The effects of denatured major bovine whey proteins on the digestive tract, assessed by Caco-2 cell differentiation and on viability of suckling mice.

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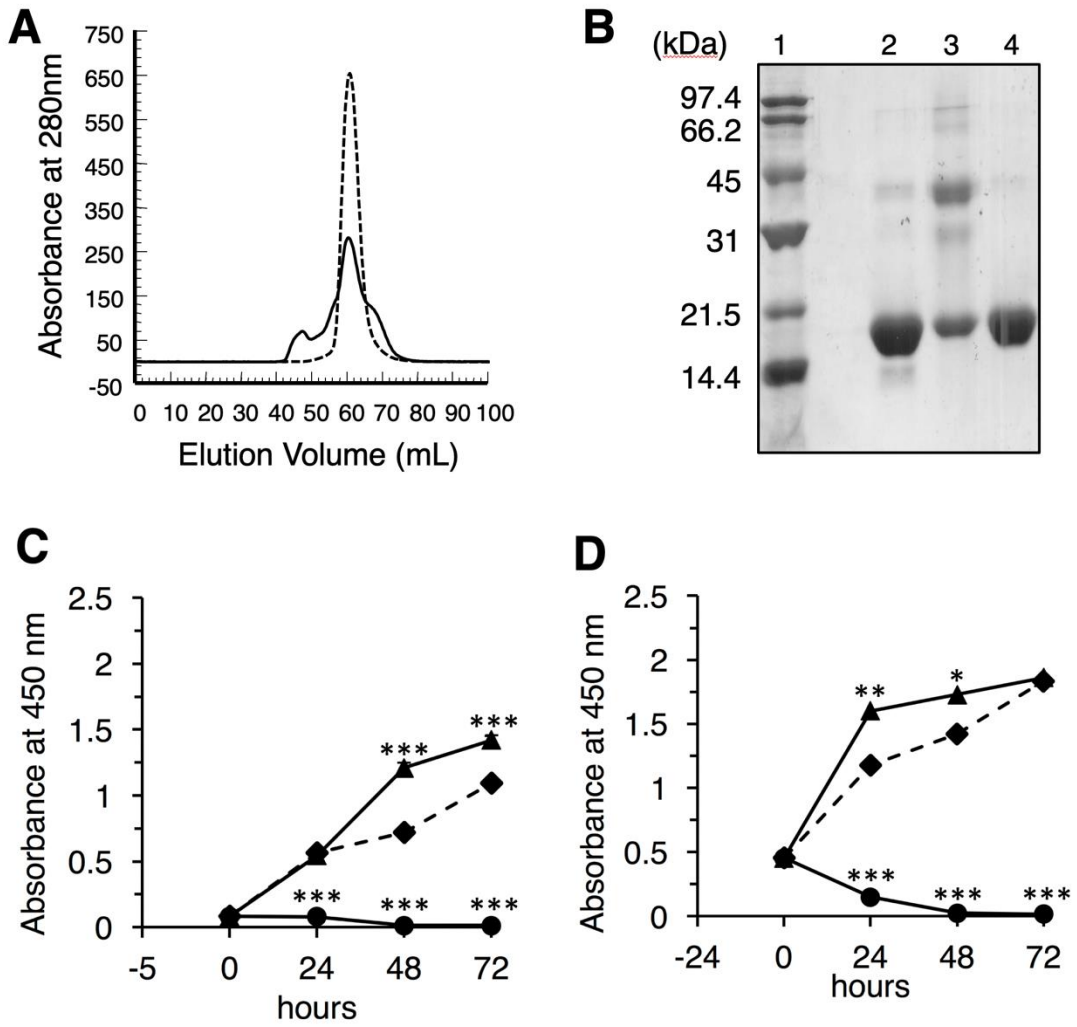
SUPPLEMENTARY FILE

Supplementary materials and methods

Purification of native α-LA and β-LG

To obtain the highly purified native α-LA and β-LG from commercial products, size exclusion chromatography was conducted using a Sephacryl S-100 HR (GE Healthcare, Chalfont St. Giles, UK) 5.0 cm × 60 cm column equilibrated with 50 mM sodium phosphate buffer (pH 6.0) connected to and controlled by the ÄKTA 10S system (GE Healthcare). Commercial α-LA and β-LG were dissolved in the same buffer at a concentration of 20 mg/mL and then loaded into the column at a flow rate of 7 mL/min. Each 2 mL fraction was monitored for protein content by using spectrophotometry at 280 nm. The main peak fraction was collected as the native form and was freeze-dried after dialysis with distilled water.

The obtained native and TFE-treated β-LGs were analyzed by high-performance liquid chromatography (HPLC) using HiLoad^{TM} 16/60 Superdex 75 prep grade column (GE Healthcare) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described Xu et al. (2005a). Milk protein concentration was determined using a Bradford Protein Assay Kit (Bio-Rad) with bovine serum albumin as the standard and by following the manufacturer's instructions.



Supplementary Figure 1 Kobayashi *et al*.

Fig. S1. Characterization of the native and TFE-treated β-LGs. (A) Size exclusion chromatography of the native and TFE-treated β-LGs using a Superdex 75 prep grade column. Native β-LG, dotted lines; TFE-treated β-LG, solid lines. (B) SDS-PAGE analysis of commercial, native, and TFE-treated β-LGs. Samples were separated in 15 % polyacrylamide gel electrophoresis, and the gel was stained in CBB. Lane 1, standard of molecular weights; lane 2, commercial β-LG; lane 3, TFE-treated β-LG; lane 4, native β-LG. (C and D) Cell growth effects of the native and TFE-treated β-LGs on IEC-6 cells. Cells were seeded and incubated for 5 or 24 h in DMEM supplemented with 10% FCS (C, 5 h pre-culture; D, 24 h pre-culture). After pre-culture, the medium was removed, and cells were added with DMEM

supplemented with 1% FCS in the absence of sample (control, diamonds and dotted line) or presence of 10 mg/ml of native β-LG (triangles and solid line), or presence of 10 mg/ml of the TFE-treated β-LG (circles and solid line), then incubated for 24, 48, and 72 h. Cell growth was detected using WST-1 assay, and absorbance was monitored at 450 nm. Each measurement was performed in quadruplicate. Each value represents the mean ± SD. ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ indicate significant difference from the respective control.

Reference

Xu M, Sugiura Y, Nagaoka S and Kanamaru Y (2005) IEC-6 intestinal cell death induced by bovine milk α-lactalbumin. *Bioscience, Biotechnology and Biochemistry* **69** 1082-1089.