

# **A protic ionic liquid attenuates the deleterious actions of urea on $\alpha$ -chymotrypsin.**

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## Experimental

### Methods

$\alpha$ -Chymotrypsin (CT) from bovine pancreas type II, essentially salt free (molecular weight: 25 kDa) was obtained from Sigma–Aldrich (USA). All materials, with high purity, were used without further purification. Buffer solution was prepared using distilled deionized water at 18.3 M $\Omega$ . All mixture samples were prepared gravimetrically using a Mettler Toledo balance with a precision of 0.0001.

### NMR studies

Spectra were recorded on a Bruker-Biospin 500 instrument. The spectra are the result of 256 scans, 1 s (delay time) at 303.4 K, and were processed with Bruker Topspin version 2.1. The concentration of CT in all solutions used for NMR spectroscopy was 40 mg/mL prepared. About 10% of  $^2\text{H}_2\text{O}$  was added to provide an internal-field-frequency lock signal. A small amount of tris buffer was added (50 mM) to stabilize the pH.

### Circular dichroism spectroscopy

CD spectroscopic studies were performed using a PiStar-180 spectrophotometer (Applied Photophysics, UK) equipped with a Peltier system for temperature control. CD calibration was performed using (1S)-(+)-10-camphorsulfonic acid (Aldrich, Milwaukee, WI), which exhibits a 34.5 M/cm molar extinction coefficient at 285 nm, and 2.36 M/cm molar ellipticity ( $\theta$ ) at 295 nm. The sample was pre-equilibrated at the desired temperature for 15 min and the scan speed was fixed for adaptative sampling (error F 0.01) with a response time of 1 s and 1 nm bandwidth. The tertiary structures of CT were monitored by using near-UV (240–300 nm), (1.0 cm path length cuvette). The CT concentration was 1 mg/ml and each spectrum was collected by averaging six spectra. Each sample spectrum was obtained by subtracting appropriate blank media without CT from the experimental enzyme spectrum. Thermal denaturation studies were carried at a heating rate of 1  $^{\circ}\text{C}/\text{min}$ . This scan rate was found to provide adequate time for equilibration. Each sample was heated from 20 to 80  $^{\circ}\text{C}$ . The change in absorbance with increasing temperature was followed at 288 nm for CT. After denaturation, the sample was immediately cooled down to measure conformational changes of the protein. The error in  $T_m$  does not exceed 0.1  $^{\circ}\text{C}$ . The estimated relative uncertainties in ( $\Delta H$ ), ( $\Delta C_p$ ) and ( $\Delta G_u$ ) are around 2-5% of the reported values.

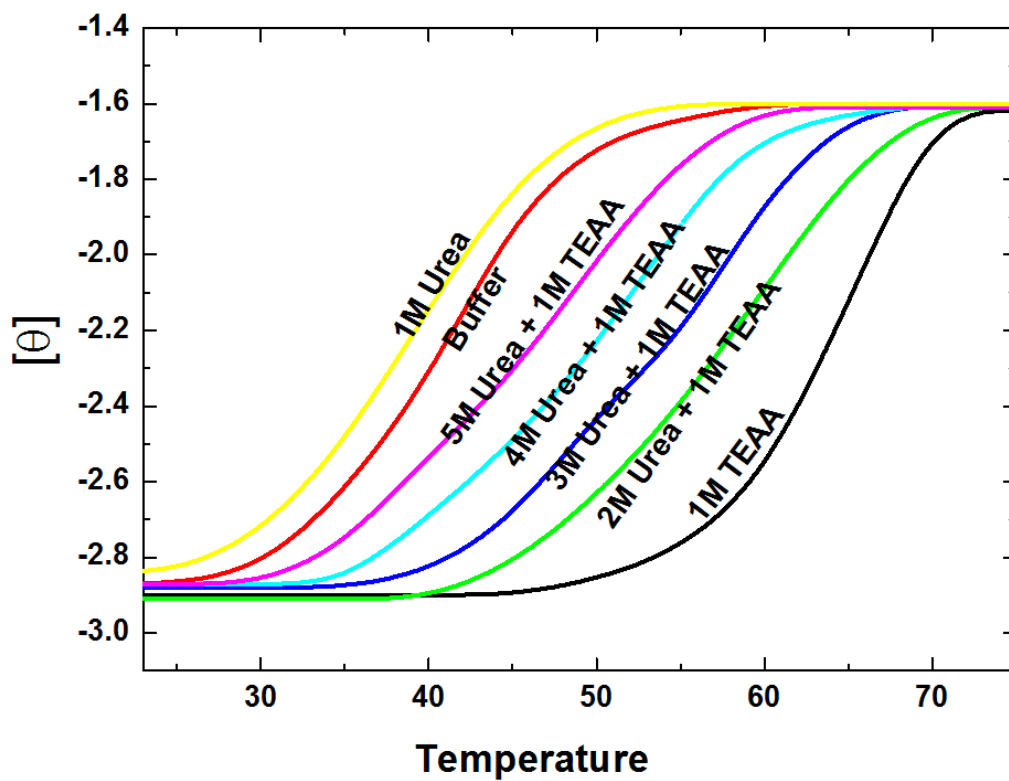
$$y(T) = \frac{y_N(T) + y_D(T) \exp \left[ -\frac{\Delta H}{R \left( \frac{1}{T} - \frac{1}{T_m} \right)} \right]}{1 + \exp \left[ -\frac{\Delta H}{R \left( \frac{1}{T} - \frac{1}{T_m} \right)} \right]} \quad (1)$$

