Supplementary Information

Title: Sugar chain-binding specificity and native folding state of lectin preserved in hydrated ionic liquids

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Materials and Methods

Cholinium dihydrogen phosphate ([ch][dhp]) was synthesised as previously described. Other ILs, i.e., 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4mim]BF_4$) and 1-ethyl-3-methylimidazolium methylphosphate ($[C_2mim][MeO(H)PO_2]$), were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). These ILs were hydrated to have three water molecules per ion pair. Other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). ConA, bovine serum albumin (BSA), and mannotriose-modified BSA (mannotriose-BSA) were purchased from Funakoshi Co., Ltd. (Tokyo, Japan). These reagents were used as received.

The solubility and folding state of ConA in buffer or hydrated ILs were analysed after centrifugation by fluorescence spectroscopy (FP-8300; Jasco Co., Japan) using excitation light at a wavelength of 280 nm.

The sugar chain-recognition ability of ConA dissolved in hydrated ILs (5 mg mL^{-1}) was investigated after dilution in the running buffer solution (1000 times) using surface plasmon resonance (SPR) spectroscopy. A BIAcore™ T200 biosensor (BIAcore, Inc., Uppsala, Sweden) was used to investigate the sugar-recognition ability (i.e., binding kinetics) of ConA for mannotriose-BSA. Mannotriose-BSA and BSA (as a reference)

were immobilised on a CM3 or CM5 sensor chip. Binding affinity was measured by running a buffer solution (HBS: 10 mmol L^{-1} HEPES, 0.15 mol L^{-1} NaCl and 0.005% Tween 20, pH 7.4) at a constant flow rate of 20 μL min^{-1}. Each binding experiment was performed for 120 s after treatment; 3.5 mol L^{-1} guanidine hydrochloride (GdnHCl) was used as a regeneration solution, and samples were then washed for 60 min with HBS.

Thermal treatment was carried out to ConA (5 mg mL^{-1}) dissolved in hydrated [ch][dhp] and phosphate buffer at 70°C for 10 min. The dissociation constant (K_D) of the dissolved ConA was calculated using association rate constant (Ka) and dissociation rate constant (Kd) from the SPR data. Samples with six different concentrations were used to calculate Ka and Kd by using a Biacore T200 Evaluation software which is based on non-linear least-squared method.

For the analysis of long-term stability, the ConA which was dissolved in hydrated [ch][dhp] and in phosphate buffer at 5mg/ml, were stored at 4 ^{o}C for 6 months. The stored samples were diluted with SPR running buffer (HBS, pH 7.4) by 1000 times and analysed with SPR measurement.

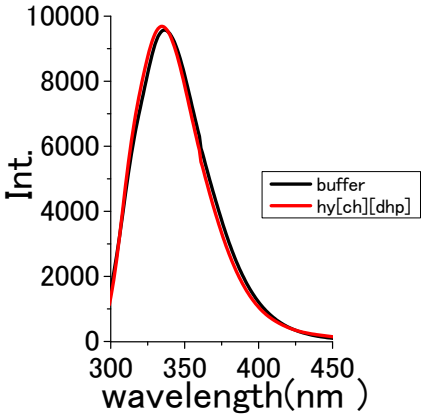

Figure S1. *Fluorescence spectra of concanavaline A dissolved in hydrated [ch][dhp] and phosphate buffer.*

The solubility and folding state of ConA in hydrated [ch][dhp] and phosphate buffer were analysed after centrifugation by fluorescence spectroscopy (FP-8300; Jasco Co., Japan) using excitation light at a wavelength of 280 nm. This suggested that ConA maintained its folding state when dissolved in hydrated [ch][dhp], as well as when dissolved in the buffer solution.

