Novel structural models of the Creatine transporter (SLC6A8) rationalize its structural determinants of binding

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Introduction

Creatine is a crucial metabolite that plays a fundamental role in ATP homeostasis in tissues with high-energy demands. The creatine transporter (CreaT, SLC6A8) belongs to the solute carrier 6 (SLC6) transporters family, and more particularly to the GABA transporters (GATs) subfamily. Understanding the molecular determinants of specificity within the SLC6 transporters in general, and the GATs in particular is very challenging due to the high similarity of these proteins.

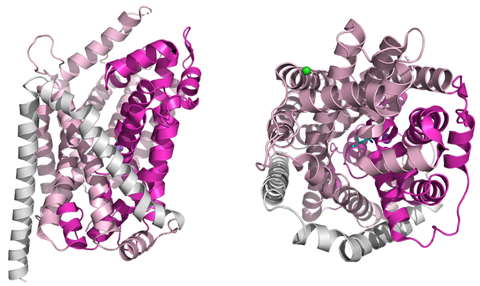
Goals and methods

- Finding key structural features involved in binding selectivity for CreaT using structure-based computational methods.
- Building reliable homology models using two templates :

⇒ the human serotonin transporter (hSERT)[1]– outward open conformation

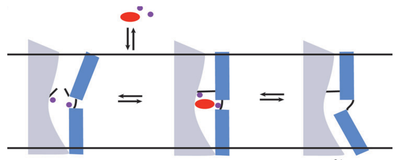
⇒ the prokaryotic leucine transporter (LeuT)[2] – occluded conformation

Templates : hSERT and LeuT



Three-dimensional structure of LeuT, (PDB ID 2A65[2]) from side and top view respectively.

- LeuT and hSERT share 21% and 44% sequence identity with human CreaT respectively
- Similar“LeuT-fold”
- Use the gated-pore mechanism



Gated-pore mechanism of transport[3]

Sequence alignments

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LeuT              396 -LNKSLDEMDFWAG-TIGVVFFGLTELIIFFWI
SLC6A4_(SERT)     484 GGAYVVKLLEEYAT-GPAVLTVALIEAVAVSWF
SLC6A3_(DAT)      467 GGIYVFTLLDHFAA-GTSILFGVLIEAIGVAWF
SLC6A2_(NET)      464 GGIYVLTLLDTFAA-GTSILFAVLMEAIGVSWF
SLC6A6_(TauT)     450 GGMYVFQLFDYYAASGVCLLWVAFFECFVIAWI
SLC6A8_(CreaT)    465 GGMYVFQLFDYYSASGTTLLWQAFWECVVVAWV
SLC6A1_(GAT1)     442 GGIYVFKLFDYYSASGMSLLFLVFFECVSISWF
SLC6A13_(GAT2)    438 GGMYVFQLFDYYAASGMCLLFVAIFESLCVAWV
SLC6A11_(GAT3)    458 GGMYIFQLFDSYAASGMCLLFVAIFECICIGWV
SLC6A12_(BGT1)    443 GGMYIFQLFDYYASSGICLLFLSLFEVVCISWV
SLC6A9_(GlyT1)    519 AGIYWLLLMDNYAA-SFSLVVISCIMCVAIMYI
SLC6A5_(GlyT2)    567 GGIYMFQLVDTYAA-SYALVIIAIFELVGISYV
SLC6A14           469 AGIYWVHLIDHFCA-GWGILIAAILELVGIIWI
SLC6A7_(PROT)     445 GGMYWLVLLDDYSA-SFGLMVVVITTCLAVTRV
SLC6A20           452 AGNYWFDIFNDYAA-TLSLLLIVLVETIAVCYV
SLC6A15_(B0AT2)   516 SGNYFVTMFDDYSA-TLPLLIVVILENIAVCFV
SLC6A17_(NTT4)    515 SGNYFVTMFDDYSA-TLPLTLIVILENIAVAWI
SLC6A19_(B0AT1)   477 SGQYWLSLLDSYAG-SIPLLIIAFCEMFSVVYV
SLC6A18_(B0AT3)   463 SGNYWLEIFDNFAA-SPNLLMLAFLEVVGVVYV
SLC6A16_(NTT5)    523 SGSYFIRLLSDYWI-VFPIIVVVVFETMAVSWA
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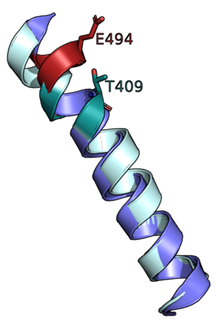
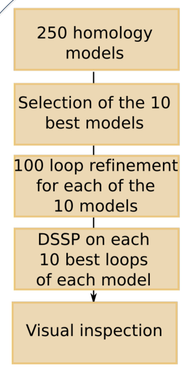
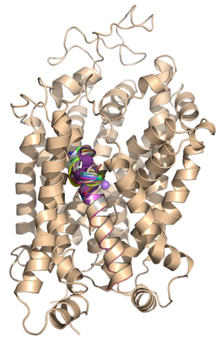
The sequence alignments of the SLC6 family reveals an additional amino acid in TM10 of all the GATs subfamily, suggesting the presence of a **π-helix.**



