Molecular studies on the bacterial nucleoside transporter NupC

Dawn Hadden, Hao Xie, Simon Patching, Steve Baldwin, Peter Henderson, Richard Herbert, Adrian Brough and Simon Phillips

Introduction

Nucleoside uptake by transport proteins is essential for the synthesis of nucleotides by salvage pathways in many mammalian cells that lack *de novo* purine biosynthetic pathways, such as bone marrow cells, enterocytes and some brain cells. By regulating the concentration of adenosine available to cell-surface receptors, nucleoside transporters in mammals also influence a wide variety of physiological processes including neurotransmission and cardiovascular activity. Moreover, the transporters represent the route of uptake for a variety of nucleoside analogue drugs used in the treatment of cancer and viral infections. Gaining a better understanding of the molecular mechanism of nucleoside transporters is therefore of both physiological and clinical interest. To this end, we and others have so far identified and cloned cDNAs encoding six different types of mammalian nucleoside transport proteins. These belong to two novel protein families, the equilibrative nucleoside transporters or "ENTs" and the concentrative nucleoside transporters or "CNTs". Unfortunately, the study of nucleoside transporters from humans and other mammals is complicated by their low natural abundance. Moreover, it has proven very difficult heterologously to express these proteins at sufficient levels for structural study. We have therefore turned to a bacterial transporter, NupC from *Escherichia coli*, as a more experimentally-amenable model for study.

Membrane topology and overexpression of NupC

NupC, first cloned by our collaborator Dr. Maurice Gallagher, is a proton-linked active nucleoside transporter that is homologous to mammalian sodium-linked nucleoside transporters of the CNT family. Our recent investigations both of NupC and of its mammalian counterparts by glycosylation scanning mutagenesis and other approaches have provided evidence for a 10 transmembrane helix (TM) topology for the bacterial protein (Fig. 1): the mammalian proteins appear to possess three additional TMs in their N-terminal region. To obtain additional direct evidence for these proposed helical arrangements of the transporters in the membrane, we have directed much effort to the overexpression and purification of NupC, so that its secondary structure can be studied by e.g. CD or FTIR spectroscopy. Overexpression of the transporter at levels up to ~25% of the total membrane protein has been successfully achieved. Excitingly, such amounts have proven sufficient for examination of the nucleoside binding site by solid-state NMR approaches. However, the unusual topology of NupC, with very short, periplasmic N- and C-terminal hydrophilic segments, has rendered purification of the overexpressed protein using conventional affinity methods difficult. For example, His-tagging the protein drastically reduces the expression level. Fortunately, a strategy of fusing the N-terminus of the transporter via a flexible linker region to maltose binding protein, which bears an export signal, has proven successful. As a result, we have been able to purify the protein to homogeneity in functional form on a large scale (tens of mg). Current efforts are aimed at producing the protein with a smaller peptide tag, to facilitate spectroscopic analysis.

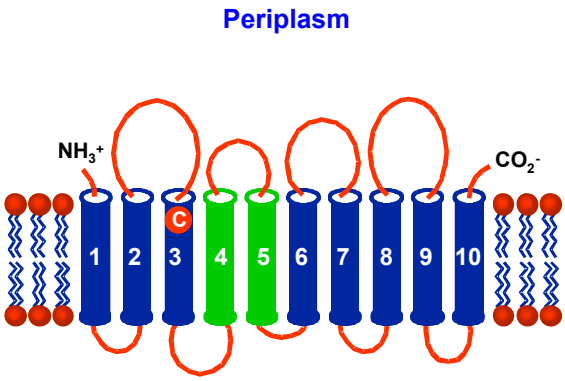

Fig. 1 Predicted membrane topology of the bacterial nucleoside / H^+ transporter NupC

Site-directed mutagenesis of NupC

NupC exhibits a number of additional features that render it a useful model system in which to explore the molecular mechanism of nucleoside transport. In particular, the transporter possesses a single cysteine residue (indicated in Fig. 1 as a red circle in TM3). We have shown that replacement of this residue by alanine has no effect on transport activity, and so have been able to use the cysteineless protein as a template for cysteine scanning mutagenesis. So far, our efforts have been directed towards TMs 4 and 5 (in green in Fig. 1). Our collaborators Professors Jim Young and Carol Cass at the University of Alberta in Edmonton showed some time ago that the corresponding regions of the mammalian homologues of NupC contain residues responsible for substrate selectivity. Cysteine mutants of NupC have been assessed for their intrinsic kinetic properties and for the ability of membrane-permeant and membrane-impermeant thiol reagents to inhibit transport. By this means we have identified a number of residues that appear to play key roles in the transport mechanism or lie on the pathway taken by substrates through the protein.

Future work

Although site-directed mutagenesis is yielding exciting information on the mechanism of transport, a complete understanding will require elucidation of the 3-D structure of the transporter at high resolution. To this end, in addition to the solid-state NMR approach mentioned above, we are shortly hoping to take advantage of the advances we have made in NupC expression and purification to begin 2-D and 3-D crystallisation trials. These will be performed both in Leeds and in collaboration with Professor Per Bullough at the University of Sheffield.

Collaborators

Maurice Gallagher; University of Edinburgh
Per Bullough; University of Sheffield
Tony Watts, Paul Spooner; University of Oxford
Jim Young, Carol Cass; University of Alberta, Edmonton, Canada

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