

University of Groningen

Purification and Reconstitution of the Glutamate Transporter of *Bacillus Stearothermophilus*.
Slotboom, D.J.; Lolkema, J.S.; Konings, W.N.

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1996

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Slotboom, D. J., Lolkema, J. S., & Konings, W. N. (1996). *Purification and Reconstitution of the Glutamate Transporter of Bacillus Stearothermophilus.*

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

C5 Membrane-bound carriers

P-C5-17

SITE-DIRECTED SPIN LABELING AND CHEMICAL CROSS-LINKING DEMONSTRATE THAT HELIX V IS CLOSE TO HELICES VII AND VIII IN THE LACTOSE PERMEASE.

WU J, VOSS J, HUBBELL WL¹, KABACK HR
Howard Hughes Medical Institute, and ¹Jules Stein Eye Institute, Univ. of Calif. Los Angeles (USA).

Purpose: To determine the structure and mechanism of the lactose permease of *E. coli*.

Methods: Permease containing a biotin domain and paired Cys residues at positions 148 (helix V) and 228 (helix VII), 148 and 226 (helix VII) or 148 and 275 (helix VIII) was affinity purified and labeled with a sulfhydryl-specific nitroxide spin label. The EPR spectra at ambient temperature and in the frozen state were analyzed to determine the proximity of each pair of tethered spin labels. Also, dibromobimane is used to crosslink paired-Cys residues.

Results: Spin-spin interactions are observed with the 148/228 and 148/275 pairs. No interaction is evident with the 148/226 pair. Analysis of broadening of the EPR spectra in the frozen state yields inter-spin distances of approximately 9 Å and 14 Å for 148/228 and 148/275, respectively. Dibromobimane crosslinks paired Cys 148/228.

Conclusions: The results provide strong support for a structure in which helix V is in close proximity to both helices VII and VIII and oriented where Cys148 is closer to helix VII.

P-C5-19

PURIFICATION AND RECONSTITUTION OF THE GLUTAMATE TRANSPORTER OF *BACILLUS STEAROTHERMOPHILUS*.

SLOTBOOM DJ, LOKKEMA JS, KONINGS WN.
Department of Microbiology, University of Groningen (NL)

Purpose: The cation selectivity of the glutamate carrier of *B. stearothermophilus* depends on the host in which it is expressed. In membrane vesicles of *B. stearothermophilus* one Na⁺ and one H⁺ are cotransported with glutamate, while in *E. coli* vesicles two protons are used. Differences in lipid environment may be responsible for this discrepancy.

Methods: The purified protein is reconstituted in liposomes composed of different phospholipids in order to study the influence of the lipid environment on the cation selectivity of the carrier.

Results: The carrier, fused to a N-terminal His-tag, was expressed in *E. coli* and could be purified from the solubilized membranes in one step using Ni²⁺-affinity chromatography. The carrier was actively reconstituted in liposomes derived from *E. coli* lipids and was shown not to use Na⁺ as coupling ion.

Conclusions: In liposomes composed of *E. coli* lipids the purified glutamate carrier from *B. stearothermophilus* uses only protons as coupling ions. Attempts are now underway to reconstitute the carrier in liposomes composed of lipids from *B. stearothermophilus*.

P-C5-18

Ca²⁺ REGULATION OF GABA TRANSPORT BY SPM VESICLES: POSSIBLE INVOLVEMENT OF CALCINEURIN ACTIVITY

GONÇALVES PP¹, CARVALHO AP², VALE MGP²

¹Dep. Biologia, Univ. Aveiro (Portugal),

²Centro Neurociências, Univ. Coimbra (Portugal)

Purpose: The transport of γ -aminobutyric acid (GABA) by synaptic plasma membrane (SPM) vesicles is inhibited by the presence of Ca²⁺ inside the vesicles. So, we investigated the possible targets for the Ca²⁺ action in the regulation of the SPM GABA transport in response to elevation of intravesicular [Ca²⁺].

Methods: SPM vesicles were isolated from osmotic disrupted sheep brain cortex synaptosomes by ultracentrifugation in a dextran T110 gradient. GABA uptake by SPM vesicles was measured isotopically by using [³H]GABA and a filtration method.

Results: We tested whether trifluoperazine and okadaic acid alter the inhibitory effect of intravesicular Ca²⁺ on the GABA transport by SPM vesicles observed in response to artificially imposed ionic gradients.

Conclusions: Since 40 μ M trifluoperazine and 10 μ M okadaic acid antagonize the Ca²⁺ effect, we conclude that calmodulin dependent phosphatase activity of calcineurin may be involved in the Ca²⁺ regulation of the neuronal GABA transport.

P-C5-20

CYCLAM LIPIDS AS MEMBRANE-BOUND CHELATORS

CHANG E L,¹ PURANIK D J,^{1,2} LIU, F,³
CHONG P L-G.³

¹Naval Research Laboratory (USA), ²Georgetown University (USA), ³Temple University (USA)

Purpose: We have synthesized N-hexadecyl cyclam (HDC) and N,N'-dihexadecyl cyclam (DHDC) and studied the lipid-mixing properties of these long-chain derivatives of cyclam.

Methods: The behavior of the mixed-lipid systems HDC or DHDC with DMPC was investigated using differential scanning calorimetry (DSC) and fluorescence spectroscopies.

Results: HDC showed no thermal transition between 5 - 95 °C, whereas DHDC had both a pre-transition and a main transition temperature.

Both fluorescence and DSC show that HDC/DMPC mixed in with DMPC with the narrowest mixed-phase region at 50 mol%. DHDC also broadened the phase transition of DMPC. Both kinds of data indicate that the derivatives are mixing into the bilayer, satisfying the criterion for membrane-bound chelation.

Conclusions: These compounds are potentially useful for selectively chelating heavy-metal cations onto lipid membrane surfaces.