Eur Biophys J (2001) 30: 42–52
DOI 10.1007/s002490100133

ARTICLE

**Maik Liebau · Annegret Hildebrand**
**Reinhard H. H. Neubert**

# Bioadhesion of supramolecular structures at supported planar bilayers as studied by the quartz crystal microbalance

Received: 27 March 2000 / Revised version: 1 December 2000 / Accepted: 4 January 2001 / Published online: 6 March 2001


**Abstract** A quartz crystal microbalance (QCM) was used to study the adhesion behavior of supramolecular aggregates at supported planar bilayers (SPBs). The QCM technique is a suitable method to detect the adsorption of biomolecules at the quartz surface owing to its sensitivity for changes in mass and viscoelastic properties. To simulate biomembranes, the quartz plates were coated with highly ordered lipid films. Therefore, a combination of self-assembled monolayers and Langmuir-Blodgett films was used. Firstly, the adsorption of liposomes coupled with the lectin concanavalin A was investigated at glycolipid-containing model membranes. Using different carbohydrates, it was possible to determine specific and nonspecific parts of the interactions. The adhesion occurred owing to specific lectin-carbohydrate interactions (about 20%) and to nonspecific interactions (about 80%). The composition of the liposomes was changed to simulate the structure of a native biomembrane consisting of the glycocalix, the lipid-protein bilayer, and the cytoskeleton. An artificial glycocalix was created by incorporating poly(ethylene glycol) into the liposomes. Liposomes which were intravesicular polymerized with polyacrylamide or polyacrylcholate simulated the cytoskeleton. It was determined that the modified liposomes had significant lower interactions with SPBs. The adsorption was reduced by approximately 80% compared to unmodified liposomes. Secondly, a model was developed for the detection of interactions between simple or mixed bile salt micelles and model membranes. It was found that simple bile salts did not adsorb at model membranes. Binary systems consisting of bile salt and phospholipid induced only small interactions. On the other hand, ternary systems consisting of bile salt, phospholipid, and fatty acid showed strong interactions. A dependence on the chain length of the fatty acid was observed. Thirdly, the interaction between ganglioside-containing model membranes and cholera toxin ($\beta$-subunit) was investigated. Different ganglioside fractions showed varying adsorption in the following sequence: $G_{M1} > G_{D1a} > G_{D1b} > G_{T1b}$.

**Key words** Quartz crystal microbalance · Liposome · Micelle · Model membrane · Bioadhesion

**Abbreviations** *ConA*: concanavalin A · *CT*: cholera toxin · *DPPC*: 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine · *DSPC*: 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine · *LA-Na*: lauric acid, sodium salt · *LB*: Langmuir-Blodgett · *MBA*: *N*,*N*′-methylene-bis-acrylamide · *NaC*: sodium cholate · *NaDC*: sodium deoxycholate · *N-glut-PE*: *N*-glutarylphosphatidylethanolamine · *OA-Na*: oleic acid, sodium salt · *PA-Na*: palmitic acid, sodium salt · *PAA*: polyacrylamide · *PAC*: polyacrylcholate · *PEG*: poly(ethylene glycol) · *PEG-PE*: lipid-coupled poly(ethylene glycol) with phosphatidylethanolamine · *QCM*: quartz crystal microbalance · *SA-Na*: stearic acid, sodium salt · *SPB*: supported planar bilayer

M. Liebau[1] · A. Hildebrand · R. H. H. Neubert (✉)
Martin-Luther-Universität Halle-Wittenberg,
Institut für Pharmazeutische Technologie und Biopharmazie,
Wolfgang-Langenbeck-Strasse 4, 06120 Halle/Saale, Germany
E-mail: neubert@pharmazie.uni-halle.de
Tel.: +49-345-5525000
Fax: +49-345-5527292

*Present address*:
[1]Department of Supramolecular Chemistry
and Technology, University of Twente, P.O. Box 217,
7500 AE Enschede, The Netherlands

## Introduction

In the past, a number of studies have been performed to understand the processes of bioadhesion. These studies were focused, on the one hand, on the investigation of physiological mechanisms and, on the other hand, to develop new drug delivery systems. However, the enormous complexity of the native processes requires suitable models.

In the present study, a quartz crystal microbalance (QCM) was used to investigate the adhesion behavior of supramolecular aggregates at model membranes as well as ligand-receptor interactions. The QCM is a method to detect mass adsorption at sensor surfaces based on the piezoelectric effect (Lu and Czanderna 1984). The loading of the quartz plate with gaseous or liquid analyte yields changes in mass and viscoelastic properties at the quartz surface owing to physi- or chemisorption. The resulting shift in resonance frequency $f$ according to Sauerbrey (1959) shows a linear dependence on the change in mass $m_Q$ of the quartz sensor:

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_Q \mu_Q}}\Delta m_Q \quad (1)$$

taking the quartz surface $A$, the basic resonant frequency $f_0$, the density $\rho_Q$ (2648 g cm$^{-3}$), and the shear modulus $\mu_Q$ ($2947\times10^{11}$ g cm$^{-1}$ s$^{-2}$) of the quartz into account.

Strictly speaking, the Sauerbrey Eq. (1) is valid only for gaseous media. In the last decade the QCM technique has become more important for measurements in liquids (Auge et al. 1994; Martin et al. 1994; Nwankwo and Durning 1998). The properties of the liquid medium, like viscosity, density, conductivity, and dielectricity, influence the piezoelectric properties of the sensor. The shear waves, spreading vertically to the quartz surface, are strongly damped. Kanazawa and Gordon (1985) explained the influence of both the density $\rho_{fl}$ and the viscosity $\eta_{fl}$ of the liquid using the mathematical expression:

$$\Delta f = -f_0^{3/2}\left(\eta_{fl}\rho_{fl}/\pi\mu_Q\rho_Q\right)^{1/2} \quad (2)$$

Different phenomena at the solid/fluid interface must be taken into account, like the thickness of the layer, the coupling or adsorption of molecules, and the surface roughness (Schumacher 1990). The influence of surface roughness and morphology of the electrode in contact with a liquid and the effects of viscous dissipation in the interfacial layer have been studied intensively (Urbakh and Daikhin 1994, 1998).

For the detection of biochemical interactions, the modification of the sensor surface is necessary. Combining "self-assembling" and the "Langmuir-Blodgett technique", highly ordered lipid bilayers are formed, the so-called supported planar bilayers (SPBs). Thiol monolayers on gold surfaces are particularly suitable owing to the strong adsorption which is associated with the formation of covalent gold-sulfur bonds (Bain et al. 1989). The adsorption of alkanethiols at gold surfaces follows a two-step process: firstly, the adsorption of thiol groups at the gold electrode and, secondly, the rearrangement of the alkyl chains (Kim et al. 1998).

The Langmuir-Blodgett (LB) technique (Blodgett and Langmuir 1937) uses the spontaneous orientation of amphiphilic molecules at the air–water interface. The lipid monolayers, formed at the water surface, lead to a reduction of the surface tension and can be transferred to solid substrates. The LB films are obtained by a film balance technique. The combination of self-assembled monolayers and LB films allows the preparation of model membranes at the sensor surface. These models simulate native biomembranes (Plant 1999).

Three model systems, investigated in our study, are aimed to demonstrate that the QCM is a suitable tool to characterize physiological processes and to create new models for the development of innovative drug delivery systems.

Recently, several studies focused their interest on the adhesion of living cells at solid surfaces. A number of investigations have been performed with cell cultures using the QCM (Gryte et al. 1993; Redepenning et al. 1993; Janshoff et al. 1996a; Rodahl et al. 1997; Frederiksson et al. 1998; Wegener et al. 1998; Ziegler 2000). In order to study and simulate cellular adhesion at biomembranes, the adsorption of liposomes at carbohydrate-containing model membranes was investigated. The liposomes were coupled with the lectin concanavalin A (ConA) (Lis and Sharon 1998). A number of investigations relating to the adsorption of liposomes at sensor surfaces have been published. Tanaka et al. (1994) studied the adsorption of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) liposomes at polymer-coated quartz plates, referring to the phase transition temperature of the phospholipid. The adsorption of liposomes at platinum surfaces was characterized by Tjärnhage and Puu (1996) in dependence on the composition and the charge of the liposomes. Keller and Kasemo (1998) found differences in the adsorption behavior and the morphology of the absorbed liposomes onto various surfaces like $SiO_2$, oxidized gold, and alkanethiol monolayers. The coating of $SiO_2$ surfaces with lipid bilayers from liposome adsorption are described by Keller et al. (2000). Pignataro et al. (2000) used the principle of molecular recognition with the investigation of the adsorption of biotin-coupled DPPC liposomes to avidin-coated quartz plates.

