Electronic Supplementary Material (ESI) for Soft Matter

Supporting Information

Adsorption of bile salts to particles allows penetration of intestinal mucus

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Materials and Methods

Bulk rheology

Rheological properties of both, the *ex vivo* mucus and the partially purified mucin, were investigated in dynamic oscillatory and rotational tests using a controlled strain AR2000 rheometer (TA Instruments, Crawley, West Sussex, UK) equipped with a cone and plate geometry (acrylic cone; 2°/40 mm, the cone angle/diameter). The following tests were performed at 37 ± 0.1 ^{o}C: (i) *a frequency sweep test*, where the applied deformation increased stepwise from 0.05 to 10 Hz over 5 min at a strain of 0.2%, (ii) *a strain sweep test* at fixed frequency (1 Hz), where the strain amplitude was increased stepwise from 0.01 to 100% over a period of ca. 4 min, and (iii) *a viscosity ramp test* for a shear rate being increased from 0.01 to 500 s^{-1} over 15 min.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR in the Attenuated Total Reflection mode (FTIR-ATR) spectra were measured for the *ex vivo* mucus and the partially purified mucin on a Bio-Rad FTS175C FTIR spectrometer equipped with an MCT detector (Bio-Rad Laboratories Inc., Cambridge, MA). Samples were analyzed in duplicate on a GoldenGate single reflection diamond ATR sampling unit (Specac Ltd, Orpington, Kent, UK). For each spectrum 128 scans at 2 cm^{-1} resolution were averaged and referenced against the empty ATR crystal. Buffer spectra recorded with the same

parameters were subtracted manually. The FTIR characterizations of the mucus and the mucin were done for untreated materials as well as for the samples that have been stained with Oregon Green (see *Specimen Preparation for Confocal Microscopy*) and/or subjected to the BS. In the latter case, the (un)stained mucus or mucin samples were thoroughly mixed with the NaCl/Bis-Tris buffer (4:1 v/v) that contained BS, incubated for 2h at room temperature and analyzed as above.

Preparation of phospholipid vesicles

The phosphatidylcholine vesicles were used in the *in vitro* model of digestion to mimic phospholipid vesicles secreted by the stomach.

The solvent was evaporated from a 0.94 mL aliquot of egg L-α-phosphatidylcholine (PC, 99% purity; Lipid Products, South Nutfield, UK) stock solution (50 mg/mL chloroform), placed in a 50-mL round bottom flask. The formed thin film of phospholipids was then suspended in 6.1 mL of 0.15 M NaCl, pH 2.5 (37^{o}C, 170 rpm for 30 min). The suspension was transferred to an ice-jacked vessel and repeatedly sonicated until it was clear, using a probe sonicator working at 40% amplitude (Digital Sonifier 250, Branson Ultrasonics Corp., CT). The vesicles were filtered through a 0.22 μm hydrophilic Durapore® syringe filter (Millipore, Bedford, MA) to remove any titanium deposited by the sonicator. The size of the PC vesicles was determined by the dynamic light scattering method using a Nano-ZS Zetasizer (Malvern Instruments Ltd, Malvern, UK) operated in a size-measure mode. The mean particle size, obtained from 3 dispersions prepared under the same conditions, was 82.4 ± 3.7 nm. The dispersion was incubated at 37^{o}C for 20 min before using in the digestion experiments.

***In vitro* gastric and duodenal digestions of NaCas stabilized emulsion**

The *in vitro* proteolysis was performed in triplicate either in the presence or absence BS in the duodenal compartment. Samples of emulsions were collected after the digestion, and analyzed using the techniques described below.

