MODELLING THE HUMAN GAMMA-AMINOBUTYRIC ACID TRANSPORTER AND FUNCTION

(Ph.D. Theses)

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INTRODUCTION

The major inhibitory neurotransmitter within the brain γ-aminobutyric acid (GABA) released during neural activity is taken up by the neuronal GAT-1 and the glial GAT-2/GAT-3 transporter subtypes translocateing GABA uphill its concentration gradient. GATs are members of the sodium and chloride ion-dependent solute carrier 6 family (SLC6). The driving force for the uphill GABA transport is the coupled movement of Na^+ ion down its concentration gradient, existing between extracellular (EC) and intracellular (IC) compartments.

GATs are potential targets for antiepileptic drugs (AEDS). Successful design has provided Tiagabine acting on hGAT-1. About 20-30 per cent of epileptics can not be treated with known AEDS. Refractoriness to AEDS prompted “original drug” discovery campaigns to develop new strategies by exploring new targets for the treatment of epilepsy, including the glial subtypes of hGATs.

Disclosure of the high resolution crystal structure of leucine transporter from *Aquifex aeolicus* (LeuT), a bacterial orthologue of hGATs and monoamine transporters in an occluded state with Leu catalyzed searches for druggability of SLC6 family transporter subtypes. The crystal structure discloses 12 transmembrane helices (TMs) which comprise two helices with unwound regions (TM1 and TM6) between which the adjacent substrate and Na^+ ion binding sites are nestled. Crystal structures of transporters disclosed so far justify the hypothetical alternate access mechanism of transport which goes back to the general theory of the mechanism of membrane transport describing protein surfaces exposed alternately from the IC or EC side of the membrane.

Here we summarize LeuT structure-based homology modelling of human GAT subtypes (hGATs) in the occluded state with GABA and different approaches developed in order to predict GABA transporter subtype-specific functions.

SPECIFIC AIMS ARE

To explore the relationship between the GABA and Na^+ ion symport process and the structure of the transporter proteins, including:

1. To build model structures of the human neuronal hGAT-1 and the glial hGAT-2/hGAT-3 subtypes, based on the crystal structure of the bacterial homologue LeuT;

2. To compare binding interactions of hGAT-1, hGAT-2 and hGAT-3 transporter subtypes including report on the binding interactions between GABA/substrate inhibitors and hGAT proteins, and the search for subtype-specific pharmacophore models and potential Zn^{2+} ion binding interactions;

3. To suggest a molecular mechanism for Na^+ ion symport on the basis of the structural description of binding between $Na^+(1)$, $Na^+(2)$, hGAT proteins and substrates.

METHODS

Homology modelling

The hGAT homology models were based on the crystal structure of LeuT (Protein Data Bank code: 2a65). The models were built using the Swiss Model server and were optimised by molecular mechanics calculations with the MMFF94 force field of the Sybyl program package.

Docking

Docking methods were optimised in order to meet the requirements concerning the experimental rank of inhibition. The GOLD program applying the GOLD scoring function was used for calculations. Based on analogy with the LeuT-Leu complex, the carboxyl group of the ligand was tethered to the TM1-bound $Na^{+}(1)$ ion by a distance constrain of 2.1-2.6 Å.

Molecular dynamics

Calculations were preformed using CHARMM program and CHARMM2 force field. During simulations the positions of those atoms which were farther than 20 Å from the ligand were fixed. For simulation of Zn^{2+} ion binding, all residues were fixed except those representing the EC part of the protein.

[^{3}H]GABA uptake experiments

Measurements were carried out using plasmamembrane vesicle fraction freshly isolated from the rat cerebral cortex. Non-specific binding was determined in the presence of 1 mM guvacine. In order to isolate transport processes mediated by GAT-2/GAT-3, hGAT-1-specific uptake inhibitor 1-(2-{[(diphenylmethylene)amino]oxy}-ethyl)-1,2,5,6-tetrahydro-3-pyridine-carboxylic acid hydro-chloride (NNC-711, 1 mM) was applied to block hGAT-1.