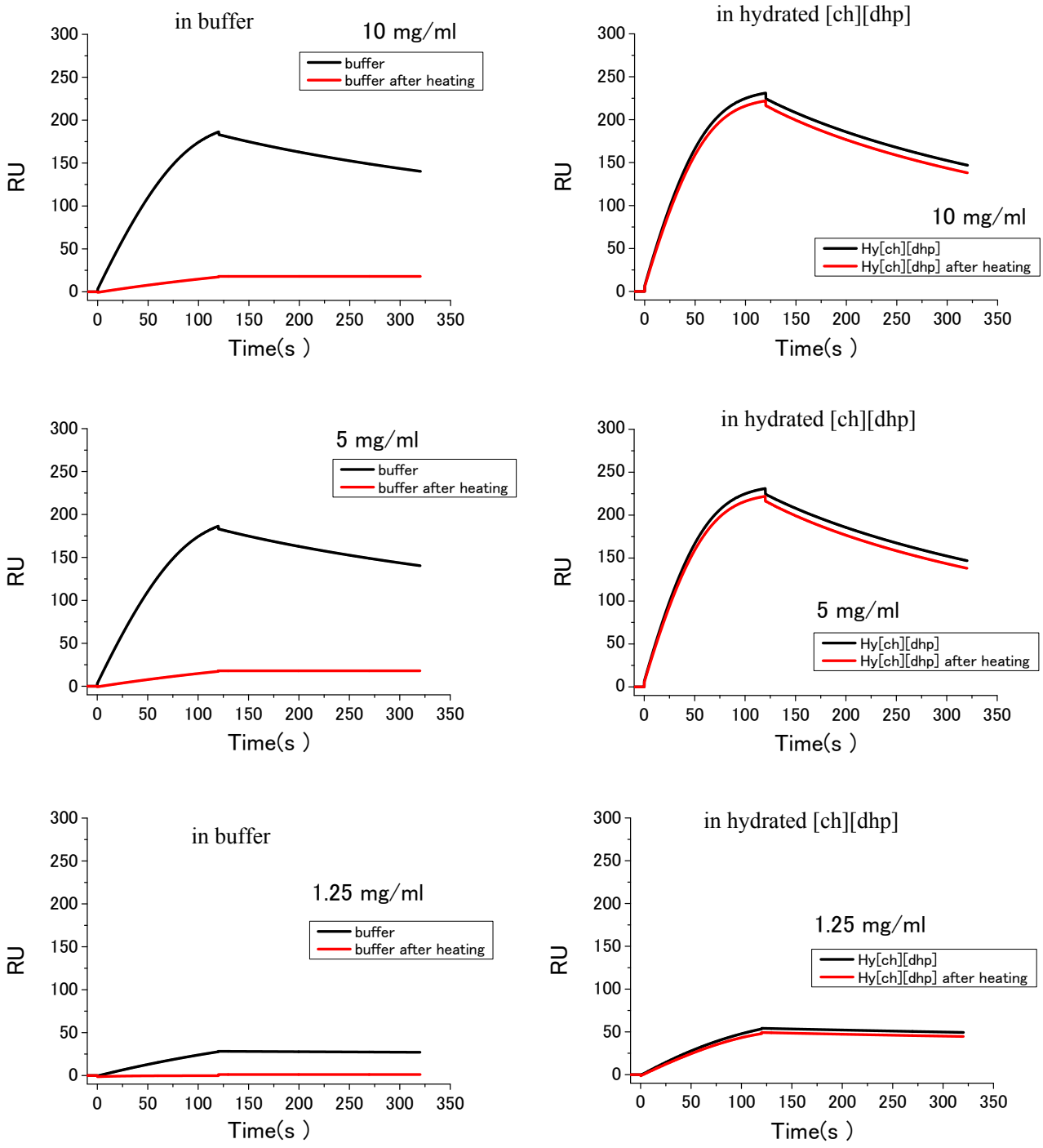

Figure S2. *SPR signals of concanavaline A after dilution with buffer, which had dissolved in buffer and hydrated [ch][dhp] with temperature treatment at 70 °C for 10 min.*

At all of the investigated concentrations, ConA maintained its original sugar chain binding affinity after thermal treatment when dissolved in hydrated [ch][dhp], but exhibited decreased sugar chain binding affinity when dissolved in only the buffer solution.

