $K_{0.5}$ for glycine, demonstrating that it has characteristics of both a competitive and non-competitive inhibitor. Mutations within S1 significantly reduced ALX1393 potency ($P < 0.05$). However, mutations within the proposed S2 site did not alter ALX1393 potency.

Our findings demonstrate that subtle changes within the S1 binding site have a significant impact on the substrate selectivity of GlyT2. We do not find any evidence in support of a S2 site and if it does exist, then it does not contribute to substrate selectivity. We also demonstrate that the binding site for the GlyT2 selective inhibitor, ALX1393, overlaps with the S1 substrate binding site.

Shi L, Quick M, Zhao Y, Weinstein H, Javitch JA. (2008) *Mol Cell* **30**, 667-73.

Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E. (2005) *Nature* **437**, 215-23.