Poster Presentations

[MS7-P07] Structural study of bile acid transporter Sekiguchi Y[2, 3], Hu NJ[2, 3], Iwata S[1, 2], Drew D[1], Cameron A[4].

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The Apical sodium dependent transporter (ASBT) transport bile acids through the apical membrane of the ileum into the portal blood. ASBT transports bile acids sodium dependently across membranes, with a stoichiometry of two sodium ions per substrate[1]. It is a pharmaceutical target for drugs and several ASBT inhibitors have been developed which can significantly lower plasma levels of cholesterol in animal models[2]. [3]. The X-ray crystal structure of a bacterial homologue of ASBT from *Neisseria meningitidis* ($ASBT_{NM}$) was solved in our lab[4]. In the structure a bile acid and two sodium ions were observed. To understand the mechanism of this protein more fully we have embarked on a program of site-directed mutagenesis in combination with crystallography. 23 mutants of $ASBT_{NM}$ were prepared including residues that interact with sodium or substrate in the crystal structure to investigate which residues were essential for activity.

We solved the structures of two of these mutants E260A and Q77A from vapor diffusion and LCP crystals respectively wih a resolution of 3.2Å and 3.0Å respectively. Both mutated residues interact with the sodium Na1 and Na2 and if these are mutated to alanine the transported activity is decreased. In these mutants structures ASBT adopts a similar conformation to the wild type. In the structure of Q77A, both two sodium ions are observed in the binding sites, however in the structure of E260A, Na1 was not observed in the Na1 binding pocket. This result coincide with their transport activities with less activity of E260A rather than Q77A. To characterize all mutants we developed substrate transport assay system using proteioliposome and it shows significant differences among each sodium binding residues in its transport activity. From these structural and functional data, contribution of respective sodium binding residues for the transport mechanism are revealed. Further detail will be described in the poster including more functional data from different techniques.

References

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