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Accumulation of phosphatidylcholine on gut mucosal surface is not dominated by electrostatic interactions

Agatha Korytowski [a], Wasim Abuillan [a], Federico Amadei [a], Ali Makky [a,1], Andrea Gumiero [b], Irmgard Sinning [b], Annika Gauss [c], Wolfgang Stremmel [c,*], Motomu Tanaka [a,d,**]

[a] Physical Chemistry of Biosystems, Institute of Physical Chemistry, Heidelberg University, D69120 Heidelberg, Germany
[b] Heidelberg University Biochemistry Center (BZH), D69120 Heidelberg, Germany
[c] Department of Internal Medicine IV, University Clinics of Heidelberg, D69120 Heidelberg, Germany
[d] Institute for Integrated Cell-Material Science (WPI iCeMS), Kyoto University, 606-8501 Kyoto, Japan

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ABSTRACT

The accumulation of phosphatidylcholine (PC) in the intestinal mucus layer is crucial for the protection of colon epithelia from the bacterial attack. It has been reported that the depletion of PC is a distinct feature of ulcerative colitis. Here we addressed the question how PC interacts with its binding proteins, the mucins, which may establish the hydrophobic barrier against colonic microbiota. In the first step, the interactions of dioleoylphosphatidylcholine (DOPC) with two mucin preparations from porcine stomach, have been studied using dynamic light scattering, zeta potential measurement, and Langmuir isotherms, suggesting that mucin binds to the surface of DOPC vesicles. The enthalpy of mucin-PC interaction could be determined by isothermal titration calorimetry. The high affinity to PC found for both mucin types seems reasonable, as they mainly consist of mucin 2, a major constituent of the flowing mucus. Moreover, by the systematic variation of net charges, we concluded that the zwitterionic DOPC has the strongest binding affinity that cannot be explained within the electrostatic interactions between charged molecules.

1. Introduction

The colonic lumen contains a large amount of bacteria that amounts to one trillion per gram of stool, colonic epithelial cells are protected from attack by the huge bacterial load by a mucus layer [1]. The protective mucus scaffolds consist of a family of highly glycosylated proteins, mucins [2]. The main intestinal secretory protein, mucin 2, is secreted by goblet cells [3] but enterocytes express transmembrane mucins 3, 12, 13 and 17 in the vicinity of apical tight junctions [2,4]. A mounting evidence suggested that the protective function of mucus against the bacterial invasion is established by phospholipids [5]. Phosphatidylcholine (PC) and lyso-phosphatidylcholine (lyso-PC) share more than 90% of the phospholipids within the intestinal mucus [6], suggesting that PC/lyso-PC are either selectively transported or bound to this compartment. Since goblet cells secreting mucin do not store phospholipids, a separate PC secretion route is postulated. A recent study unraveled that the selective transport of PC/lyso-PC is mediated via paracellular transport through tight junction to the apical side [7].

There have been several reports suggesting that the depletion of phospholipid coating and thus the disruption of mucosal barrier has been suggested as underlying cause of disease, such as ulcerative colitis [8]. For example, the colon intestine surface of rats orally treated with detergents exhibited a decrease in both water contact angles and barrier capability against dextran sodium sulfate [5]. Actually, in human ulcerative colitis, PC and lyso-PC molecules in the intestinal mucus are reduced by 70% [6,8]. From the very simple viewpoint of interfacial free energy, it is plausible that the mucus layer and wet lumen or biofilms should be interfaced by the formation of a lipid bilayer, while the mucosal layer in contact with the dry (ambient) atmosphere should be stabilized by a lipid monolayer (Scheme 1).

In this study, we shed light on the mechanism how PC/lyso-PC molecules are selectively accumulated on mucus surfaces, where a barrier against colonic microbiota is generated. To address this question, we studied the interactions of lipids and two mucin preparations from the flowing mucus, whose main constituent is mucin 2. The systematic

* Corresponding author.
** Correspondence to: M. Tanaka, Physical Chemistry of Biosystems, Institute of Physical Chemistry, Heidelberg University, D69120 Heidelberg, Germany.
E-mail addresses: Wolfgang.Stremmel@med.uni-heidelberg.de (W. Stremmel), tanaka@uni-heidelberg.de (M. Tanaka).
[1] Present address: CNRS UMR 8612, Institut Galien Paris-Sud, Faculté de Pharmacie, 5 rue J.B. Clément, 92296 Châtenay-Malabry, France.

