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Supplementary Data

Supplementary Methods

MALDI-TOF MS

To estimate the removal yield of BGP-C7 by the dialysis, the treated serum samples added with BGP-C7 at the final concentration of 50 μg/mL were dialyzed at 4ºC using a Slide-A-Lyzer 3K MWCO or incubated at 4ºC. After overnight dialysis or incubation, BGP-C12 peptide was added to the serum samples as an internal standard. For dried-droplet preparations, aliquots of 1 μL were applied onto the target plate and immediately mixed with an equal volume of 10 mg/mL α-hydroxycinnamic acid in 50% (v/v) acetonitrile, 50%(v/v) water and 0.1% (v/v) trifluoroacetic acid, and dried. The target plate was loaded into Voyager DE-STR MALDI TOF mass spectrometer (Applied Biosystems, Framingham, MA), and mass spectra of the samples were obtained in the reflector mode. A total of 500 single-shot spectra were accumulated from each sample.

To investigate the degradation rate of BGP-C7 in serum, the treated and non-treated sera were added with a 10-fold volume of PBS containing 50 μg/mL of BGP-C7, incubated at room temperature. After 0, 12 and 24 h, aliquots from the serum solutions were used for mass spectrometry analysis as described above.

Western Blotting

The digestion of MBP-V_L in serum was investigated by Western blotting. The treated and non-treated human sera were added with a 100 vol of PBS containing 50 μg/mL of MBP-V_L, and analyzed after incubation at room temperature for 1, 3, 10 and 15 h. The samples were separated by the 10% SDS-PAGE and transferred to a PVDF membrane (BioRad). HRP

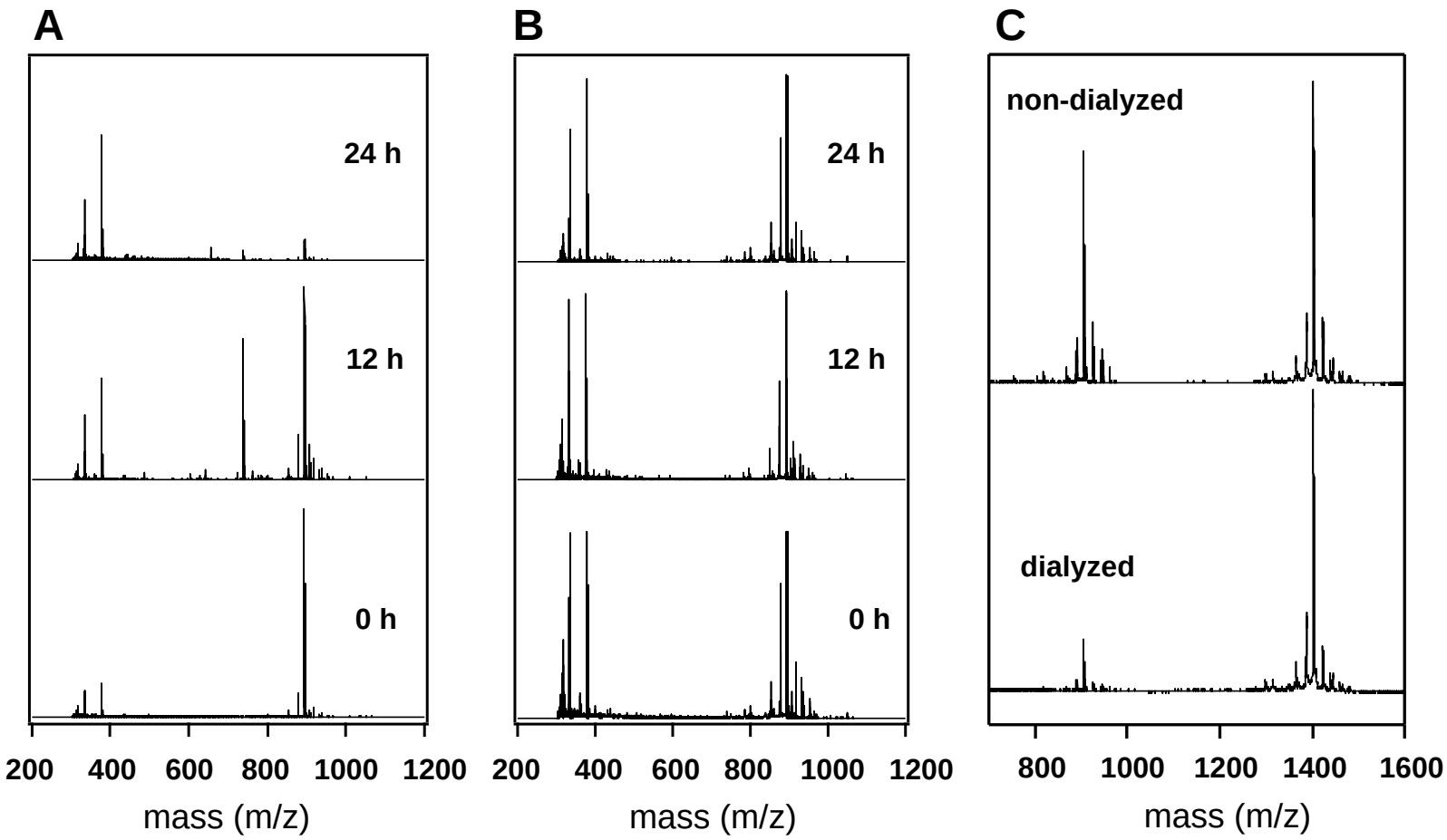
conjugated anti-His tag antibody (Penta-His HRP Conjugate, QIAGEN GmbH, Hilden, Germany) was used to detect MBP-V_L and reacted with chemiluminogenic reagent ECL plus (GE healthcare). The images were acquired with a LAS-4000 imager (FUJIFILM Co. Tokyo, Japan).