Proteins, incorporated into the membranes or bound to the surface, and phospholipids, which form liposomes of different size, charge, aggregation structure, and number and fluidity of bilayers in aqueous media, are the major components of biological membranes (Marsh 1996). To simulate this native arrangement, unilamellar liposomes were coupled with the protein ConA (Bendas et al. 1997). The lectin ConA, isolated from *Canavalia ensiformis* (jack bean), contains 237 amino acids per monomer ($M_r = 26{,}500$) and shows an affinity to α-D-mannose and α-D-glucose (Becker et al. 1975; Bouckaert et al. 1995). Interactions between pure ConA and glycolipid coated surfaces were investigated using the QCM by Okahata et al. (1995). The lectin affinity was clearly recognizable, because adsorption was only detected with α-D-glucopyranoside-containing lipid films. Coating with β-D-galactopyranoside lipid layers or

pure 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine (DSPE) films showed hardly any frequency changes.

A native cell membrane consists of the glycocalix, the lipid-protein bilayer, and the cytoskeleton. To simulate this system, the composition of the liposomes can be changed (Ringsdorf et al. 1988). The glycocalix is imitated using so-called "stealth" or "sterically stabilized" liposomes (Lasic 1994) by attaching the hydrophilic polymer poly(ethylene glycol) covalently to the outer liposome surface. The simulation of the cytoskeleton, the three-dimensional network in the cell interior, succeeded by intravesicular polymerization with polyacrylamide or polyacrylcholate (Ringsdorf et al. 1988).

As mentioned above, the field of drug delivery systems has been intensively investigated in recent years. These systems are supposed to be able to transport and release different agents at specific target structures. Among the variety of delivery systems, liposomes are of importance owing to their ability to incorporate lipophilic substances and the physiological origin of the liposome components (Storm and Crommelin 1998).

Another class of drug delivery systems of recent interest are micellar systems because of their excellent solubilization capacity in physiological media. The formation of micelles as well as liposomes is caused by the "hydrophobic effect". This self-aggregation of amphiphilic molecules is associated with a loss of hydrocarbon/water interfacial energy since the hydrophobic chains are in contact with other chains and mainly sequested from water (Tanford 1980).

In this study, a second model was developed to investigate the adsorption behavior of simple and mixed micellar bile salt systems using the QCM. Bile salts are physiological detergents in humans and play an important role in intestinal lipid digestion and absorption (Tso and Fujimoto 1991). In contrast to classical detergents, where the hydrophilic headgroup and the lipophilic flexible aliphatic chains are clearly separated, bile salt molecules have a lipophilic surface, which is the convex side of the rigid steroid ring system, and a hydrophilic surface, which is the polyhydroxylated concave side of the molecule. Owing to their structure and rigidity, the aggregation properties are completely different compared to "normal" surfactants (Lichtenberg 1993). The most widely accepted shape for the molecular organization of bile salts are spherical micelles. In the field of binary mixtures consisting of bile salt and phospholipid, a number of studies using different methods have been performed to investigate the size, shape, and structure of the aggregates, as well as thermodynamics and kinetics of the liposome-micelle phase transition (Lasch 1995; Lichtenberg 1996). The structure of the mixed micelles is still controversially discussed to be disk-like (Mazer et al. 1980) or rod-like (Cohen et al. 1998). In contrast, only a few studies have dealt with ternary mixed micellar systems consisting of bile salt, phospholipid, and fatty acid (Schwarz et al. 1998). Relating to the QCM technique, the interactions between bile acids and cholestyramine were investigated by Chance and Purdy (1996). The adsorption of a few other surfactants was studied by means of the QCM. Lee et al. (1997) used sodium dodecyl sulfate. Caruso et al. (1995, 1996) focused their interest on poly(ethylene glycol) monododecyl ethers. The partitioning of sodium dodecyl sulfate in DPPE layers was determined by Colberg et al. (1998) as well as the solubilization. The adsorption behavior of different surfactants at 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine (DPPE) layers was examined by Okahata and Ebato (1991).

In a third approach, the QCM was used to detect specific ligand-receptor interactions between gangliosides and cholera toxin ($\beta$-subunit). Gangliosides are glycolipids which are located in cell membranes. High concentrations of gangliosides are contained in brain tissue. They are double-tailed amphiphilic molecules in which a ceramide lipid part, composed of a sphingosine and a fatty acid, is linked by an oligosaccharide chain containing one or more sialic acid residues (Corti et al. 1996). The gangliosides used in this study are schematically shown in Fig. 4 below. The oligosaccharide structures are exposed to the cell environment and are responsible for the molecular recognition of different ligands like toxins or viruses (Müthing and Unland 1994; Kuziemko et al. 1996).

Cholera toxin (CT), produced by the bacterium *Vibrio cholerae*, is a 87 kDa protein consisting of two subunits with the composition $AB_5$, i.e. five B ($\beta$)-subunits enclose one A ($\alpha$)-subunit. The $\alpha$-subunit (27 kDa) is responsible for the activation of the adenylate cyclase and consequently for the production of excessive amounts of cAMP, which triggers the release of an excess of digestive fluid into the intestine. The nontoxic $\beta$-subunit (12 kDa) initiates the binding to the gangliosides, especially to $G_{M1}$, at the cell surface (Fishman 1982; Terrettaz et al. 1993).

Gangliosides are intensively investigated by means of the QCM. The binding of $G_{M3}$-containing lipid matrices to wheat germ agglutinin was studied by Sato et al. (1994, 1996). These investigations were extended to other gangliosides, $G_{M1}$, $G_{M2}$, and $G_{M4}$, and the influence of the composition of the lipid matrix (Sato et al. 1998). The fusion of ganglioside $G_{M1}$-containing liposomes at quartz plates and their interactions with CT were studied by Ohlsson et al. (1995). The peanut agglutinin affinity to $G_{M1}$-phospholipid monolayers was characterized by Janshoff et al. (1996b). Interactions between gangliosides and different bacterial toxins have been investigated with the QCM technique by Janshoff et al. (1997). In this study, the binding of CT to $G_{M1}$, $G_{M3}$, and asialo-$G_{M1}$ was the object of the measurements. Spangler and Tyler (1999) used the ganglioside $G_{M1}$ as a capture agent for CT and an enterotoxin from *Escherichia coli* to determine the binding of antibodies. In the work of Matsubara et al. (1999), $G_{M1}$ served for the selection of ganglioside binding peptides.

## Materials and methods

### QCM apparatus

The experimental set-up used is shown in Fig. 1. AT-cut quartz crystals with a basic resonant frequency of 10 MHz were purchased from Quarzkeramik (Stockdorf, Germany). The quartz plates (15 mm diameter) with gold electrodes (6 mm diameter) were fixed in the measuring cell (Fig. 1), exposing only one side of the slide to the liquid medium. The QCM cell, made of polyacrylate, with an interior volume of 200 μL, was developed at the University of Magdeburg. The oscillator, supplied by ifak (Magdeburg), was mounted outside the chamber. The frequency data were recorded with special DOS-based software, developed by ifak, and analyzed using Origin software (version 5.0) as provided by MicroCal (Northampton, Mass., USA).

### Supported planar bilayers

A combination of "self-assembling" and the "LB technique" was introduced for the simulation of the biomembranes. Firstly, the quartz surface was coated with a self-assembled monolayer of an alkanethiol and, secondly, with a phospholipid LB film. In this way, supported planar bilayers were obtained (Fig. 2A).

The self-assembling of an alkanethiol was carried out with 1 mM 1-hexadecanethiol (Fluka, Neu Ulm, Germany) solutions in chloroform (Sigma, Deisenhofen, Germany). The saturation of the quartz surface with alkanethiol was reached after 4 h.

