BIOFUNCTIONAL ASSEMBLIES AT LIPID INTERFACES THROUGH RECOMBINANT SPHERICAL BACTERIAL PROTEIN

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A novel strategy to construct biomimetic assemblies of bio macromolecules on supported membranes is presented. It is based on the anchoring of a 15 nm ball-like recombinant protein, lumazine synthase, on a phospholipid membrane. These proteins have functional groups pointing in solution. They are coupled to phospholipids in solution exhibiting different functional headgroups like biotin and Ni chelator complexes. In this feasibility study we probe the adsorption dynamics of LuSy molecules on phospholipid membranes functionalised with different key-lock like binding mechanisms. The density of adsorbed protein is followed by neutron reflectivity experiments. The influence on the adsorption process of the concentration of a competing inhibiting lipopolymer (phospholipid with polyethylene-glycol headgroup called PEG) at the interface was also measured.

Introduction

The interest in microstructured biofunctional surfaces has increased dramatically during the last years owing to numerous potential practical applications as for example the design of smart biosensors on electrooptical devices (Fromherz [1] , Sackmann [2]) and the surface anchoring of proteins under non-denaturing conditions e.g. for applications in the rapidly developing field of proteomics or to stimulate tissue growth on transplants (Kanthlehner [3]).

These biofunctional surfaces have also a fundamental interest. They allow the study of the physical basis of self-assembly of biomembranes and lipid/protein interaction mechanisms by various surface sensitive techniques. They also allow the design of mimetics of cell and tissue surfaces which permit to study the regulation of cell adhesion by the interplay of receptor mediated lock-and-key forces which are universal interfacial forces. Finally, it plays a key role in the understanding of the cell membrane elasticity (Sackmann [4]).

Lumazine Sythase

In the present work, we introduce a versatile new tool for the design of smart biomimetic solid surfaces based on a large recombinant protein: the lumazine synthase (LuSy) from Bacillus subtilis. Lusy is composed of 60 β subunits and has a ball-like structure with a diameter of 15 nm and a hole of 5 nm in it.

The Lusy proteins are prepared by genetic engineering. They can thus be easily modified and selectively conditioned by coupling of functionalized groups. Lumazine Synthase can exist in a number of different oligomerization states[5-9] and has been studied in considerable detail [8,10-11]. An outstanding feature of Lusy is that the amino as well as the carboxyl end of each subunit are located on the outer surface of the protein, enabling the recombinant coupling of anchoring groups (such as biotin, histidin tags or even other proteins like hisactophilin).

A major advantage of this coupling techniques is that direct contact of the bound protein with the surface is largely avoided and thus non-specific binding is minimised. By using proteins with histidin tags the reversible binding and unbinding of, for example, actin can be induced by small changes in pH (as shown in Behrisch 1995) or by chelator antagonists such as EDTA or Imidazole.

Neutron reflectivity experiments

Over the last years, it has been shown that neutron reflectivity is a very powerful technique to characterise bio-active surfaces with grafted films (Deme [12]). It opens new possibilities to study the interaction of water-soluble amphiphilic proteins with lipid membranes and to observe protein-protein recognition processes.

The experiments were performed at the liquid-air interface using the EROS reflectometer. A teflon tray of a size of 4 cm by 10 cm and 1 mm deep containing 13 ml of D_2O buffer was used. A waterbath was used to control the temperature. To prevent exchange of D_2O with H_2O, a lid with quartz glass windows for the neutron beam was

used to cover the whole cell. To measure and control the surface pressure, a Wilhelmy plate attached to a spring balance was used.
The neutron reflectivity technique allows us to measure, in real time, the amount of the protein adsorbed to a lipid surface.
We present here the evolution with time of the structure of the protein layer for two different functional binding groups: histidin and biotin. In the case of histidin, the effect of the concentration of inhibiting polymers (*i.e* PEG) at the lipid interface has also been tested.
This study shows that different binding behaviour must be considered depending on the functional groups of the protein and the phase of the lipid interface (crystalline or liquid).

NTA protein and PEG

Reflectivity curves have been obtained after adsorption of LuSy functionalized with histidin tags, to the lipid interfaces. These interfaces consist of 90 mol% of DMPC lipids and 10 mol% of DOGS-NTA-Ni lipids which can bind histidin. Curves are represented on figure1. The lipid layer was spread to a surface pressure of about 30 mN/m, where DMPC is known to be still in a liquid phase. The fit enables us to obtain the evolution of the absorbed quantity of protein to the interface as a function of time and to determine the saturation value (Figures 3 and 2).

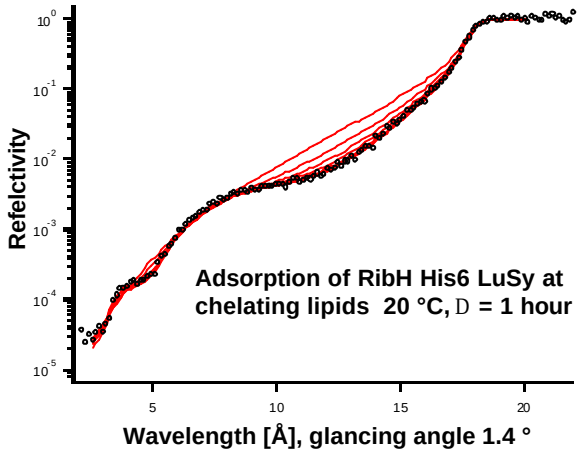

Figure 1. Reflectivity data obtained during the adsorption of LuSy to a Ni Chelator covered surface. The lines correspond to the best fits of the neutron reflectivity data sets, plotted over wavelength. The time distance between two sets of data is one hour.

