Ion channel

Ion channels are an intensively investigated protein class in drug discovery. Being membrane proteins, their expression and isolation in a functional state is a demanding task and the use of affinity tags is not well established.

The *Strep*-tag proved already in several cases to be suitable for the functional purification of membrane proteins (see below for references). As an example for ion channels, the work of Rübenhagen et al., 2000, is presented in more detail in this application note. Rübenhagen et al. puified the secondary glycine betaine uptake system BetP of *Corynebacterium glutamicum* from *Escherichia coli* membranes in *Strep*-tagged form after heterologous expression of the *betP* gene (Figure 1) and reconstituted it in *E. coli* lipids. BetP retained its kinetic properties (*V*max and *K*m for betaine and Na^+) as compared with intact cells (Figure 2) demonstrating the *Strep*-tag being compatible with and being valuable for the purification of fully active transmembrane proteins. BetP is predicted to be a typical 12-transmembrane segment transporter carrying cytoplasmically exposed domains of 50–60 amino acids at its N- and C-terminal parts. Therefore, it can be regarded as a representative example for ion channels relevant in drug discovery.

Figure 1

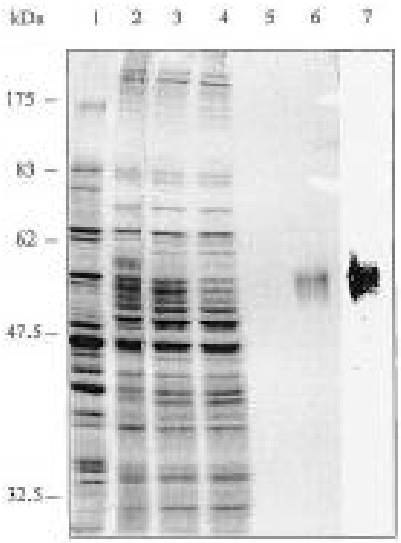

Figure 1: Purification of *Strep*-BetP. Proteins were separated by 12.5% SDS-polyacrylamide gel electrophoresis and stained with silver (*lanes 1–6*), and *Strep*-BetP was immunodetected with *Strep*-Tactin alkaline phosphatase conjugate (*lane 7*). *Lane* 1, induced cells (15 µg protein); *lane 2*, membrane vesicles (15 µg); *lane 3*, solubilized membrane proteins (15 µg); *lane 4*, flow through (15 µg); *lane 5*, last washing fraction (0.1 µg); and *lanes 6 and 7*, elution fraction (1 µg).

Figure 2

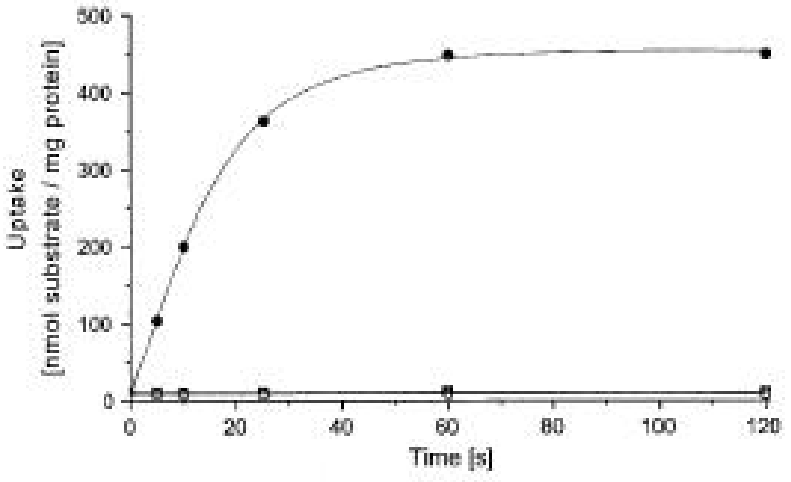

Figure 2: Transport specificity of *Strep*-BetP in proteoliposomes. 15 µM of ^{14}C-radiolabeled compatible solutes were added to proteoliposomes, glycine betaine (*solid circles*), proline (*squares*), or ectoine (*open circles*). For establishing hyperosmotic conditions, 250 mM NaCl was added to the uptake buffer. Determined KM and Vmax were similar to those found for the same transporter in cells of C. glutamicum or *E. coli*. Thus, the reconstituted carrier protein fully retained its functional properties with respect to substrate specificity and affinity toward its substrates betaine and Na^+. Furthermore, after homologous expression in *C. glutamicum* the N-terminal *Strep*-tag was shown to have no significant influence on the regulatory properties of BetP.

References where *Strep*-tag was used for the functional purification of membrane proteins

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