Structure-activity relationship of the bacterial galactose-H+ symport protein: homologue of the human GLUT transporters

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Introduction

Membrane transport proteins are encoded by 5-15% of the genome in all organisms from microbes to man and play a crucial role in the metabolism of living cells by mediating the influx of nutrients and the efflux of toxins and other waste products. Over 600 families of membrane proteins have been identified by extensive sequence analysis and out of these the major facilitator superfamily (MFS) of transport proteins is one of the largest. However, with very few exceptions, little three-dimensional information has been obtained. MFS proteins are generally deduced to have 12-membrane spanning α-helices comprised predominantly of hydrophobic residues, with helices 6 and 7 connected by a cytoplasmic domain containing 60-70 hydrophilic amino acids. The galactose-H^+ symport protein of *Escherichia coli* is one such transport protein and is importantly, a structural and functional homologue of the family of mammalian passive facilitated glucose transporters (GLUT). It is also homologous to the L-arabinose-H^+ symporter and the D-xylose-H^+ symporter of *E.coli* with 34 and 64% identity respectively. The sugar specificities of the *E.coli* transporters vary in the sense that GalP primarily transports hexoses and AraE and XylE transport pentoses. Nevertheless, the sugar specificities of GalP, GLUT1 (found in the human erythrocyte) and GLUT4 (rat adipocytes) are similar, suggesting that GalP is a bacterial equivalent of GLUT1. This proposition is further strengthened by the observation that the antibiotics cytochalasin B and forskolin, which are proven to be potent inhibitors of glucose transport in GLUT1, GLUT2, GLUT3 and GLUT4 also inhibit sugar transport by GalP. GalP is therefore an ideal model for studies of the structure/function relationship of GLUT1 and sugar transporters in general.

Although atomic resolution crystal structures of soluble proteins have been reported in an increasing number, such progress has not been made in terms of transporters and other membrane proteins, which have proven extremely difficult to crystallize. GalP has also resisted rigorous crystallisation attempts for many years, which could partly be due to its inherent conformational flexibility that makes it difficult to obtain stable crystals. The aim of the work here was to identify specific residues of GalP that influence substrate/inhibitor recognition and increase the overall stability of the protein, favourable for crystallisation. Up to 23 different mutants were constructed in order to analyse the role of individual residues in the structure and function of the protein. We describe the characteristics of one such mutant that has glycine in the place of native aspartate (Asp312Gly) at the interface of TM8 and TM9, so changing the residue in GalP to that found at the corresponding position in the inositol transporter, IolF, from *Bacillus subtilis*.

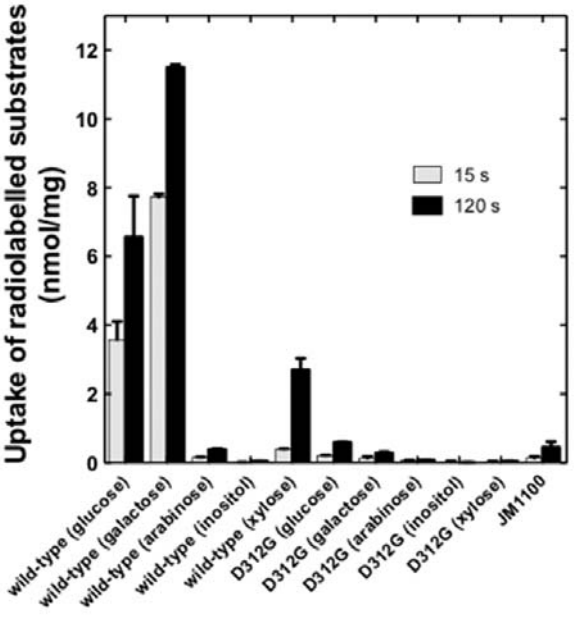

Figure 1: sugar transport assay.

Results

The ability to transport sugars was abolished in the GalP protein with an Asp312Gly mutation (Figure 1). However, fluorescence measurements showed that the protein was still able to bind galactose or glucose substrate. The specificity for other sugars was lost (Figure 1). Furthermore, in the mutant the binding of the inhibitors, cytochalasin B and forskolin, was actually enhanced (Figure 2). Reconstitution of this protein into liposomes also revealed the loss of counterflow activity (Figure 2).

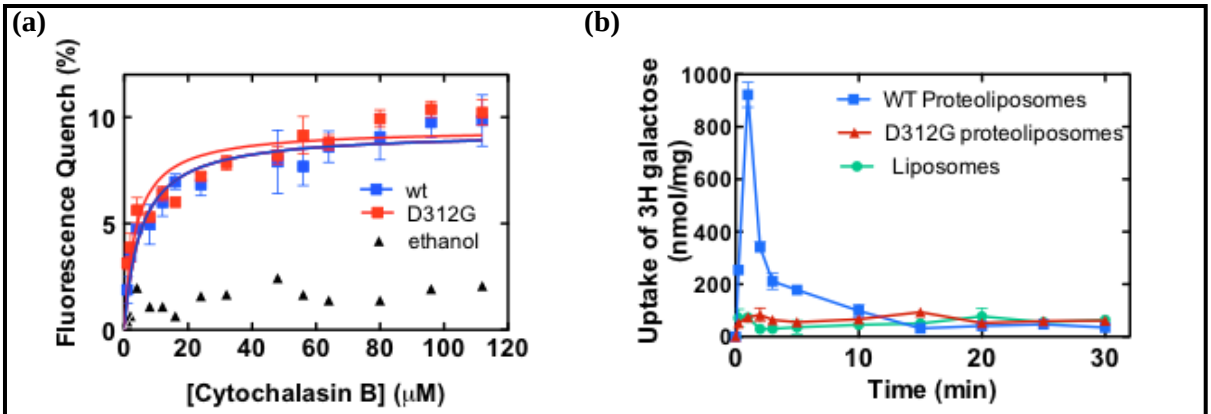

Figure 2: (a) fluorescence binding assay for wild-type and D312G GalP. **(b)** activity of wild-type and D312G GalP reconstituted into liposomes.

The detergent-solubilised and purified mutant protein exhibited much greater thermal stability than the wild-type, especially in maltoside detergents (not shown), and is being taken forward into crystallisation trials.

The results show that the Asp312Gly mutation specifically blocks the structural changes necessary for substrate translocation with little or no effect on ligand binding, creating a stabilised protein more suitable for crystallisation trials than the wild-type.

Publications

Findlay, H., Rutherford, N., Henderson, P. & Booth, P. (2010) Unfolding free energy of a two-domain transmembrane sugar transport protein. *Proc. Natl. Acad. Sci. USA* **107:** 18451-18456.

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Collaborators

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