In order to quantify the influence of the inhibiting polymers at the lipid interface, the same measurements were performed while introducing increasing concentration of PEG lipid in the DMPC matrix (still containing 10 mol% of DOGS-NTA). The results are reported on Figure 2, and show clearly that the amount of LuSy bond to the surface is reduced. The steep decrease of LuSy adsorption between 0.8 and 1.7 mol% PEG can be explained with a phase transition of PEG from a pancake to mushroom like phase.

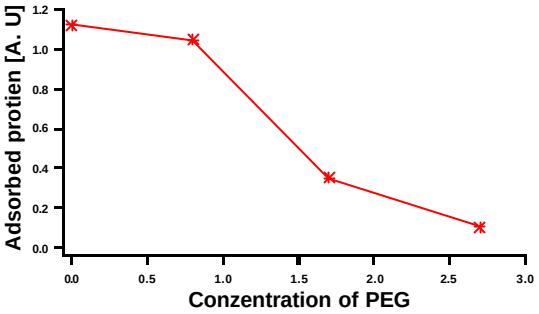

Figure 2. Saturation values of adsorbed LuSy depending on PEG concentration at the lipid layer.

NTA protein and Biotin

The binding dynamics also depends on the phase of the lipid layer or binding interface. To check that fact we choose to measure also the adsorption kinetics of LuSy using another locking process to the interface. This new locking process consist of LuSy functionalized with biotin and streptavidin in solution, a protein which can strongly bind biotin. The binding constant of the streptavidin-biotin binding is much stronger than the one of the histag-Ni chelator binding used previously. Therefore a faster binding process (and eventually a denser protein layer) was expected. In fact, the opposite was observed (see figure 3). One possible reason for this is, that streptavidin forms at the interface a crystallised layer with much less in plane diffusion than the previous liquid DMPC resulting in a slower process. Another possible reason is that the free streptavidin available in the solution, results in aggregation of LuSy. These big aggregates have a much lower diffusion constant in the subphase and this results also in a slower adsorption process leading to a lower effective LuSy concentration.

The presence of a streptavidin mediated interaction between LuSy molecules also gives rise to a new situation at the surface. The LuSy adsorbs to the interface not in the form of a monolayer but in a more complicated fashion (figure 4). This is shown by the fact that it is no longer possible to adequately fit the data with a monolayer model. The correct model consists of a dense monolayer followed by progressively more dilute protein layer.

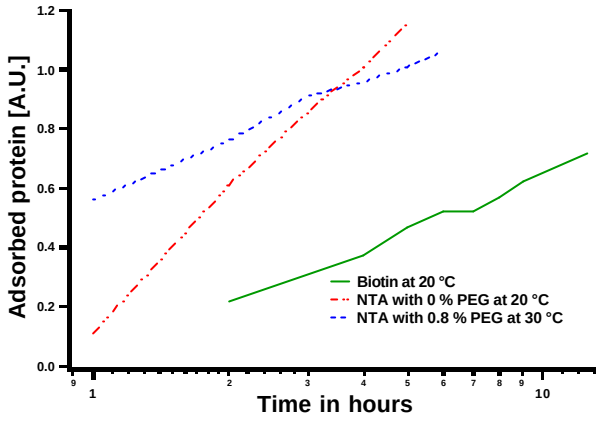

Figure 3. Dynamic adsorption of protein to the interface. The graph shows different dynamical adsorption processes: the adsorption of histidin tagged LuSy without and with 0.8 mol% PEG lipid and compares the two processes with the adsorption of a biotin coated LuSy to a streptavidin interface. Considering the fact that the streptavidin biotin binding is much stronger than the NTA Ni-Chelator binding, this dynamic behaviour needs further explanation (see text).

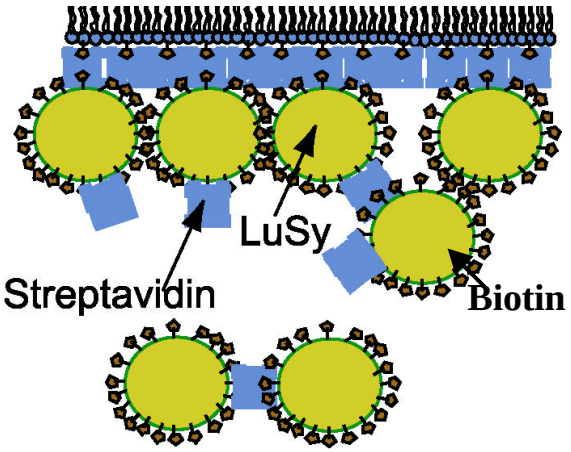

Figure 4. Model of multilayers to fit the data of biotin Lusy adsorbed to a streptavidin interface

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