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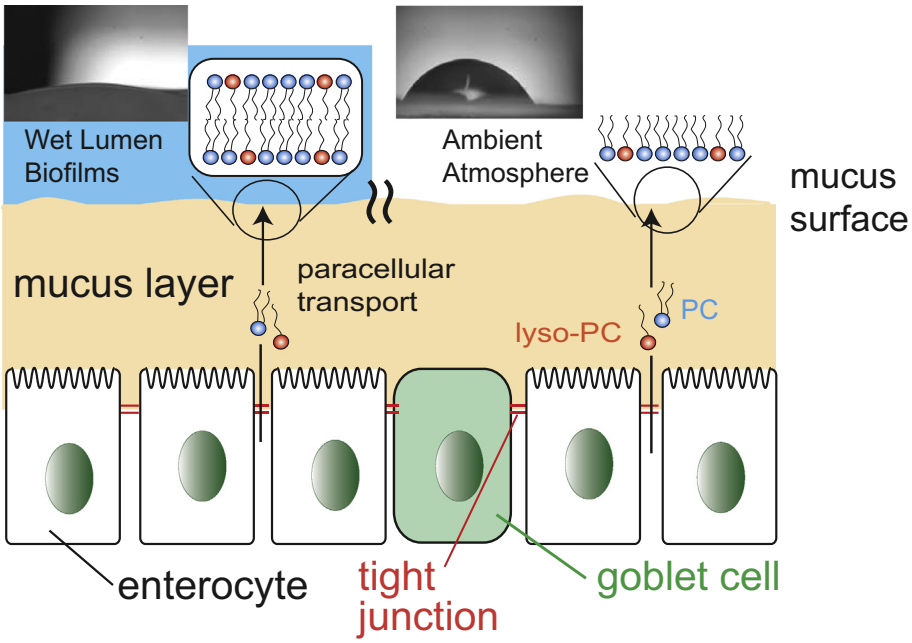

Scheme 1. Transport of phospholipid through the tight junction in colon epithelia (goblet cells, enterocytes) and accumulation to the mucus layer surface. Establishment of the protection layer by amphiphilic lipids can be evidenced by simple contact angle measurements. The explant from rat colon epithelial tissue pre-treated with water (left) showed a very low contact angle ($\theta < 20°$), suggesting the protection by a lipid bilayer in contact with wet lumen or biofilms. On the other hand, the same tissue exposed to an ambient atmosphere (right) exhibited a much higher contact angle ($\theta \sim 70°$), implying the formation of a lipid monolayer.

combination of several experimental techniques unraveled the molecular parameters that dictate the significance of lipid-mucin interactions.

2. Experimental section

2.1. Materials

Deionized water from a Milli-Q device (Millipore, Molsheim, France) was used throughout this study. In this study we used two types of mucin products from porcine stomach (Sigma-Aldrich, Munich, Germany): mucin type II (MS2378) is a crude preparation of mucin, while mucin type III (M1778) is a partially purified preparation following the previously reported protocol [9]. It should be noted that the nomenclature, following that of the manufacturer, has no correlation with mucin 2 and mucin 3. As the unidentified impurities would influence some of the results, we confirmed the reproducibility of the results by repeating experiments using samples from two different batches. Chloroform solutions of lipids were purchased from Avanti Polar Lipids (AL, USA) throughout this study. As the lipid model, we used four lipids that possess identical hydrocarbon chains but different head groups:: DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) and DOPE (1,2-dioleoyl-*sn*-glycero-3–phosphoethanolamine) as zwitterionic (±), DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) as cationic (+), and DOPG (1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol)) as anionic (−) lipids. Unless stated otherwise, all other chemicals were purchased either from Sigma-Aldrich (Munich, Germany) or Carl Roth (Karlsruhe, Germany), and were used without further purification. As the buffer, HEPES buffered saline containing 150 mM NaCl, 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), and 0.1 mM ethylenediaminetetraacetic acid (EDTA) adjusted to pH 7.4 was used throughout this study.

The following static light scattering, dynamic light scattering and zeta potential measurements were performed in HEPES buffer using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK) equipped with a He-Ne laser with a wavelength of 632.8 nm with a backscattering geometry at a constant scattering angle of 173°. Values for viscosity, refractive index and dielectric constant of HEPES buffer were chosen from the manufacturer's database (viscosity 1.0021 cP, refractive index 1.330 and relative dielectric constant 80.4). A refractive index of 1.45 was used for mucin proteins.

2.2. Static Light Scattering (SLS)

A 800 μL portion of mucin solution was filled in a square glass cuvette, and the scattering intensity from different concentrations was measured. The SLS measurements were repeated 6 times each consisting of 10 runs with a single run duration of 10 s. A refractive index increment of 0.1 mL/g was used for mucin solution.

2.3. Surface activity

Critical aggregation concentration (c^*) of mucin was calculated from the surface tension of 60 μL suspensions (concentrations: 20 μg/mL–10 mg/mL) using a Kibron Micro TroughX (Kibron Inc., Espoo, Finland). Each data point corresponds to a mean value of at least three independent measurements.

2.4. Dynamic Light Scattering (DLS)

A 100 μL portion of mucin solution (10 mg/mL) was added to a 300 μL portion of vesicle suspension (1 mM), prepared by extrusion through a polycarbonate membrane with a pore size of 100 nm (Avestin, Mannheim, Germany). As the apparent molecular weight of mucin obtained from SLS does not correspond to the native one due to the preparation protocols (Footnote: information from the manufacturer), the weight concentration of mucin was kept constant to compare mucin type II and mucin type III. DLS experiments were carried out at 25°C. DLS measurements on pure mucin were repeated 3 times each consisting of 100 to 500 runs with a single run duration of 30 s while that on mucin and vesicle suspensions were repeated more than 5 times with a single run duration of 60 s. The raw data were analyzed as distribution by intensity with Igor PRO (WaveMetrics, Portland, USA) software using a log-normal function $f(x,K) = K_0 + K_1 * \exp -[\ln(x/K_2)/K_3]^2$ yielding the position of the maximum from K_2 and the full width at half maximum (FWHM)