Glutamate transport in thermophilic and mesophilic bacteria

Tolner, Berend

Summary

So far, it has been difficult to obtain 3-dimensional crystals of integral membrane proteins which are suitable for structural analysis at high resolution. Membrane (associated) proteins from thermophilic organisms may offer the advantage that solubilized proteins are more stable than proteins from mesophilic organisms, which may be very helpful in crystalization attempts.
In *chapter 1* an overview is given of the special properties of membranes and enzymes from thermophilic microorganisms relative to those of mesophiles. Thermophiles are adapted to life at higher temperature by having special membrane lipids and a higher thermo-*stability* and thermo-*activity* of the proteins. Other adaptations that have been described include high turnover rates of the energy transducing enzymes and the use of Na^+ rather than H^+ as coupling ion in energy transduction.
The aim of the present project was to characterize bacterial glutamate transporters from related bacterial species which differ with respect to the optimal temperature of growth (T_{opt}). For this purpose, *Bacillus caldotenax* (T_{opt} of 70°C), *Bacillus stearothermophilus* (T_{opt} of 63°C), *Escherichia coli* (T_{opt} of 37°C) and *Bacillus subtilis* (T_{opt} of 37°C) were selected.
The cloning of the genes encoding the Na^+/H^+/L-glutamate symport proteins, and characterization of the respective gene products, of *B. stearothermophilus* (*gltT*$_{Bs}$), *B. caldotenax* (*gltT*$_{Bc}$), and the H^+/L-glutamate symport protein of *B. subtilis* (*gltP*$_{Bsu}$) is described in *chapters 2* and *4*, respectively. The genes were cloned by complementation in *E. coli* JC5412 by selecting for growth on glutamate as sole source of carbon, energy and nitrogen. The *gltT*$_{Bs}$, *gltT*$_{Bc}$, and *gltP*$_{Bsu}$ genes encode homologous proteins that are also homologous to transporters for structurally related compounds such as aspartate, fumarate, malate and succinate. Both, sodium- and proton-coupled transporters, belong to this family of dicarboxylate transporter proteins. Hydropathy profiling and multiple alignment of the family of carboxylate transporters suggest that each of the proteins spans the cytoplasmic membrane 12-times with both the amino-acid and carboxy-terminus on the inside (*Chapter 2* and *4*). Comparison of the primary structure of GltT$_{Bs}$ and GltT$_{Bc}$, with that of the H^+/glutamate transport protein of E. coli K-12 (GltP$_{Ec}$) revealed regions of high similarity among regions which differed completely. However, by translating the nucleotide sequence of *gltP*$_{Ec}$ in different reading frames and by comparing the translated sequences with those of GltT$_{Bs}$ and GltT$_{Bc}$, it

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became apparent that the divergence of the sequence was caused by sequencing errors. This was confirmed by resequencing $gltP_{Ec}$ which is described in *chapter 3*. The correct open reading frame encodes a protein of 437 (instead of 395) amino acids, and hydropathy analysis predicts 12 rather than 4 previously suggested membrane spanning α-helical regions.
The substrate and cation-selectivity of $GltP_{Bsu}$ is described in *chapter 4*. Transport of acidic amino acids in *B. subtilis* is an electrogenic process in which L-glutamate or L-aspartate is symported with at least two protons. This is shown by transport studies in membrane vesicles in which a proton motive force is generated by oxidation of ascorbate / phenazine methosulfate or by artificial ion-gradients. An inwards-directed sodium gradient had no (stimulatory) effect on proton motive force driven L-glutamate uptake. The transporter is specific for L-glutamate and L-aspartate, and is inhibited by β-hydroxyaspartate and cysteic acid, but not by α-*methyl*-glutamate.
In *chapter 5* the substrate and cation-selectivity of the H^+/glutamate and Na^+/glutamate symport proteins of *E. coli* K-12 ($GltP_{Ec}$ and $GltS_{Ec}$, respectively), and the Na^+/H^+/glutamate symport proteins of *B. stearothermophilus* ($GltT_{Bs}$) and *B. caldotenax* ($GltT_{Bc}$) are described. L-glutamate transport by these proteins was studied in membrane vesicles derived from cells in which the proteins were either homologously or heterologously expressed. For this purpose, a glutamate transport deficient strain (*gltS*$^-$, *gltP*$^-$) of *E. coli* (ECOMUT1) was constructed. Transport of L-glutamate by each of the proteins was shown to be electrogenic and characterized as high affinity L-glutamate cation-symport. The $GltP_{Ec}$, $GltT_{Bs}$ and $GltT_{Bc}$ transporters translocate L-glutamate and L-aspartate, whereas $GltS_{Ec}$ only facilitates the transport of L-glutamate. Transport of L-glutamate by $GltP_{Ec}$, $GltT_{Bs}$ and $GltT_{Bc}$ is inhibited by β-hydroxyaspartate and cysteic acid, but not by the $GltS_{Ec}$ inhibitor α-*methyl*-glutamate. $GltS_{Ec}$ symports L-glutamate with (at least) two sodium ions. When the L-glutamate transporters $GltP_{Ec}$, $GltT_{Bs}$ and $GltT_{Bc}$ are expressed in *E. coli* transport of L-glutamate was found to be electrogenic and to occur in symport with ≥ 2 H^+. This finding is surprising since earlier studies in membrane vesicles of *B. stearothermophilus* and *B. caldotenax* indicated a Na^+/H^+/L-glutamate symport for both $GltT_{Bs}$ and $GltT_{Bc}$. The sodium ion dependency of these GltT transporters was observed to increase with temperature. These observations suggest that the coupling ion-specificity is influenced by the conformation of the transport proteins, which is different in *E. coli* and the *Bacillus* membranes.