The LB film was transferred onto the quartz plate with in-house-made LB equipment consisting of a temperature-controlled water bath, a Wilhelmy pressure sensor, and a movable barrier, at a lateral pressure of 30 mN $m^{-1}$ and at room temperature. LB films of 0.3 mM 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine (DSPC) (Sigma) in chloroform, containing 10% alkylmannoside, were used for the liposome experiments. The alkylmannosides 1-*O*-(3,6,9-trioxapentacosyl)-α-D-mannopyranoside (mannoside III) and 1-*O*-(3,6,9,12,15,18,21,24-octaoxatetracontyl)-α-D-mannopyranoside (mannoside VIII) (shown schematically in Fig. 3) were synthesized according to Ogawa et al. (1981). However, for the micelle experiments the quartz plates were coated with pure DSPC LB films (0.3 mM in chloroform).

The CT experiments required the incorporation of different gangliosides into the DSPC LB matrix. The LB films were transferred in a molar DSPC/ganglioside ratio of 9:1. The ganglioside fractions $G_{M1}$, $G_{D1a}$, $G_{D1b}$, $G_{T1b}$, and asialo-$G_{M1}$, shown schematically in Fig. 4, were purchased from Pallmann (Munich, Germany).

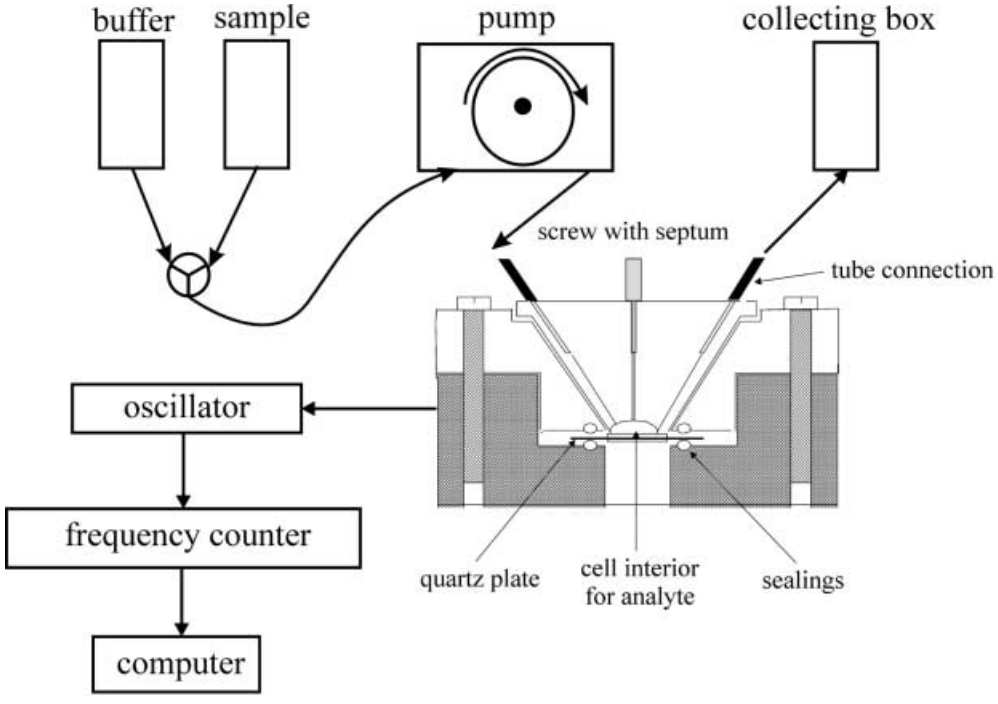



**Fig. 1** Schematic experimental set-up for the quartz crystal microbalance (QCM) measurements with the QCM cell used (made of polyacrylate, with a diameter of about 5 cm) in detail

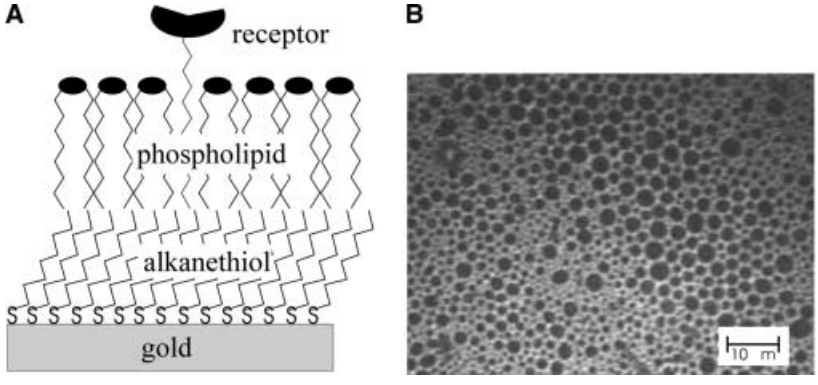



**Fig. 2** A Scheme of a supported planar bilayer (SPB) consisting of a alkanethiol layer and a phospholipid LB film including receptor molecules, e.g. mannosides or gangliosides; **B** Fluorescent image of a DSPC/$G_{M1}$ (90:10) LB layer at a lateral pressure of 30 mN $m^{-1}$ and at room temperature with dark crystalline domains, enlarged 775-fold; the *bar* represents 10 μm

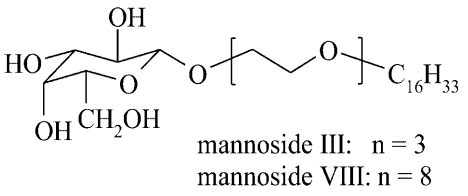



**Fig. 3** Structures of mannoside III and mannoside VIII, the target structures incorporated into the DSPC LB films for the investigation of the adsorption of liposomes

A combination of the LB technique and fluorescence microscopy (fluorescence microscope: Olympus, Proxy Cam 200) enabled the simultaneous measurement of the isotherms and the domain growth of the ganglioside-containing LB films. Therefore, the fluorescence marker NBD-PC (1-hexadecanoyl-2-[*N*-nitrobenz-2-oxa-1,3-diazol-4-yl)aminohexanoyl]-*sn*-glycero-3-phosphocholine, Sigma) was used (1 mol%). The NBD-PC marker was excited with laser light of 488 nm; the emission was detected at 520 nm.

Before each measurement the quartz plates had to be cleaned thoroughly. This was guaranteed by repeated washing with methanol (Brenntag Chemiepartner, Mülheim, Germany), followed by rinsing with piranha solution [1/4 30% $H_2O_2$ + 3/4 $H_2SO_4$ (both Merck, Darmstadt, Germany)] over a period of 15 s.

## Liposome experiments

Large unilamellar liposomes (15 mol) were prepared by suspending the different lipid components, 60% soy bean phosphatidylcholine, 30% cholesterol (both Sigma), and 10% *N*-glutarylphosphatidylethanolamine (N-glut-PE) according to Weissig (1991), in 1 mL 0.15 M Tris buffer (pH 7.4) (Fluka) at 60 °C, followed by extrusion through 400 nm polycarbonate membranes (extruder: Lipex Biomembrane, Vancouver, Canada). The liposome size (about 250 nm) was determined by light scattering with a Malvern Autosizer IIc (Malvern Instruments, Malvern, UK).

All water used in this study was ultrapure as obtained from a Milli-Q system (Millipore, Molsheim, France).

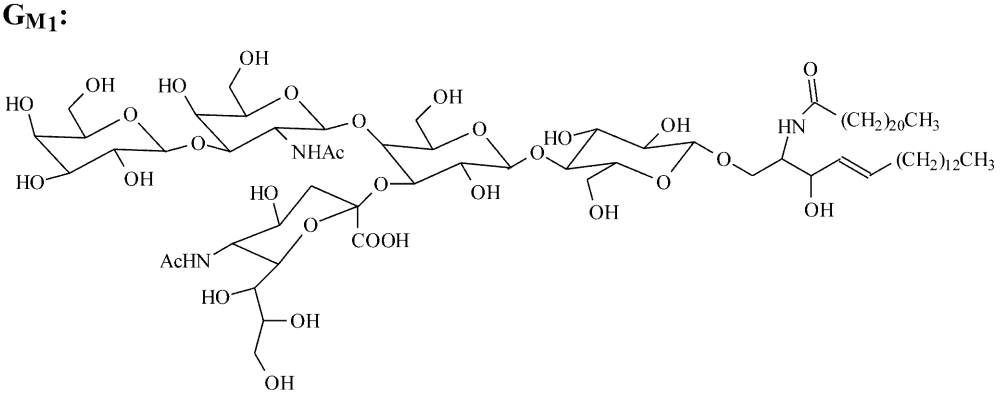
N-glut-PE served as a hydrophobic anchor for the lectin ConA (Sigma). The coupling procedure of ConA to the liposomes was performed as described elsewhere (Liebau et al. 1998).