Each heat-induced transition curve was analyzed for  $T_m$  (midpoint of denaturation) and  $\Delta H$  (denaturational enthalpy change at  $T_m$ ) using a non-linear least squares method according to the equation 1. In equation 1,  $y(T)$  is the optical property at temperature  $T$ ,  $y_N(T)$  and  $y_D(T)$  are the optical properties of the native and denatured protein molecules at  $T$ , respectively, and  $R$  is the gas constant. In the analysis of the transition curve, it was assumed that a parabolic function describes the dependence of the optical properties of the native and denatured protein molecules (i.e.  $y_N(T) = a_N + b_N T + c_N T^2$  and  $y_D(T) = a_D + b_D T + c_D T^2$ , where  $a_N$ ,  $b_N$ ,  $c_N$ ,  $a_D$ ,  $b_D$ , and  $c_D$  are temperature-independent coefficients). A plot of  $\Delta H$  versus  $T_m$  at each concentration of ILs provides the value of  $\Delta C_p$ , the constant-pressure heat capacity change.

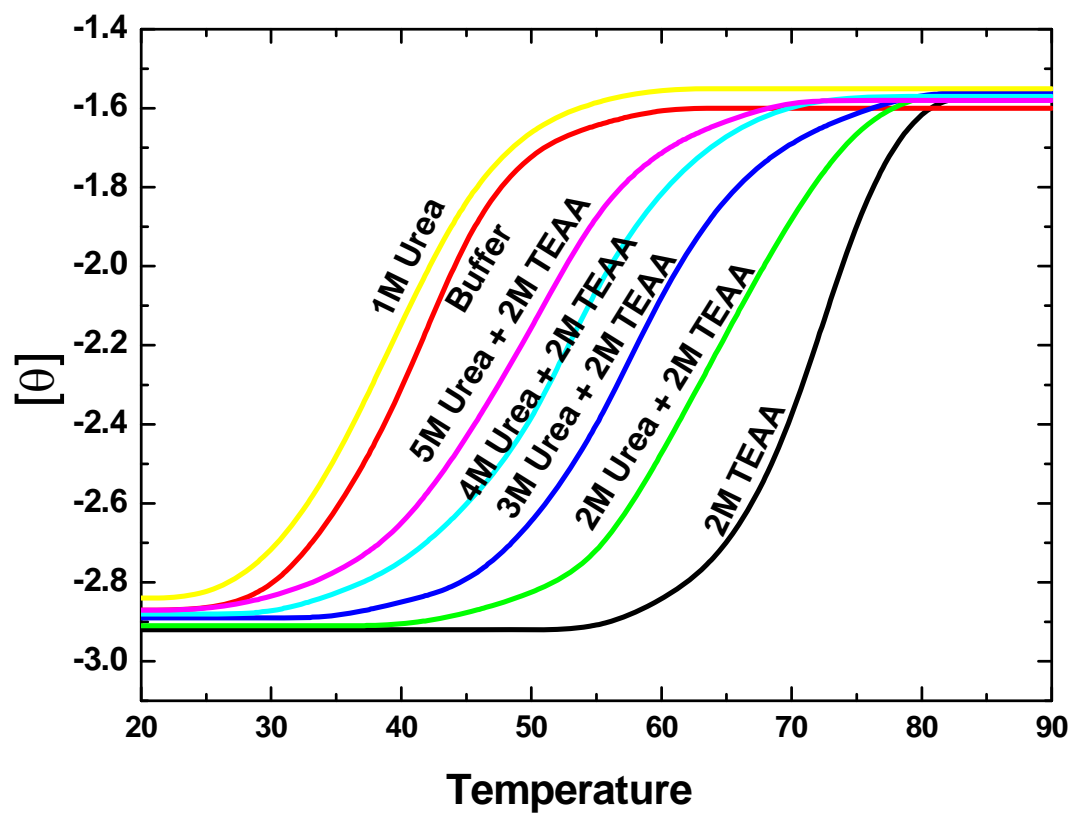
$$\Delta C_p = \left( \frac{\Delta H}{\Delta T_m} \right)_p \quad (2)$$

