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Rhodopsin and Other Proteins in Artificial Lipid Membranes*

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Abstract. Some basic aspects of incorporation of hydrophobic peptides and proteins in artificial lipid membranes are discussed. As examples valinomycin as a carrier model and gramicidin *A* as a channel former in lipid vesicles and in planar lipid membranes are presented.

In the second part of the lecture some examples of incorporation of membrane proteins into lipid vesicles and planar lipid membranes are reported. The interaction with artificial lipid membranes of the Ca^{++} ATPase from the sarcoplasmic reticulum, of Rhodopsin, and of Bacteriorhodopsin is presented.

Key words: Lipid bilayer membranes – Membrane proteins on reconstituted systems.

Studies on reconstituted membrane systems are fruitful because it is possible to elucidate the molecular interaction between membrane bound proteins on the lipid phase on well defined systems. Also it should be possible to study the ion transfer across lipid membranes induced by isolated transport systems.

At first some basic aspects of incorporation of hydrophobic ionophoric peptides in artificial membranes are discussed. Valinomycin stands for an example as a carrier molecule and gramicidin *A* for a channel former.

Valinomycin creates on natural membranes and artificial lipid membranes a high potassium selective permeability, whereas gramicidin *A* forms hydrophobic transmembrane channels, which are permeable for monovalent cations.

The molecular interaction of valinomycin with the lipid phase of artificial membranes was investigated by Hsu and Chan [1].

They measured by the C^{13}NMR method in presence and without valinomycin the phase transition properties of dimyristoyllecithin (PC 14 : 0) liposomes and dipalmitoyllecithin (PC 16 : 0) liposomes. As result a marked influence on the phase transition of dimyristoyllecithin liposomes in presence of valinomycin was found, whereas the phase transition of dipalmitoyllecithin-liposomes was unaffected by the

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antibiotic. It is concluded that valinomycin should be located near the interface in the lipid membrane.

These findings are in agreement with the carrier model proposed by Stark et al. [2]. These authors proposed on the basis of electrical relaxation experiments on planar lipid bilayer membranes that valinomycin as a translocation carrier is mainly located near the membrane interface.

A rate number of the loaded and the free carrier respectively was calculated to $10^4\ s^{-1}$.

In contrast to valinomycin gramicidin *A* totally eliminates the phase transition of dipalmitoyllecithin liposomes [3]. These findings confirm the hypothesis that gramicidin *A* forms transmembrane channels.

On planar lipid bilayer membranes it could be shown that gramicidin *A* induces current fluctuation, which indicate that its action consists of a channel mechanism [4]. Urry [5] postulated that such a ionic channel consists of a dimer. This hypotheses is experimentally confirmed by findings of Veatch et al. [6] and Appell et al. [7].

The current fluctuation of a single channel event allows to calculate the transport rate of a cation through the pore.

Under the same conditions, where for the valinomycin potassium system the turnover number was $10^4\ s^{-1}$, a rate of $10^7\ s^{-1}$ was found for the potassium ion through the gramicidin *A* channel.

In the second part of the lecture examples for reconstitution of membrane bound proteines with lipids are presented.

1) One of the best elaborated systems in this field is the Ca^{++} ATPase from the sarcoplasmic reticulum (SR). It was found by several authors that the SR Ca^{++} ATPase can be recombined with liposomes made from defined [8] or natural lipids [9].

Such a Ca^{++} ATPase doped lipid systems show a Ca^{++} sensitive transport activity.

The same protein was investigated in lipid bilayer membranes. Shamoo et al. [10, 11] could show in a series of different papers that the protein after succynilation induces on planar lipid bilayers a large Ca^{++} selective conductance. The molweight of the succynilated protein was 100,000 Dalton. Then, the 100,000 Dalton component was trypsinated to different fractions. After this procedure the smallest fraction, which showed Ca^{++} selective ionophoric behaviour was a 20,000 Dalton component. It is concluded by the authors that this 20,000 Dalton component is the ionophoric part of the SR Ca^{++} ATPase protein.

2) Hong and Hubbell [12] were able to incorporate Rhodopsin in lipid vesicles. The molar ratio between protein and lipid was 1 : 500. After adding of 11-cis retinal it was possible to regenerate bleached Rhodopsin in the vesicles. Furthermore it was shown that Rhodopsin makes the lipid phase more rigid similar to the effect of cholesterol.

The ionic transport through lipid membranes was studied by Darszon and Montal [13]. They showed that the ionic flux for monovalent cations is induced by light. The presence of Ca^{++} amplifies markedly this effect.

On planar lipid membranes, made from two Rhodopsin containing monolayers [14] it was possible to introduce a large light sensitive conductance change. This

effect is presumably connected with the appearence of ionic channels induced by Rhodopsin.

3) It was shown by several authors [15, 16] that purple membrane fragments containing Bacteriorhodopsin can be incorporated in to liposomes made from synthetic well defined and natural lipids. Apparently after illumination the membrane fragments pump protons into the vesicles.

Recently it was shown by Drachev et al. [17] that membrane fragments containing Bacteriorhodopsin can be incorporated in planar lipid membranes. It was shown that after illumination a large proton sensitive potential arises, which becomes to zero after doping the membrane with proton carriers like uncouplers of the oxidative phosphorylation.

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