For the incorporation of poly(ethylene glycol) (PEG) (Avanti Polar Lipids, Alabaster, Ala., USA) into the liposomes, 2.5% or 5% PEG with phosphatidylethanolamine (PEG-PE; molecular weight 2000) was added to the lipid suspension, diminishing the fraction of soy bean phosphatidylcholine. For the preparation of polymerized liposomes, the lipids were suspended with the appropriate monomer solution of 2.5% or 5% acrylamide and *N*,*N*′-methylene-bis-acrylamide (MBA) (Sigma) in a molar ratio of 19:1 or 10% acrylcholate (Polyscience, Warrington, Pa., USA) and 3% or 5% MBA. Riboflavin (0.5 mg $mL^{-1}$) (Sigma) was used as radical starter. Excess monomers were removed by gel permeation chromatography (Sepharose 4B; Sigma). The polymerization occurred under UV irradiation over a period of 12 h.

The experiments were performed in stand-by (i.e. no flow) and flow mode with a flow rate of 1 mL $min^{-1}$. On the pre-condition that a stable baseline was available, the sample was pumped in the flow mode or injected in the stand-by mode into the cell. After adding the analyte, the frequency decrease was monitored over a period of 30–60 min until a stable frequency was reached. Finally, the cell was rinsed with buffer and the frequency after the washing procedure was determined.

## Micelle experiments

The bile salts sodium cholate (NaC) and sodium deoxycholate (NaDC), the phospholipid DPPC, and the sodium salts of the fatty acids [lauric (LA-Na), palmitic (PA-Na), stearic (SA-Na), and oleic acid (OA-Na)] were purchased from Sigma. The phospholipid was pure, as checked by thin-layer chromatography. The purity of the



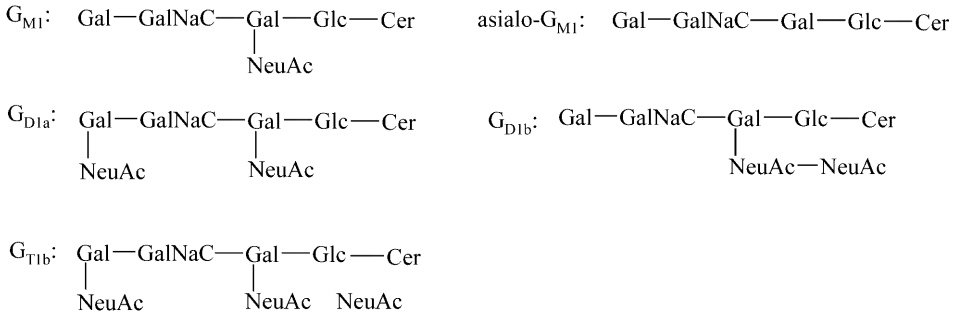




**Fig. 4** Structures of the gangliosides (*Cer*, ceramide; *Gal*, galactose; *GalNac*, *N*-acetylgalactose amine; *Glc*, glucose; *NeuAc*, *N*-acetylneuraminic acid), used as the target structures incorporated into the DSPC LB films for the investigation of the adsorption of cholera toxin

bile salts was tested by mass spectrometry (Finnigan LCQ, Thermoquest, San Jose, Calif., USA). The components of the phosphate (mono- and disodium phosphate) buffer were purchased from Merck. All substances were used without further purification.

For the simple bile salt solutions, NaC (20 mM) or NaDC (20 mM) were dissolved in phosphate puffer (pH 7.3, 20 mM). Mixed bile salt (20 mM)-phospholipid (5 mM) or bile salt (20 mM)-phospholipid (5 mM)-fatty acid, sodium salt (10 mM) solutions were prepared by dissolving a certain amount of the substances in phosphate buffer (pH 7.3, 20 mM), followed by ultrasonication for 20 min at 50 °C. All systems were stirred continuously for 5 h at 50 °C in a water bath. The samples were filtered through a membrane filter of 0.2 μm pore size. All samples were freshly prepared.

The QCM experiments for the micellar systems were carried out in the same way as for the liposomal systems.

### CT experiments

The CT ($\beta$-subunit) was used as received by Sigma. The CT solutions (0.1%) were prepared by dissolving the substance in phosphate buffer (pH 7.3, 20 mM). All experiments were performed in stand-by mode. After reaching a stable baseline, 2.5 μL of the CT solution were injected into the cell with a microliter syringe. To remove remaining CT material, a washing procedure with buffer solution followed after saturation. Then the frequency change was determined.

## Results

### Liposome experiments

The QCM technique was used to study specific adhesion processes of lectin-coupled liposomes at carbohydrate-containing model membranes. The lectin ConA, linked by the hydrophobic anchor N-glut-PE to the liposomes, reacted with the carbohydrate mannoside III which was embedded in the LB matrix. The frequency changes, correlating to the amount of the adsorbates, were determined for the samples with ConA-coupled and -uncoupled liposomes. No adsorption was detected using uncoupled liposomes. In contrast, the ConA-coupled liposomes induced a drastic frequency decrease of $1204 \pm 238$ Hz. The frequency course for the adsorption process is shown in Fig. 5. The saturation of the quartz surface was reached after 30 min. The following rinsing procedure with buffer yielded only a slight frequency increase of 17 Hz due to the removal of nonspecific bound molecules. The ConA-coupled liposomes could not be washed off, which points to specific lectin-carbohydrate interactions. All values for the liposome experiments are given as the mean of five measurements under the same conditions.

By modifying the SPB it is possible to differentiate between specific ConA-mannoside interactions and nonspecific interactions of the phospholipid headgroups of the LB film and the liposomes. Therefore, mannoside III was exchanged by mannoside VIII, an alkylmannoside with eight ethoxy units, i.e. with a longer spacer between carbohydrate and lipid anchor. The frequency decreased only by $987 \pm 82$ Hz, which indicates a reduced mass loading at the quartz surface of approximately 20%. The conclusion is that the amount of the nonspecific head group interaction is 20% minimum.

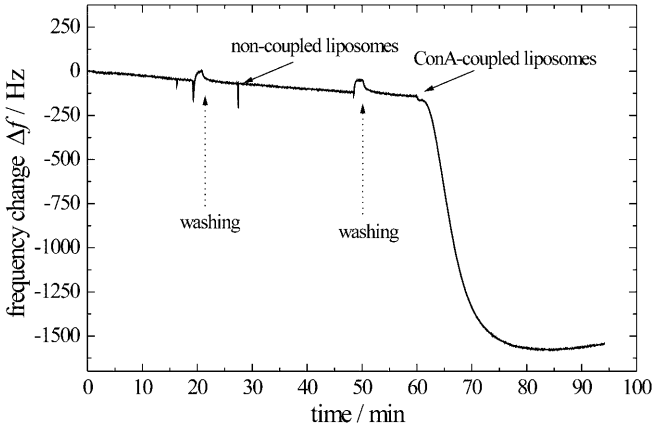



**Fig. 5** Time course of the frequency decrease $\Delta f$ of a 10 MHz quartz crystal for the adsorption of ConA-coupled and non-coupled liposomes at mannoside III-containing SPB (1-hexadecanethiol and DSPC with 10% mannoside III) in stand-by mode and rinsing with buffer solution

Inhibition experiments were performed with the competitive effecting methyl α-D-mannopyranoside to differentiate between specific lectin-carbohydrate interactions and nonspecific headgroup interactions. The methyl α-D-mannopyranoside-containing liposome dispersion was brought into the QCM cell. These preincubated liposomes adsorbed at mannoside-containing model membranes as described before (Fig. 5). A significant difference was obtained after washing. In the case of preincubated liposomes, a desorption of approximately 20% was measured.