Supplementary Figures

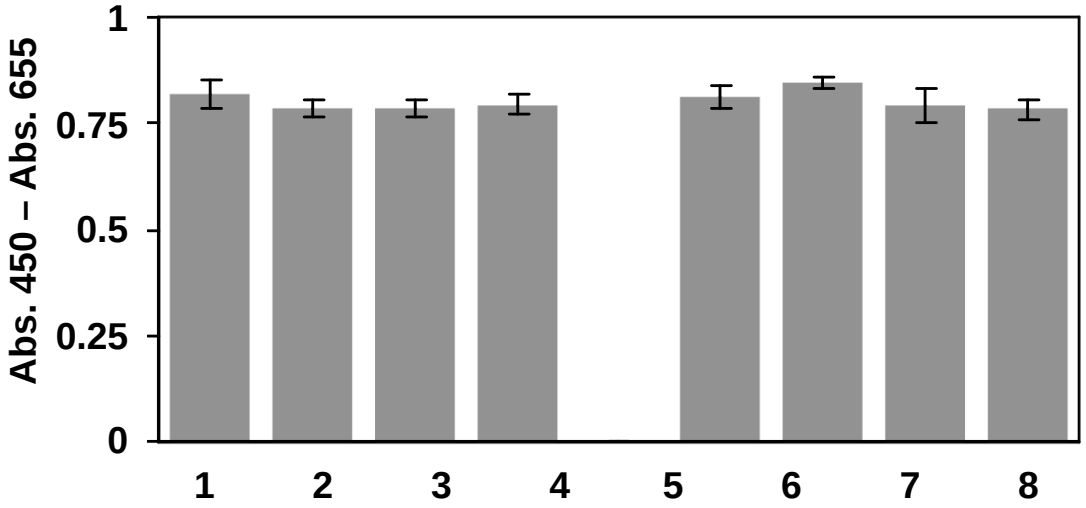
Fig. S1. Mass spectra of BGP-C7. BGP-C7 in PBS (50 μg/mL, 90 μL) was added with 10 μL of non-treated (A) and pretreated (B) human sera, and incubated at room temperature for 0, 12 and 24 h. (C) To estimate the yield of the dialysis, the treated human sera added with BGP-C7 at the final concentration of 50 μg/mL were dialyzed at 4ºC using a Slide-A-Lyzer 3K MWCO or incubated at 4ºC. After overnight dialysis or incubation, BGP-C12 peptide was added to the serum samples as an internal standard.

Fig. S2. The effect of the pre-treatments. (1)-(4) OS-ELISA for Sample 1-4. Sample 1 is 20% human serum in PBS containing 10 μM of BGP-C7. Sample 2 is the BlueGel-treated sample 1. Sample 3 is the sample 2 added with 8x PIC solution. Sample 4 is the sample 3 heated at 65°C for 10 min. Because the BlueGel treatment and the addition of PIC changed the volume to approximately 1.1-fold, the changes were compensated by adding PBS to the non-treated samples. (5)-(8) Sandwich-ELISA for Sample 5-8. Sample 5 is PBS containing 10 μM of BGP. Sample 6 is the BlueGel-treated sample 5. Sample 7 is the sample 6 added with 8x PIC solution. Sample 8 is the sample 7 heated at 65°C for 10 min. The compensation of the sample volume was performed in a same way as described above.

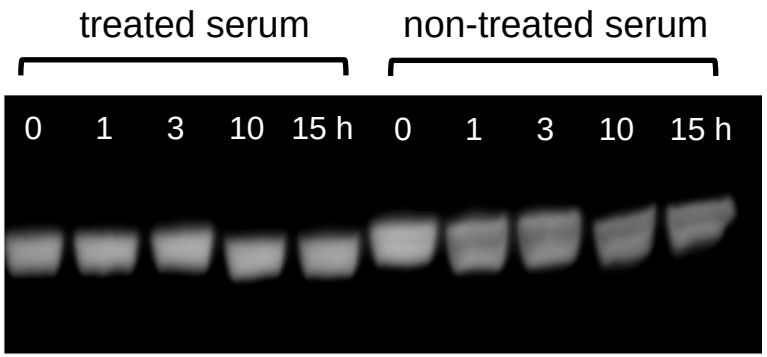
Fig. S3. The digestion of MBP-V_L in serum investigated by Western blotting.



Ihara et al. Figure S1



Ihara et al. Figure S2



Ihara et al. Figure S3