***In vitro* gastric digestion**

Before digestion, 6 mL of the emulsion was diluted with 3 mL of the PC dispersion. The pH of the system was adjusted to 2.5 with 0.1 M HCl/NaOH and the volume further made up to 9.9 mL with 0.15 M NaCl (pH 2.5). After 10 min preincubation at 37^{o}C, 100 µL of the solution of porcine pepsin (P6887, Sigma, Poole, UK; activity: 3,300 U/mg of protein calculated using haemoglobin as substrate) in 0.15 M NaCl (pH 2.5) was added to give a concentration of 50

μg/mL. The final concentration of Na-Cas in the digestion mix was 1mg/mL (pepsin:Na-Cas ratio of 1:20, w/w), PC of 2.32 mg/mL and the oil content of 18% (w/w). The digestion was done under continuous moderate agitation (170 rpm) at 37^{o}C for 60 min. After this time, the pepsinolysis in the gastric mixture was terminated by increasing the pH to 7.5 with 0.1 M NaOH and kept at 37^{o}C for 15 min to inactivate pepsin. The resulting gastric emulsion was transferred directly to the duodenal compartment of digestion.

***In vitro* duodenal digestion**

The gastric emulsion was made up with a 0.5 M Bis-Tris buffer (pH 6.5; Sigma, Poole, UK) to a final Bis-Tris concentration of 25 mM. A mixture of bile salts (BS), comprising equimolar quantities of sodium taurocholate (T4009, Sigma, Poole, UK) and sodium glycodeoxycholate (G9910, Sigma, Poole, UK), was added to a final total concentration of 7.4 mM. The pH of the system was adjusted to 6.5 with 0.1 M NaOH and the digestion followed for 30 min at 37^{o}C (170 rpm), mimicking the transfer of the gastric digesta into the duodenal conditions and its further degradation. Control experiments were also done in the absence of BS. The digestion was performed with duodenal proteases, porcine trypsin (activity: 13,800 U/mg of protein calculated using BAEE as substrate) and bovine α-chymotrypsin (activity: 40 U/mg of protein calculated using BTEE as substrate). Both enzymes were purchased from Sigma (T0303 and C7762 respectively). The enzymes were dissolved in 0.15 M NaCl (pH 6.5) and added to the digestion mixture to give final enzyme:Na-Cas ratios of 1:400 (w/w) for trypsin and 1:100 (w/w) for chymotrypsin. After 30-min digestion, the enzymes were inactivated by mixing the emulsion with Bowman-Birk trypsin-chymotrypsin inhibitor solution (T9777 Sigma, Poole, UK) at the concentration calculated to inactivate twice the amount of the duodenal enzymes used. The emulsion obtained finally after the duodenal compartment is referred to as a gastro-duodenal emulsion (GDE) throughout this paper.

Emulsion droplet size measurements

The droplet size was determined using a LS-230 laser-diffraction particle sizer (Beckman Coulter Ltd, High Wycombe, UK). The measurements were made for three replicate emulsions obtained under the same conditions.

ζ-potential measurements

The ζ-potential of dispersions of particles (i.e. dispersed mucus/mucin, latex beads, emulsion droplets, bacteria) was obtained from dynamic light scattering measurements using

a Nano-ZS Zetasizer (Malvern Instruments Ltd, Malvern, UK). Prior to analysis, dispersions were diluted with 0.15 M NaCl, 25 mM Bis-Tris buffer (pH 6.5; either without or with the BS, 7.4 mM) to the concentrations given for different types of analyzed systems in the Materials and Methods section of the manuscript. Diluted dispersions were then injected into a DTS1060 folded capillary cell (Malvern Instruments Ltd). Each sample was analyzed at least 20 times and the results displayed as a mean. Data shown are the average and standard deviation from three dispersions prepared under the same conditions.