In addition to the modification of the SPB, the composition of the liposomes was varied for a further characterization of the adsorption behavior of liposomal systems. For the experiments the LB matrix contained mannoside VIII. An artificial glycocalix was created incorporating PEG into the liposomes. Amounts of 2.5% or 5% PEG-PE were added. In contrast to pure ConA-coupled liposomes, the frequency change decreased to $516 \pm 104$ Hz for 2.5% PEG-PE and to $213 \pm 9$ Hz for 5% PEG-PE. Owing to the steric shielding of the liposomes, the adsorption could be reduced: for 2.5% PEG-PE by 48% and for 5% PEG-PE by 79%. Therefore, it can be concluded that the extension of the distance between the LB film and the liposomes reduces the amount of nonspecific headgroup interactions by up to approximately 80%.

Otherwise, with polyacrylamide (PAA) or polyacrylcholate (PAC), intravesicular polymerized liposomes simulate the cytoskeleton. The polymer concentrations were chosen in such a way that, in size and permeability, reproducible liposomes could be made. For the PAA liposomes, 2.5% or 5% acrylamide monomers were used. Both kinds yielded a reduced adsorption at the SPB, but no significant difference could be established for the different acrylamide

amounts (2.5% PAA: 199 ± 69 Hz; 5% PAA: 208 ± 82 Hz). As a result, the frequency changes were reduced by approximately 80% in contrast to pure ConA-coupled liposomes ($f = 987 \pm 82$ Hz). The measurements with the PAC-containing liposomes (10%) were carried out with 3% and 5% of the copolymer MBA. The PAC liposomes with 3% MBA yielded a frequency change of only 47 ± 17 Hz and with 5% MBA a frequency change of 153 ± 69 Hz, which indicates a reduced adsorption at the SPB by 95% and 85%, respectively.

The influence of the liposome structure on the adhesion behavior of the investigated liposomal systems is summarized in Table 1.

## Micelle experiments

Further investigations were carried out using the QCM to study the interactions between simple and mixed bile salt micellar systems and lipid bilayers. The adsorption behavior of different micellar systems, pure NaC or NaDC bile salt micelles, binary NaC/DPPC or NaDC/DPPC mixed micellar systems, and ternary NaDC/DPPC/fatty acid (sodium salt) mixed micellar systems, was studied at model membranes.

For both pure bile salt micellar solutions, no adsorption processes were detectable using the QCM. The binary systems showed only slight frequency changes, which, however, indicate existing interactions. No differences were found for the NaC ($f = 26 \pm 12$ Hz) or NaDC ($f = 25 \pm 16$ Hz) containing solutions. In contrast, for the ternary systems, significant adsorption was observed. The frequency course for the ternary mixtures is shown in Fig. 6.

In dependence on the fatty acid component, the following frequency changes were determined for the systems containing a saturated fatty acid: NaDC/DPPC/LA-Na, 55 ± 9 Hz; NaDC/DPPC/PA-Na, 108 ± 22 Hz; NaDC/DPPC/SA-Na, 123 ± 37 Hz. Therefore, a linear dependence (linear regression: $R = 0.994$) on the alkyl chain length of the saturated fatty acid was established. In addition, measurements were performed with the sodium salt of oleic acid, a single unsaturated fatty acid. There a lower frequency decrease of 36 ± 14 Hz was obtained. All results for the micellar experiments are given as the mean of five measurements under the same conditions. The results of the experiments with different bile salt micellar systems are shown in Table 2.

## CT experiments

The binding behavior of CT ($\beta$-subunit) at ganglioside-containing SPBs was examined using the QCM, with 10% of the gangliosides embedded into the LB matrix. This amount was chosen as a maximum frequency change was obtained, in contrast to higher amounts (30% ganglioside), pure ganglioside, or pure DSPC films. To investigate the morphology of the mixed LB films, the transfer of the monolayers was combined with fluorescence microscopy (Fig. 2B). The fluorescence marker NBD-PC (1 mol%) is localized in the bright liquid analogous phase. Dark lipid domains with a size of about 1.5–3 μm are clearly visible.

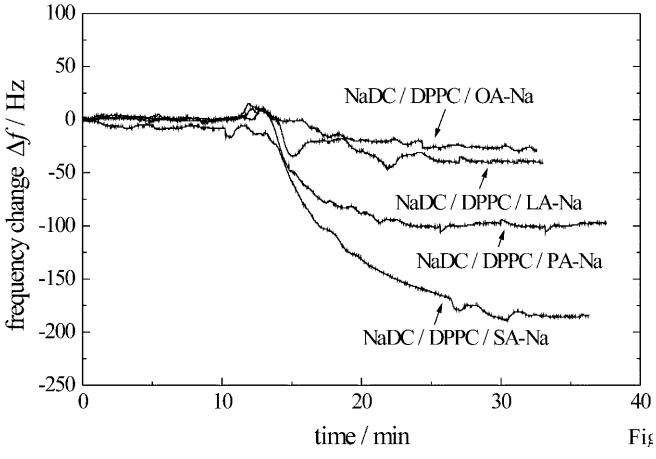



**Fig. 6** Time course of the frequency decrease $\Delta f$ of a 10 MHz quartz crystal for the adsorption of ternary mixed micellar bile salt systems consisting of the bile salt NaDC (20 mM), the phospholipid DPPC (5 mM), and the sodium salt of a fatty acid (10 mM); *OA-Na*, oleic acid; *LA-Na*, lauric acid; *PA-Na*, palmitic acid; *SA-Na*, stearic acid) at SPBs (1-hexadecanethiol and pure DSPC) in flow mode

**Table 1** Frequency decreases $\Delta f$ of a 10 MHz quartz crystal (stand-by mode), standard deviation (SD), and amount of binding for the adsorption of pure ConA (standard) and modified ConA-liposomes (2.5% or 5% PEG-PE; 2.5% or 5% PAA; 10% PAC with 3% or 5% MBA) at mannoside VIII-containing SPBs (first layer, 1-hexadecanethiol; second layer, DSPC with 10% mannoside VIII)

| Liposomal system | $f$ (Hz) | SD (Hz) | Binding (%) |
|---|---|---|---|
| Standard | 987 | 82 | 100 |
| PEG-PE (2.5%) | 516 | 104 | 52 |
| PEG-PE (5%) | 213 | 9 | 21 |
| PAA (2.5%) | 199 | 69 | 20 |
| PAA (5%) | 208 | 82 | 21 |
| PAC/MBA (3%) | 47 | 17 | 5 |
| PAC/MBA (5%) | 153 | 69 | 15 |

**Table 2** Frequency decreases $\Delta f$ of a 10 MHz quartz crystal (flow mode) and SD for the adsorption of simple bile salt micellar solutions, binary, and ternary mixed micellar systems at SPBs (first layer, 1-hexadecanethiol; second layer, pure DSPC)

| Micellar system | $f$ (Hz) | SD (Hz) |
|---|---|---|
| NaC | 1 | – |
| NaDC | 2 | – |
| NaC/DPPC | 26 | 11 |
| NaDC/DPPC | 25 | 13 |
| NaDC/DPPC/LA-Na | 55 | 9 |
| NaDC/DPPC/PA-Na | 108 | 12 |
| NaDC/DPPC/SA-Na | 137 | 31 |
| NaDC/DPPC/OA-Na | 36 | 11 |

The interactions occurred within a short period (saturation after 10 min). The strongest interactions were observed with $G_{M1}$ ($f = 58 \pm 14$ Hz). The ganglioside fractions showed varying frequency changes in the following sequence: $G_{M1}$ ($58 \pm 14$ Hz) > $G_{D1a}$ ($42 \pm 9$ Hz) > $G_{D1b}$ ($19 \pm 5$ Hz) > $G_{T1b}$ ($11 \pm 5$ Hz). No change in frequency was determined for asialo-$G_{M1}$, which was measured as reference. The results for the CT experiments, all as the mean of five measurements under the same conditions, are summarized in Table 3.

