1P353**Ion transport across a bilayer lipid membrane facilitated by gramicidin A-Effect of counter anions on the cation transport-**

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Gramicidin A (GA) is a linear polypeptide antibiotic and forms specific ion channels within the BLM for the transport of monovalent cations. There are many studies on the facilitated transport of monovalent cations by GA. Most of research groups regarded the influence of a counter anion on the transport of cations as negligible. On the other hand, a few research groups reported that the ion permeability of liposomes containing GA was affected by anionic species. It is not clear, however, why the counter ion affects the ion permeability. In the present work, the influence of the counter anion on the facilitated transport of K⁺ from one aqueous phase (W1) to another (W2) across a BLM containing GA is investigated by cyclic voltammetry and the ion transport mechanism was elucidated by considering the hydrophobicity of both K⁺ and counter ions and the formation constant of the complex of GA and K⁺. Cyclic voltammograms for the ion transport across the BLM were symmetrical about the origin (0V, 0A) and the ion transfer current increased with an increase in the applied membrane potential. The current height at a definite membrane potential depended on the hydrophobicity of anion species and was proportional to the squared value of the GA concentration in the BLM. It was found that the positive current flowed from W1 to W2 across the BLM was attributable to not only the transport of K⁺ ion from W1 to W2 but also that of the counter anion from W2 to W1. The negative current was then attributed to the opposite reaction.

1P355**ATP cannot be an energy source for the P-type ATPase-mediated cation transport in the Post-Albers scheme**

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The free energy released from the ATP hydrolysis has long been believed to be source of energy for the function of ATPases. The Post-Albers scheme of ion transport in P-type ATPases consists of steps that involve phosphorylation and dephosphorylation of the ATPase, and a transporting cation-dependent conformational change in the ATPase. In a classic experiment Post et al. (1, 2) showed that the scheme is reversible, and synthesized ATP by sequentially treating the Na⁺,K⁺-ATPase with Pi and Na⁺. They pointed out that the interaction free energy of Pi and Na⁺ are equal. Although their results in general support the reaction sequence, they do not necessarily mean that the ATP is an energy source for the Na⁺-transport. Rather, they indicate that it is the Na⁺ binding that activates the ATPase, and the ATP simply releases the energy from the activated ATPase. This contradicts the current concept of ATP as an energy source. Using a different P-type ATPase, the Ca²⁺-ATPase of the sarcoplasmic reticulum, we have demonstrated that the Ca²⁺-bound ATPase contains enough free energy to make ATP. We found that the ATPase formed the ATP by transferring the phosphate group from the pNPP to ADP under the condition where the ΔG° value of the pNPP hydrolysis is less than that of the ATP. Obviously, the ATP plays a role other than being the source of energy in cation transport. References: (1) R. L. Post et. al (1975) J. Supramol. Structure, 3, 479-497. (2) K. Taniguchi, R. L. Post (1975) J. Biol. Chem., 250, 3010-3018.

1P354**Electrochemical elucidation on ion transport across a bilayer lipid membrane -The role of hydrophobic ions as carrier of the counter ion-**

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A bilayer lipid membrane (BLM) can be regarded as the simplest model of biomembranes. There are many studies on ion transport from one aqueous phase (W1) to another (W2) across the BLM facilitated by ion channels or carrier compounds. The current due to the transfer of ions across the BLM is also observed when a hydrophobic ion is added into W1 and/or W2. It has been assumed that the current is responsible for the transfer of the hydrophobic ion, but authors have already reported that the hydrophilic counter ion distributes spontaneously into the BLM with the hydrophobic ion in order to keep electroneutrality in the membrane and predominantly moves when hydrophobicity of the additive ion is very high. On the other hand, mitochondrial uncouplers, such as dinitrophenolate, etc. are well known as negatively charged carriers transporting cations in the mitochondrial inner membrane. The fact supports the authors' idea because uncouplers are classified as hydrophobic ions. In this research, the detailed mechanism of the ion transport across the BLM is elucidated by voltammetry in consideration of the hydrophobicity and the concentration of the additive ion and the hydrophilicity of the counter ion. The role of uncouplers as hydrophobic ions is also discussed.

1P356**Crystallization and X-ray diffraction of SecYE translocon from *Thermus thermophilus* with a Fab fragment**

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The Sec translocation machinery provides a channel-like pathway for the movement of newly synthesized secretory and membrane proteins across and into the plasma membrane (in prokaryotic cells) or the endoplasmic reticulum membrane (in eukaryotic cells). The SecYEG complex in prokaryotes functions as the core component of the protein-conducting channel, called translocon. Prompted by possible advantages of thermal stability of proteins from thermophilic bacteria, we are attempting at determining the structure of the *Thermus thermophilus* Sec machinery. Here, we report crystallization of *T. thermophilus* SecYE complex (TSecYE) complexed further with a specific Fab fragment. Initially, we had crystallized TSecYE alone after thorough optimization of the sample preparation-crystallization conditions, but the crystals never diffracted beyond 6 Å. Attempts were then made to improve the diffraction quality by co-crystallization of TSecYE with an antibody Fab fragment that provides additional hydrophilic surfaces for crystal contacts. We have thus succeeded in obtaining TSecYE/Fab crystals of greatly improved quality that diffracted X-ray at least at 4 Å, revealing a space group of C2.