Results

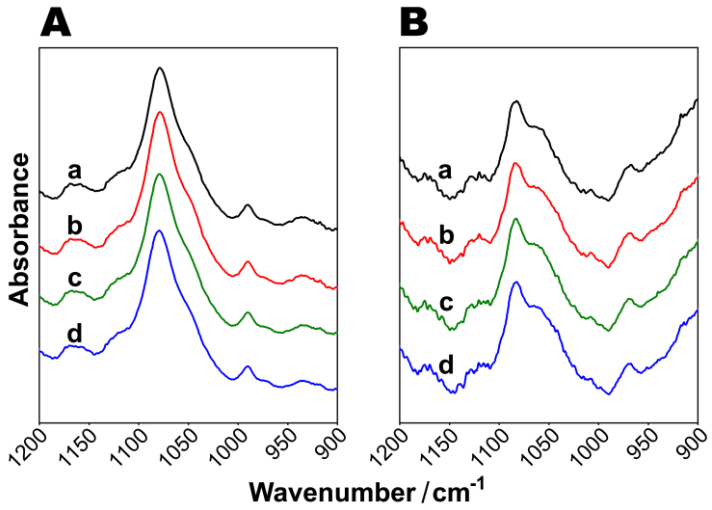

Figure S1

FTIR spectra of the carbohydrate region for (A) the *ex vivo* mucus and (B) the partially purified mucin. The following samples were analyzed: (a) control material (no treatment with either the bile salts (BS) or the Oregon Green stain (OG)), (b) after 2h incubation with the BS, (c) after 30 min staining with OG, (d) after 30 min staining with OG followed by 2h incubation with the BS.

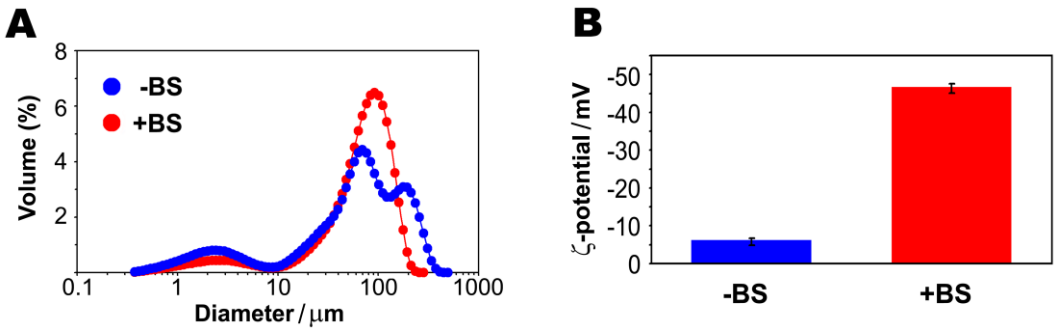

Figure S2

(A) Droplet size distributions and (B) ζ-potential mean and standard deviation of emulsions obtained just after simulated gastro-duodenal proteolysis of NaCas stabilizing the emulsions. The digestion experiments were carried out in the presence or absence of BS.

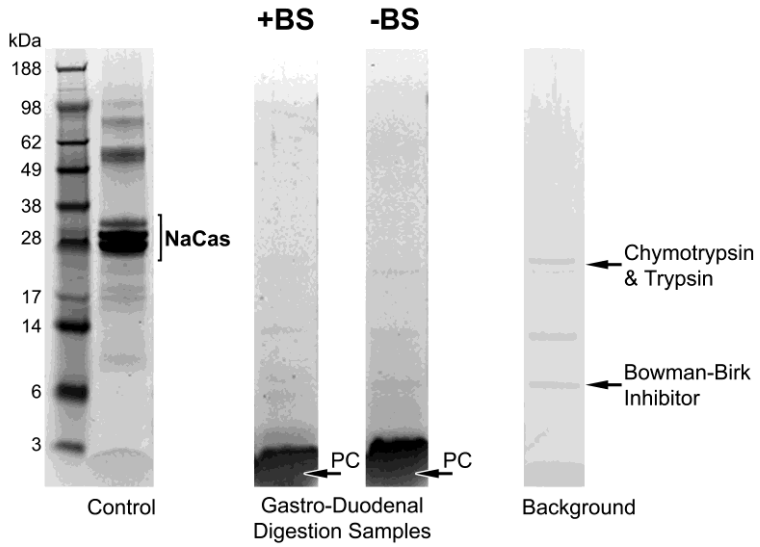

Figure S3

SDS-PAGE analysis (reducing conditions) of NaCas from emulsion before the digestion experiments (control), and the results of gastro-duodenal digestions carried out in the presence or absence of BS. The background sample comprised trypsin and chymotrypsin, used at the same concentrations as in digestion, and Bowman-Birk inhibitor as used in the digestion samples to stop proteolytic activity of the enzymes.