MAIN RESULTS AND THESES

1. The structure of hGAT-1 model is similar to that of LeuT, substrate binding core and Na^+ ion binding sites are close to each other.

2. Docking and molecular dynamics calculations disclosed that the amino function of the effective inhibitors favours binding to the Tyr60 on the unwound region of TM1 helix and to the Ser396 on TM8. High docking scores and the favoured binding mode are sufficient for predicting whether a ligand were to inhibit GABA transport or not.

3. A pharmacophore model for hGAT-1 was suggested: effective inhibitors adopt a semi-extended conformation with a distance between amino and carboxyl functions $\delta_{(N\text{-}C1)}$ of 4,1 ± 0,4 Å and the optimal value for GABA torsion angle closest to the carboxyl function C(1)-C(α)-C(β)-C(γ) (τ_C) is 185 ± 15° (Figure 1, left). By comparison, the $GABA_A$ receptor-bound conformation of GABA is extended when related to that of the GABA transporter, similarly to the excitatory neurotransmitter Glu.

4. The GABA binding cores of hGAT-2 and hGAT-3 are different from that of hGAT-1 and similar to each other, therefore cannot be used for predicting glial subtype-selective substrate inhibitors.

5. Major differences among subtypes are found in the second EC loop. Location of the hGAT-3 subtype-selective Zn^{2+} ion binding sites suggests allosteric inhibition mechanism in which the second EC loop is involved.

6. Docking and molecular dynamics revealed the existence of semi-extended (Figure 1, left) and ring-like (Figure 1, right) conformations of GABA-Na^+(1) complex within the binding core, suggesting that the pharmacophore conformation is different from the trafficking conformation of GABA. These findings may anticipate the way Na^+ symport proceeds within the family of SLC6 transporters.

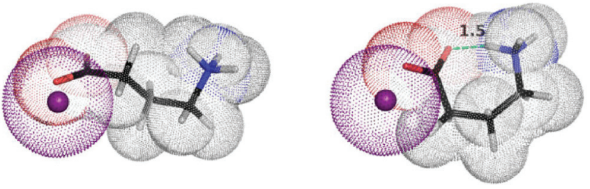

Figure 1. Semi-extended and ring-like conformations of GABA in complex with Na^+(1) in the substrate binding crevice of the occluded state of hGAT.

CONCLUSION

Docking GABA and substrate inhibitors into the substrate binding crevice of the occluded state hGAT-1 homology model, and the pharmacophore established this way are proposed to be effective tools predicting potential substrate inhibitors at hGAT-1. In contrast, glial hGAT-2 and hGAT-3 subtypes cannot be distinguished by their substrate binding cores, however by their allosteric Zn^{2+} ion binding sites. The formation of GABA-Na^+(1) complexes nestled by the unwound regions of the TM1 and TM6 helices in the occluded conformation of hGATs may provide mechanistic clues for Na^+ ion-coupled amino acid symport. LeuT structure-based homology modelling of hGATs disclosed a platform of new approaches with the potential of more effective medicinal chemistry campaigns in the future.

THESES RELATED PUBLICATIONS

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3. Kardos J, Palló A, Bencsura Á, Simon Á. Assessing structure, function and druggability of major inhibitory neurotransmitter γ-aminobutyrate symporter subtypes. Current Medicinal Chemistry, submitted

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ADDITIONAL PUBLICATIONS

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2. Kardos József, Harmat V, Palló A, Barabás O, Szilágyi K, Gráf L, Náray-Szabó G, Goto Y, Závodszky P, Gál P. Revisiting the mechanism of the autoactivation of the complement protease C1r in the C1 complex: structure of the active catalytic region of C1r. Mol Immunol 45, 1752-1760 (2008) IF: 3.555