π-helices were identified at distinct positions in the templates LeuT (cyan) and SERT (red).

Homology modeling



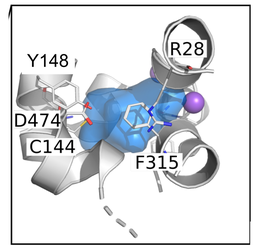


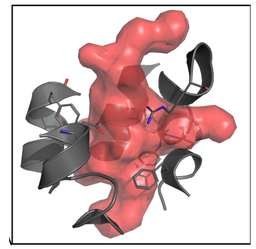
- Generation of a 3D model of a protein with an unknown structure (‘target’) based on an experimentally determined structure of a homolog protein (‘template’)[3].
- The protocol generally includes several steps (c.f. flow chart) ranging from template selection to model validation.
- The process is iterative until a suitable model is obtained.
- We modeled the CreaT transporter in the outward open and occluded conformation
- We included a loop refinement procedure to optimize this TM10 insertion.

Main differences between the two conformations :

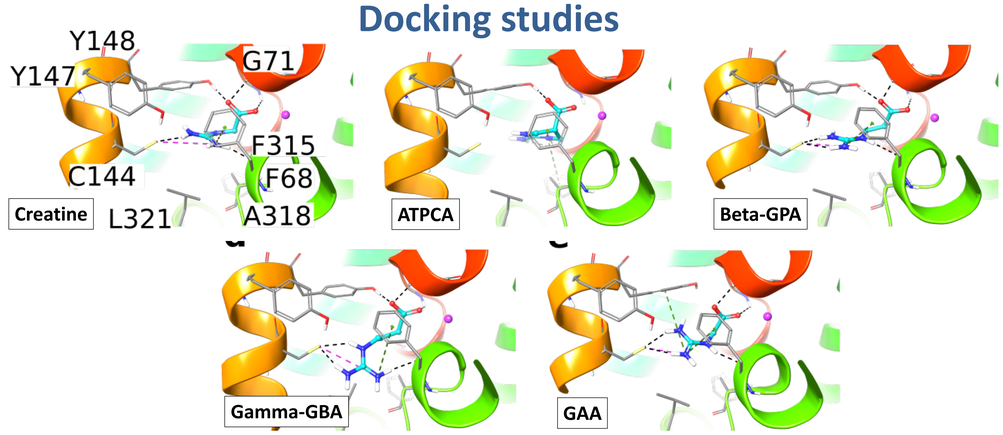
- a tilting of two broken helices TM1 and 6 on the extracellular side,
- a flipping of the conserved Tyrosine 148 (hydrophobic extracellular lid).

=> Significant increase of the binding site volume,[4] i.e. 285 Å^3 in the outward occluded conformation *vs.* 1293 Å^3 in the outward open conformation.





Docking studies



- Induced fit docking of known CreaT ligands in the occluded conformation to rationalize biological activities.
- An optimal length of carbon linker (4.5-5 Å) seems necessary between the guanidine and carboxylate groups to establish hydrogen bonds with respectively Cys 144 and Gly 71 and the Na^+ .

Conclusions

- Our homology models provide structural insight into the structural determinants characterizing the substrate selectivity of CreaT.
- The presence of a π-helix in TM10 provides a specific packing of the binding site.
- Screening large virtual libraries would allow to validate our binding mode hypothesis.

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3. Colas, C., Ung, P. M. & Schlessinger, A. SLC Transporters: Structure, Function, and Drug Discovery MedChemComm 7(6):1069-1081 (2016)
4. Smith, R. H. B., Dar, A. C. & Schlessinger, A. PyVOL: a PyMOL plugin for visualization, comparison, and volume calculation of drug-binding sites. *bioRxiv* (2019).

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