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Tryptophan Fluorescence Spectroscopy, Fluorescent Impurities & Membrane-Bound Proteins

Robillard, G.T.; Swaving-Dijkstra, D.; Broos, J.

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P–C5–13

UNIDIRECTIONAL RECONSTITUTION OF A SECONDARY TRANSPORT PROTEIN

POOLMAN, B., KNOL, J., VEENHOFF, L., AND SULTER, G.
Dept. Microbiology, Univ. Groningen, Haren (NL); internet: B. Poolman@biol.rug.nl

The mechanism of membrane reconstitution of the lactose transport protein (LacS) of *Streptococcus thermophilus* was studied systematically by stepwise solubilization of preformed liposomes and protein incorporation at the different stages of liposome solubilization. The detergents were removed by adsorption onto polystyrene beads. For reconstitutions mediated by *n*-dodecyl-β-D-maltoside, $C_{12}E_8$, and to a lesser extent Triton X-100, the highest transport activities were obtained when the liposomes were titrated with low amounts of detergent. Importantly, under these conditions LacS was reconstituted in a inside-out orientation, as suggested by the outside labelling of a single cysteine mutant with a membrane impermeable biotin-maleimide. The results are consistent with a mechanism of reconstitution in which the hydrophilic regions on the cytoplasmic surface of LacS prevent a random insertion of the protein into the membrane

P–C5–14

TRYPTOPHAN FLUORESCENCE SPECTROSCOPY, FLUORESCENT IMPURITIES & MEMBRANE-BOUND PROTEINS

ROBILLARD,G.T., SWAVING-DIJKSTRA, D. and BROOS, J., GBB/BIOSON, University of Groningen, Groningen (NL)

Purpose: Fluorescent impurities spectroscopically indistinguishable from tryptophan accumulate in detergent-containing enzyme solutions to levels far above the background and prevent detailed analysis of tryptophan fluorescence spectra. The goal was to identify all sources of these impurities and develop methods to eliminate them.
Methods: A tryptophan-minus mutant of the membrane-bound EII^{mtl} was constructed and tyrosine fluorescence was monitored.
Results: An enzyme isolation procedure was developed to eliminate fluorescence impurities arising from all sources. EII^{mtl}(Trp-) and single tryptophan mutant enzymes were then isolated in which the impurities no longer interferred with the tryptophan emission signal.
Conclusion: Tryptophan fluorescence maxima, quantum yields, lifetimes and anisotropies can now be measured for membrane-bound proteins in detergent solutions if the proper precautions are followed in the handling of the protein.

P–C5–15

COTRANSPORT OF H^+, LACTATE AND H_2O IN MEMBRANE PROTEINS.

ZEUTHEN T, HAMANN S, LACOUR, M.
Panum Institute, University of Copenhagen DK

Purpose: Cotransport of H_2O has been demonstrated in the K^+/Cl^- cotransporter (Zeuthen T, J.Physiol.1994,478,203-219). Here it was shown that about 500 H_2O molecules were transported for each pair of ions, i.e. a ratio of 110 mmol l^{-1}. For analogy, we tested another electroneutral symporter: H^+/lactate.
Methods: Microelectrodes recorded rapid changes in cellular H^+-activities and cell volume in the retinal pigment epithelium from Bullfrog. We used TMA^+ as volume marker. The fluxes of H^+ and H_2O across the retinal membrane were correlated to abrupt changes in mannitol and lactate concentrations in the retinal solution.
Results: Retinal hyperosmolarities caused an uphill efflux of H^+ and an efflux of H_2O in a ratio of 105 mmol l^{-1}. Isosmotic increases of lactate caused influxes of H^+ and H_2O in a ratio of 106 mmol l^{-1}. This influx of H_2O could proceed despite opposing osmotic differences of 50 mosm l^{-1}. The fluxes exhibited saturation. Unstirred layer artefacts can be excluded.
Conclusions: Energy can be transferred between the fluxes of H^+, lactate and H_2O in a manner which defines cotransport in a membrane protein.

P–C5–16

HELIX PACKING IN THE LACTOSE PERMEASE OF *ESCHERICHIA COLI* AS DETERMINED BY USE OF DESIGNED METAL ION BINDING SITES AND SITE-DIRECTED SPIN LABELING.

VOSS J*, HUBBELL WL†, KABACK HR*.
*Howard Hughes Medical Institute, and †Jules Stein Eye Institute, Univ. of Calif. Los Angeles (USA).

Purpose: The magnetic dipolar interaction between site-directed metal/nitroxide pairs is used to elucidate helix packing in the lactose permease of *E. coli*, a paradigm for polytopic membrane proteins. Specifically, the goal is to determine the distance and orientation of helix VII with respect to a bound metal in the transmembrane domain of the permease.
Methods: Individual Cys residues were introduced into a lactose permease mutant devoid of native Cys residues for site-directed spin-labeling. The mutants also contained a high-affinity divalent metal ion binding site in the transmembrane domain. The effect on the electron paramagnetic resonance (EPR) spectrum of Cu(II) binding to the engineered binding site was analyzed in terms of Redfield theory for each of the site-directed nitroxides.
Results: The broadening due to bound Cu(II) of the EPR spectrum for nitroxides attached at positions S233C, C234 or T235C of transmembrane helix VII was used to calculate inter-spin distances, between the nitroxide and the metal, of 12.5, 16.1 and >22 Å, respectively.