### Fluorescence spectroscopy

Steady-state fluorescence measurements were conducted with a Cary Eclipse spectrofluorimeter (Varian optical spectroscopy instruments, Mulgrave, Victoria, Australia) equipped with thermostated cell holders and temperature was kept constant by a circulating water bath using a peltier device attached to the sample holder of the fluorimeter. The excitation wavelength was set at 295 nm in order to calculate the contribution of the tryptophan residues to the overall fluorescence emission. The experiments were performed at 25 °C by using a 1cm sealed cell and both excitation and emission slit width were set at 5 nm, and corrected for background signal. Both the change in fluorescence intensity and the shift in fluorescence maximum wavelength were recorded to monitor the unfolding transition.



**Figure S1** The variation in  $T_m$  values of CT in buffer (control), 1 M urea, 1 M TEAA and various urea concentrations (2-5 M) in the presence 1 M of TEAA.



**Figure S2** The variation in  $T_m$  values of CT in buffer (control), 1 M urea, 2 M TEAA and various urea concentration (2-5 M) in the presence 2 M of TEAA.

**Table 1** Transition temperature ( $T_m$ ), calorimetric enthalpy changes ( $\Delta H$ ), Gibbs free energy ( $\Delta G_u$ ) and heat capacity change ( $\Delta C_p$ ) determined by CD analysis.

Solvent	$T_m$ ( $^{\circ}\text{C}$ )	$\Delta H$ ( $\text{kJ}\cdot\text{mol}^{-1}$ )	$\Delta G_u$ ( $\text{kJ}\cdot\text{mol}^{-1}$ )	$\Delta C_p^a$ ( $\text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ )
Buffer	42	424	18.15	10.0±0.09
1M TEAA	63	1302	102.5104	20.0±0.06
2M TEAA	72	1742	156.331	24.10±0.09
1M Urea	38	312	10.89453	8.0±0.10
2M Urea	33	255	6.174369	7.0±0.06
3M Urea	30	217	3.347569	7.0±0.20
4M Urea	27	195	1.618159	6.0±0.06
5M Urea	25	101	0.100936	3.9±0.09
1M TEAA: 1M Urea	58	962	67.78648	16.5±0.08
1M TEAA: 2M Urea	54	938	60.62168	17.0±0.03
1M TEAA: 3M Urea	50	909	52.44951	18.0±0.18
1M TEAA: 4M Urea	47	899	47.07451	19.0±0.12
1M TEAA: 5M Urea	44	875	41.38384	19.0±0.8
2M TEAA: 2M Urea	66	1402	114.6942	21.20±0.04
2M TEAA: 3M Urea	60	1379	101.2157	22.90±0.08
2M TEAA: 4M Urea	55	1339	88.02574	24.30±0.04
2M TEAA: 5M Urea	52	1287	78.38153	24.70±0.05

<sup>a</sup> plot of  $\Delta H$  versus  $T_m$  at each concentration mixture of TEAA and urea provides the value of  $\Delta C_p$