## Discussion

The QCM is a very effective method to study ligand-receptor interactions or adhesion processes at biomimetic surfaces. In the present study, different adsorption models were created. This was reached by coating the quartz plates with a lipid bilayer consisting of a self-assembled alkanethiol monolayer and a phospholipid LB film. In order to imitate native membranes, the LB film was equipped with special recognition structures. Mannosides were used for the liposome experiments to investigate the lectin-carbohydrate interaction, and gangliosides for the CT experiments to study ganglioside-toxin interactions.

In a first model for the simulation of cell adhesion, the adsorption of liposomes coupled with the lectin ConA was investigated. For this, mannoside III was incorporated into the LB layer, making use of lectin-carbohydrate binding. An intense adsorption confirmed this theory compared to liposomes which were not coupled with the lectin. It can be assumed thereby that the frequency change is not only caused by specific mannoside-ConA interactions but also by nonspecific interactions between the phospholipid headgroups of the LB film and the liposomes. To differentiate between both interactions, mannoside VIII with a longer ethoxy spacer was embedded into the LB matrix. In this way the distance between the model membrane and the adsorbed liposomes could be extended. As a result, the suppression of the approach of the phospholipid headgroups and consequently of the nonspecific effects succeeded. A desorption of approximately 20% of nonspecific bound material proves this. Therefore, it can be concluded that at least 20% nonspecific headgroup interactions are involved in the whole adsorption process.

**Table 3** Frequency decreases $\Delta f$ of a 10 MHz quartz crystal (stand-by mode) and SD after injection of a 0.1% cholera toxin solution, owing to interactions with different ganglioside fractions incorporated into the SPBs (first layer, 1-hexadecanethiol; second layer, DSPC with 10% ganglioside)

| Ganglioside fraction | $f$ (Hz) | SD (Hz) |
|---|---|---|
| Asialo-GM1 | 2.5 | – |
| $G_{M1}$ | 58 | 14 |
| $G_{D1a}$ | 42 | 9 |
| $G_{D1b}$ | 19 | 5 |
| $G_{T1b}$ | 11 | 5 |

The composition of the liposomes was changed for a more detailed characterization of the liposome adhesion. The measurements were performed using PEG-linked stealth liposomes. This enables the imitation of the glycocalix. The surface of the liposomes is sterically shielded. By adding 2.5% PEG-PE the frequency change was decreased by 48% in contrast to standard liposomes (only ConA coupled); the addition of 5% PEG-PE yielded a reduction of 79%. So a higher amount of PEG at the liposomes surface, associated with a stronger shielding of the liposomes, caused a smaller frequency decrease. Therefore, it can be derived that a tight contact of the phospholipid headgroups is prevented. This suppression of the headgroup interactions to approximately 80% of the whole adhesion process leads to the conclusion that the liposome adsorption occurs to 80% due to nonspecific interactions between the phospholipid headgroups and to 20% due to specific lectin-carbohydrate interactions. However, one cannot rule out the possibility of a certain steric hindrance by the PEG incorporated into the liposomes and an associated change in the fixed aqueous layer around the liposomes (Shimada et al. 1995). This steric barrier function of PEG-linked stealth liposomes is of special interest for the development of drug delivery systems with long circulation times in the bloodstream (Allen 1994; Storm and Crommelin 1998). There exists a commercial product based on PEG liposomes with the drug doxorubicin for the indication of AIDS-related Kaposi's sarcoma (Caelyx in Europe) (Gabizon and Muggia 1998).

This result was confirmed by the experiments with intravesicular polymerized liposomes. The artificial cytoskeleton, created by the incorporation of polyacrylamide or polyacrylcholate, caused an increased rigidity of the liposomes. "Normal" liposomes, contacting the LB-coated quartz surface, undergo a deformation during the adsorption, spontaneously break up, and finally fuse to form multilamellar lipid bilayers. Owing to their enormous rigidity, the polymerized liposomes keep their spherical shape. That is why they do not fuse at the modified surface. For the PAA liposomes, the adsorption was reduced by approximately 80% compared with the "normal" liposomes. No difference could be found between 2.5% and 5% PAA. In contrast, the MBA amount played a crucial role for the addition of PAC. For 3% MBA a reduced frequency decrease of approximately 95% was observed, whereas 5% MBA yielded a reduction of the frequency change of about 85%. Consequently, the degree of polymerization and the crosslinking are of importance. Both measures for the modification of the liposome composition and structure lead to the result that the fusion of the phospholipid bilayers is hindered by the minimization of headgroup interactions.

In a second model the adsorption behavior of simple and mixed bile salt micellar systems to DSPC LB membranes was investigated. For simple bile salt micelles, no adsorption at SPBs was observed. The negatively charged bile salt micelles do not interact with

the phosphatidylcholine headgroups of the LB film. A longer contact of the detergent with the phospholipid layer showed no effect. In contrast, the use of the strong detergent sodium dodecyl sulfate (SDS) in a micellar concentration range yielded an increase in frequency, i.e. a removal of the LB film, caused by the solubilization of the phospholipid. This result agrees with the experiments from Colberg et al. (1998). They characterized the behavior of DPPE films in dependence on the SDS concentration. Monomeric detergent solutions with a SDS concentration of 10 or 20% of the critical micellization concentration (cmc) resulted in a rapid mass uptake owing to the partitioning of the detergent into the lipid layer. However, higher concentrations (50 or 90% of the cmc) yielded a frequency increase due to the solubilization of the lipid. The DPPE layer is completely removed from the quartz plate under the formation of mixed micelles. The binary systems (bile salt and DPPC) indicated slight frequency changes without recognizable differences for the systems with the trihydroxy bile salt NaC or the dihydroxy bile salt NaDC. The ternary systems (bile salt, phospholipid, and sodium salt of a fatty acid) showed significant frequency changes. A linear dependence of the frequency change on the fatty acid chain length was determined. Systems with the saturated lauric acid or the unsaturated oleic acid caused less strong frequency changes, like systems with the saturated long-chain fatty acids palmitic and stearic acid. The shorter lauric acid with 12 carbon atoms, as well as oleic acid with a double bond, form micelle-like structures in aqueous media. However, palmitic and stearic acid belong to the class of bilayer-forming amphiphiles. It is assumed that mixed micellar aggregates which are formed by detergents and a higher amount of bilayer-forming amphiphiles have not a classical, spherical shape but a rod- or disk-like one (Cohen et al. 1998). Therefore, they lose the minimal surface of a sphere and the contact area increases for rods or disks. As a result, a stronger interaction of these micellar systems with the phospholipid layer is obtained. Since simple bile salt micelles did not interact with the DSPC LB film, but binary micellar solutions with DPPC showed frequency changes, the effect must have been caused by interactions between the phospholipid headgroups, as already described for the liposome experiments. From these results it cannot be concluded whether mixed micellar aggregates or individual molecules are responsible for the observed interaction with the lipid-coated QCM surface. In any case, the addition of a phospholipid, and especially of a fatty acid, causes detectable effects resulting from the interaction with the model membrane.

As mentioned above, the measured frequency decrease is not only caused by mass adsorption at the quartz surface but also by the viscosity of the liquids. For the liposome experiments, the uniformity of the samples relating to the viscosities was given by the similar composition and the equal preparation procedure. Owing to the different composition of the micellar systems for these experiments, viscosity measurements were performed (results not given). The viscosity values for the micellar solutions at 20 °C were in the range of pure water.

Mixed micellar systems are becoming more important as new drug delivery systems. A number of drugs can be found in liposomal formulations. However, there are only a few conventional formulations containing drugs in micellar solutions, like diazepam (Valium) and phytomenadion (Konakion 10 mg) for parenteral application or for oral administration (Konakion 2 mg). These mixed micellar systems consist of glycocholic acid, soybean PC, and other excipients. Recent results showed a better stability for ternary mixed micelles (bile salt + phospholipid + fatty acid) compared to binary mixtures (bile salt + phospholipid) (Schwarz et al. 1998; Hildebrand A, Schädlich A, Hartmann M, Neubert RHH, unpublished results) Therefore, the detailed characterization of these ternary systems is of special interest to create new vehicles for drugs.