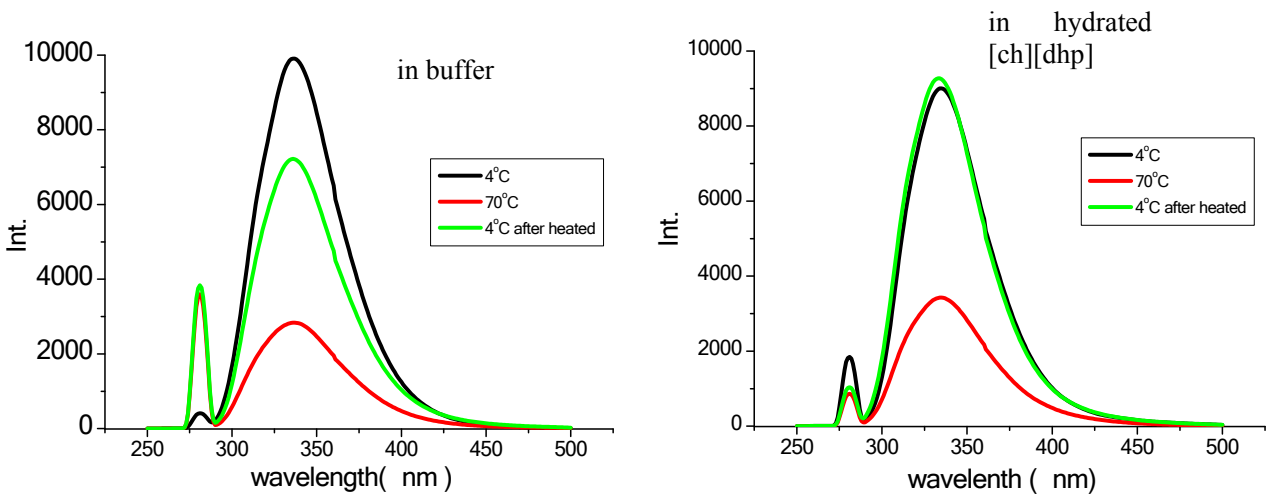

Figure S3. *Fluorescence spectra of concanavaline A dissolved in buffer and hydrated [ch][dhp] with temperature treatment.*

The folding state of ConA was investigated by determining the fluorescence spectral shift and spectral intensity during thermal treatment. Before thermal treatment, similar fluorescence spectra were observed in buffer and hydrated [ch][dhp] at 4°C. Under thermal treatment at 70°C, a decrease in spectral intensity was observed both in the buffer solution and the hydrated [ch][dhp], suggesting a change in the folding state. However, when the solution was then cooled down to 4°C, the spectrum intensity observed in hydrated [ch][dhp] showed recovery. When ConA was dissolved in the buffer solution, the spectral intensity around 340 nm was not recovered. Additionally, the spectral intensity following excitation at 280 nm significantly increased as compared with that observed without heat treatment, suggesting the formation of aggregates during heat treatment in the buffer solution.

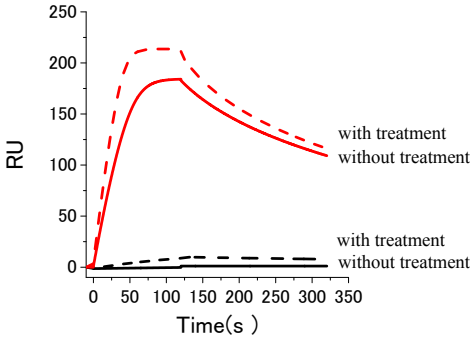

Figure S4. *Effect of long term storage (6 months at 4 °C) on the binding ability to sugar chain of con A dissolved in buffer (black line) and hydrated [ch][dhp] (red line). The sample were stored without (solid line) and with (dashed line) temperature treatment at 70 °C for 10 min.*

The ConA stored in hydrated [ch][dhp] for 6 months maintained its sugar chain binding affinity, even after heat treatment. On the other hand, almost no sugar chain binding affinity was observed when ConA was stored in the buffer solution regardless of thermal treatment.