SecA Exposes a Carboxy-Terminal Domain to the Periplasm

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P–C5–01

SECA EXPOSES A CARBOXY-TERMINAL DOMAIN TO THE PERIPLASM.

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Purpose : To elucidate the structure/function of the bacterial preprotein translocase consisting of the membrane bound components SecY, SecE and SecG, and the peripheral ATPase SecA.

Methods and Results : SecY, SecE and SecG were overexpressed from a synthetic operon. SecYEG$^+$ vesicles showed an increase in translocation and preprotein stimulated translocation ATPase activity, demonstrating the functional overexpression. Furthermore, overexpression of SecYEG leads to increased SecA membrane binding, mainly at the expense of the cytosolic SecA pool. The membrane associated conformation of SecA was analysed by trypsin digestion, immune precipitation, and maleimide labeling of SecA in right-side out and inside out membrane vesicles. These experiments show that SecA, under non translocating conditions exposes a 5 kD domain to the periplasmic side. This 5 kD domain was identified as the carboxy-terminus.

Conclusions : SecA is an intrinsic subunit of the *E. coli* preprotein translocase and protrudes, even under non translocating conditions, the membrane with its carboxy-terminal tail.

P–C5–02

SOLUBILIZATION AND PURIFICATION OF THE ADP/ATP-CARRIER FROM CHICKEN HEART MITOCHONDRIA

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Purpose: Current protocols for the purification of mitochondrial carriers including the ADP/ATP-carrier (AAC) involve chromatography of Triton X-100 extracts on hydroxyapatite (HA). We have analyzed the efficiency of other non-ionic detergents for AAC solubilization. In addition, binding of protein-detergent micelles to different ion-exchange resins was analyzed. The goal is to purify AAC in detergents suitable for protein crystallization.

Methods: Mitochondria were solubilized with a variety of detergents and the extracts analyzed for protein and AAC content. Solubilized proteins were applied to different ion-exchange columns. Binding and elution of AAC was monitored.

Results: The most powerful detergents for AAC solubilization were Triton X-100, dodecyl- and decylmaltoside. With both maltosides and octyl-POE, AAC bound irreversibly to HA but could be eluted and purified by chromatography on DEAE- or CM-Sepharose.

Conclusions: The binding and elution characteristics of AAC to HA are strongly determined by the detergent. In contrast, weak anion- or cation-exchangers provide a tool for carrier purification which is largely independent of the detergent.

P–C5–03

EFFECT OF POLYPRENYL DIPHOSPHATE ON PERMEABILITY AND STABILITY OF MEMBRANES

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Purpose: Phosphopolyisoprenols function as a hydrophobic carrier of glycosyl units across membranes during glycosylation reactions in cells. The influence of hexadecaprenyl diphosphate (C80-PP) on permeability and stability of bilayer lipid membranes made from dioleoylphosphatidylcholine or its mixture with C80-PP was studied.
Methods: Current-voltage characteristics, conductance-temperature relationships, membrane capacitance and membrane breakdown voltage have been studied. Membrane hydrophobic thickness and the activation energy for ion migration have been determined.
Results: C80-PP increases the activation energy for ion migration and the membrane electromechanical stability, and slightly changes the membrane hydrophobic thickness.
Conclusions: We conclude that C80-PP can modulate the permeability and stability of model lipid membranes. (supported by grant SCSR no. 6 P04A 014 10)

P–C5–04

TRANSPORT OF IONS ACROSS MODEL MEMBRANES MODIFIED BY POLYPRENYL MONOPHOSPHATE

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Purpose: Polyprenyl phosphates are involved in glycosylation reactions as a transmembrane carrier lipid. The influence of polyprenyl monophosphate on ion permeability of bilayer lipid membranes made from dioleoylphosphatidylcholine or its mixture with polyprenyl monophosphate was studied.
Methods: Steady-state diffusion potentials, current-voltage characteristics and conductance-temperature relationships have been studied. Membrane permeability coefficients for sodium and chloride ions and the activation energy for ion migration have been determined.
Results: Polyprenyl monophosphate: -decreases membrane permeability coefficients both for sodium and chloride ions; -increases the transference number for sodium ions and the activation energy for ion migration across lipid bilayer.
Conclusions: The results indicate that polyprenyl monophosphate molecules can modulate the transport properties of membranes.
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