The third model dealt with interactions between CT and ganglioside-containing model membranes. Various ganglioside fractions in the LB matrix resulted in different frequency values with the following sequence: $G_{M1} > G_{D1a} > G_{D1b} > G_{T1b} >$ asialo-$G_{M1}$. As expected, $G_{M1}$ showed the strongest interaction with CT, because it is the native recognition structure for CT at the cell surface. Janshoff et al. (1997) detected a strong interaction between CT and $G_{M1}$ as well, but only a small binding to $G_{M3}$ and asialo-$G_{M1}$. In contrast, Sato et al. (1998) found a high affinity of wheat germ agglutinin to $G_{M3}$ and $G_{M4}$, but no affinity to $G_{M2}$ and $G_{M1}$ owing to a steric hindrance of the side residues. The results for the influence of the lipid layer composition are in agreement with those from Sato et al. In this study, lipid layers with a ganglioside content of 10% were used (Sato et al. used 20%), because pure ganglioside monolayers yielded lower affinities due to the absence of a certain space around the sialic acid group which is required for binding. The importance of the oligosaccharide residues for the binding behavior is expressed in the negligible frequency change of asialo-$G_{M1}$, which is marked by the absence of the sialic acid residue. The existence of any sialic acid residues for the gangliosides $G_{D1a}$, $G_{D1b}$, and $G_{T1b}$ causes not an increase in the binding but a decreased interaction due to the deviation from the native recognition structure. The QCM enabled the detection of specific CT-ganglioside interactions in dependence on the ganglioside fraction. The results obtained are in close agreement with previous ones by Kuziemko et al. (1996), determined by surface plasmon resonance.

## Conclusions

The application of the QCM technique in liquid media enables a detailed characterization of biochemical processes. A wide variety of investigations imitating native interactions can be performed by creating model membranes with special recognition structures at the quartz surface.

Three different approaches were described. At first it was demonstrated that lectin-coupled liposomes adsorb at carbohydrate-containing model membranes, caused by specific lectin-carbohydrate interactions and nonspecific ones. The nonspecific headgroup interactions can be reduced by modifying the lipid bilayer by incorporating a longer ethoxy spacer or changing the liposome composition by means of intravesicular polymerization or sterical stabilization. Furthermore, the adsorption of simple and mixed bile salt micelles was characterized. It was established that ternary micellar systems of bile salt, phospholipid, and fatty acid (sodium salt) have the strongest affinity to SPBs, whereas simple or binary systems do not adsorb. The influence of the fatty acid component was found to play a crucial role. Finally, the binding of CT ($\beta$-subunit) at ganglioside-containing lipid bilayers was described. The strongest interaction was found for $G_{M1}$; asialo-$G_{M1}$ showed no recognizable frequency change.

For future investigations, the combination of the QCM technique with other methods is planned, particularly relating to a concrete separation between mass and viscosity effects.

The performed investigations contribute to the explanation of biological processes, such as cell adhesion and toxin-ganglioside binding. The development of new drug delivery systems, which are able to transport and release drugs at specific target structures, is another aim of the present study. Liposomal and micellar systems of physiological origin are suitable for lipophilic drugs owing to their excellent solubilization capacity.

**Acknowledgements** This work was supported by the Deutsche Forschungsgemeinschaft (DFG; project no. NE 427/8-3), the Institut für Automation und Kommunikation (ifak), Magdeburg, and the Otto-von-Guericke-Universität Magdeburg, Institut für Prozessmesstechnik und Elektronik. We thank J. Müthing from the Universität Bielefeld, Technische Falkultät, for the assistance with the cholera toxin binding studies.

## References

Allen TM (1994) The use of glycolipids and hydrophilic polymers in avoiding rapid uptake of liposomes by the mononuclear phagocyte system. Adv Drug Deliv Rev 13:285–309

Auge J, Hauptmann P, Eichelbaum F, Rösler S (1994) Quartz crystal microbalance sensor in liquids. Sens Actuators B 18–19:518–522

Bain CD, Troughton EB, Tao YT, Evall J, Whitesides GM, Nuzzo RG (1989) Formation of monolayer films by the spontaneous assembly of organic thiols onto gold. J Am Chem Soc 111:321–335

Becker JW, Reeke GN, Wang JL, Cunningham BA, Edelman GM (1975) The covalent and three-dimensional structure of concanavalin A. J Biol Chem 250:1513–1524

Bendas G, Vogel J, Bakowski U, Krause A, Müller J, Rothe U (1997) A liposome-based model system for the simulation of lectin-induced cell adhesion. Biochim Biophys Acta 1325:297–308

Blodgett K, Langmuir I (1937) Built-up films of barium stearate and their optical properties. Phys Rev 51:964–982

Bouckaert J, Loris R, Poortmans F, Wyns L (1995) Crystallographic structure of metal-free concanavalin A at 2.5 Å resolution. Proteins Struct Funct Genet 23:510–524

Caruso F, Serizawa T, Furlong DN, Okahata Y (1995) Quartz crystal microbalance and surface plasmon resonance study of surfactant adsorption onto gold and chromium oxide surfaces. Langmuir 11:1546–1552

Caruso F, Rinia HA, Furlong DN (1996) Gravimetric monitoring of nonionic surfactant adsorption from nonaqueous media onto quartz crystal microbalance electrodes and colloidal silica. Langmuir 12:2145–2152

Chance JJ, Purdy WC (1996) Bile acid measurements using a cholestyramine-coated TSM acoustic wave sensor. Anal Chem 68:3104–3111

Cohen DE, Thurston GM, Chamberlin RA, Benedek GB, Carey MC (1998) Laser light scattering evidence for a common wormlike growth structure of mixed micelles in bile salt- and straight-chain detergent-phosphatidylcholine aqueous systems: relevance to the micellar structure of bile. Biochemistry 37:14798–14814

Colberg MT, Carnes K, Sáez AE, Grant CS, Hutchinson K, Hesterberg D (1998) Hydration and removal of supported phospholipid films in aqueous surfactant solutions. Thin Solid Films 327–329:247–251

Corti M, Cantu L, Del Favero E (1996) Self-aggregation properties of gangliosides, glycosidic surfactants of biological origin. Tenside Surf Deterg 33:214–219

Fishman PH (1982) Role of membrane gangliosides in the binding and action of bacterial toxins. J Membr Biol 69:85–97

Frederiksson C, Kihlman S, Rodahl M, Kasemo B (1998) The piezoelectric quartz crystal mass and dissipation sensor: a means of studying cell adhesion. Langmuir 14:248–251

Gabizon A, Muggia FM (1998) Initial clinical evaluation of pegylated-liposomal doxorubicin in solid tumors. In: Woodle MC, Storm G (eds) Long circulation liposomes: old drugs, new therapeutics. Springer, Berlin Heidelberg New York, pp 165–174

Gryte DM, Ward MD, Hu WS (1993) Real-time measurement of anchorage-dependent cell adhesion using a quartz crystal microbalance. Biotechnol Prog 9:105–108

Janshoff A, Wegener J, Sieber M, Galla HJ (1996a) Double-mode impedance analysis of epithelial cell monolayers cultured on shear wave resonators. Eur Biophys J 25:93–103

Janshoff A, Steinem C, Sieber M, Galla HJ (1996b) Specific binding of peanut agglutinin to $G_{M1}$-doped solid supported lipid bilayers investigated by shear wave resonator measurement. Eur Biophys J 25:105–113

Janshoff A, Steinem K, Sieber M, el Bayâ A, Schmidt MA, Galla HJ (1997) Quartz crystal microbalance investigation of the interaction of bacterial toxins with gangliosides containing solid supported membranes. Eur Biophys J 26:261–270

Kanazawa KK, Gordon JG (1985) The oscillation frequency of a quartz resonator in contact with liquid. Anal Chim Acta 175:99–105

Keller CA, Kasemo B (1998) Surface specific kinetics of lipid vesicle adsorption measured with a quartz crystal microbalance. Biophys J 75:1397–1402

Keller CA, Glasmästar K, Zhdanov VP, Kasemo B (2000) Formation of supported membranes from vesicles. Phys Rev Lett 84:5443–5446

Kim HK, Kwak S, Kim YS, Seo BI, Kim ER, Lee H (1998) Adsorption kinetics of alkanethiols studied by quartz crystal microbalance. Thin Solid Films 327–329:191–194

Kuziemko GM, Stroh M, Stevens RC (1996) Cholera toxin binding affinity and specificity for gangliosides determined by surface plasmon resonance. Biochemistry 35:6375–6384

Lasch J (1995) Interactions of detergents with lipid vesicles. Biochim Biophys Acta 1241:269–292

Lasic D (1994) Sterically stabilized vesicles. Angew Chem Int Ed Engl 33:1685–1698

Lee KT, Raghavan S, Mathew V (1997) Detection of surfactants in rinse water using a quartz crystal microbalance technique. Electrochem Soc Proc 96–21:126–139

Lichtenberg D (1993) Micelles and liposomes. In: Shinitzky M (ed) Biomembranes – physical aspects. VCH, Weinheim, pp 63–96
Lichtenberg D (1996) Liposomes as a model for solubilization and reconstitution of membranes. In: Lasic D, Barenholz Y (eds) Handbook of nonmedical applications of liposomes, vol. II. CRC Press, Boca Raton, Fla., pp 199–218
Liebau M, Bendas G, Rothe U, Neubert RHH (1998) Adhesive interactions of liposomes with supported planar bilayers on QCM as a new adhesion model. Sens Actuators B 47:239–245
Lis H, Sharon N (1998) Lectins: carbohydrate-specific proteins that mediate cellular recognition. Chem Rev 98:637–674
Lu C, Czanderna AW (1984) Applications of piezoelectric quartz crystal microbalances. Elsevier, Amsterdam, pp 1–61
Martin SJ, Frye GC, Wessendorf KO (1994) Sensing liquid properties with thickness-shear mode resonators. Sens Actuators A 44:209–218
Marsh D (1996) Physical characterization of liposomes for understanding structure-function relationship in biological membranes. In: Lasic D, Barenholz Y (eds) Handbook of nonmedical applications of liposomes, vol. II. CRC Press, Boca Raton, Fla., pp 1–19
Matsubara T, Ishikawa D, Taki T, Okahata Y, Sato T (1999) Selection of ganglioside GM1-binding peptides by using a phage library. FEBS Lett 456:253–256
Mazer NA, Benedek GB, Carey MC (1980) Quasielastic light scattering studies of aqueous biliary lipid systems. Mixed micelle formation in bile salt-lecithin solutions. Biochemistry 19:601–615
Müthing J, Unland F (1994) A comparative assessment of TLC overlay technique and microwell adsorption assay in the examination of influenza A and Sendai virus toward oligosaccharides and sialic acid linkages of gangliosides. Glycoconjug J 11:486–492
Nwankwo E, Durning CJ (1998) Mechanical response of thickness-shear mode quartz-crystal resonators to linear viscoelastic fluids. Sens Actuators A 64:119–124
Ogawa T, Beppu K, Nakabayashi S (1981) Trimethylsilyl trifluoromethanesulfonate as an effective catalyst for glycoside synthesis. Carbohydr Res 93:C6–C9
Ohlsson PA, Tjärnhaage T, Herbai E, Löfås S, Puu G (1995) Liposome and proteoliposome fusion onto solid substrates studied using atomic force microscopy, quartz crystal microbalance, and surface plasmon resonance. Biological activities of incorporated components. Bioelectrochem Bioenerg 38:137–148
Okahata Y, Ebato H (1991) Absorption behaviors of surfactant molecules on a lipid-coated quartz crystal microbalance. An alternative to eye-irritant tests. Anal Chem 63:203–207
Okahata Y, Ebara Y, Sato T (1995) The quartz crystal microbalance study of protein binding on lipid monolayers at the air–water interface. MRS Bull 20:52–56
Pignataro B, Steinem C, Galla HJ, Fuchs H, Janshoff A (2000) Specific adhesion of vesicles monitored by scanning force microscopy and quartz crystal microbalance. Biophys J 78:487–498
Plant AL (1999) Supported hybrid bilayer membranes as rugged cell membrane mimics. Langmuir 15:5128–5135
Redepenning J, Schlesinger TK, Mechalke EJ, Puleo DA, Bizios R (1993) Osteoblast attachment monitored with a quartz crystal microbalance. Anal Chem 65:3378–3381
Ringsdorf H, Schlarb B, Venzmer J (1988) Molecular architecture and function in polymeric oriented systems: models for the study of organization, surface recognition, and dynamics in biomembranes. Angew Chem Int Ed Engl 27:113–158
Rodahl M, Höök F, Fredriksson C, Keller CA, Krozer A, Brzezinski P, Voinova M, Kasemo B (1997) Simultaneous frequency and dissipation factor QCM measurements of biomolecular adsorption and cell adhesion. Faraday Discuss 107:229–246
Sato T, Serizawa T, Okahata Y (1994) Recognition of monosialoganglioside ($G_{M3}$) reconstituted in sphingomyerin and glucosylceramide membranes against wheat germ agglutinin: quantitative analysis by a quartz crystal microbalance. Biochem Biophys Res Commun 204:551–556
Sato T, Serizawa T, Okahata Y (1996) Organization and recognition function of gangliosides in lipid monolayers. Front Biomed Biotech 3:145–155
Sato T, Serizawa T, Ohtake F, Nakamura M, Terabayashi T, Kawanishi Y, Okahata Y (1998) Quantitative measurements of the interaction between monosialoganglioside monolayers and wheat germ agglutinin (WGA) by a quartz crystal microbalance. Biochim Biophys Acta 1380:82–92
Sauerbrey G (1959) Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung. Z Phys 155:206–222
Schumacher R (1990) The quartz microbalance: a novel approach to the in-situ investigation of interfacial phenomena at the solid/liquid interface. Angew Chem Int Ed Engl 29:329–343
Schwarz MA, Raith K, Dongowski G, Neubert RHH (1998) The effect on the partition equilibrium of various drugs by the formation of mixed bile salt/phosphatidylcholine/fatty acid micelles – a characterization by micellar affinity capillary electrophoresis, part IV. J Chromatogr A 809:219–229
Shimada K, Miyagishima A, Sadzuka Y, Nozawa Y, Mochizuki Y, Ohshima H, Hirota S (1995) Determination of the thickness of the fixed aqueous layer around polyethyleneglycol-coated liposomes. J Drug Targ 3:283–290
Spangler BD, Tyler BJ (1999) Capture agents for a quartz crystal microbalance-continuous flow biosensor: functionalized self-assembled monolayers on gold. Anal Chim Acta 399:51–62
Storm G, Crommelin JA (1998) Liposome: quo vadis? Pharm Sci Technol Today 1:19–31
Tanaka S, Iwasaki Y, Ishihara K, Nakabayashi N (1994) Assessment of adsorption of liposomes on a phospholipid polymer surface using a quartz crystal microbalance. Macromol Rapid Commun 15:319–326
Tanford C (1980) The hydrophobic effect. Formation of micelles and biological membranes. Wiley New York, pp 42–95, 106–138
Terrettaz S, Stora T, Duschl C, Vogel H (1993) Protein binding to supported lipid membranes: investigations of the cholera toxin-ganglioside interaction by simultaneous impedance spectroscopy and surface plasmon resonance. Langmuir 9:1361–1369
Tjärnhage T, Puu G (1996) Liposome and phospholipid adsorption on a platinum surface studied in a flow cell designed for simultaneous quartz crystal microbalance and ellipsometry measurements. Colloids Surf 8:39–50
Tso P, Fujimoto K (1991) The absorption and transport of lipids by the small intestine. Brain Res Bull 27:477–482
Urbakh M, Daikhin L (1994) Influence of the surface morphology on the quartz crystal microbalance response in a fluid. Langmuir 10:2836–2841
Urbakh M, Daikhin L (1998) Surface morphology and the quartz crystal microbalance response in liquids. Colloids Surf A 134:75–84
Wegener J, Janshoff A, Galla HJ (1998) Cell adhesion monitoring using quartz crystal microbalance: comparative analysis of different mammalian cell lines. Eur Biophys J 28:26–37
Weissig V (1991) Methoden zur Darstellung funktionalisierter Liposomen mit Adjuvanseffekt. Postdoctoral thesis, Martin-Luther-Universität Halle-Wittenberg
Ziegler C (2000) Cell-based biosensors. Fresenius J Anal Chem 366:552–559