Supplimentary Informations

A comparative study of the Hofmeister series of anions of the ionic salts and ionic liquids on the stability of α-chymotrypsin

Awanish Kumar, Anjeeta Rani and Pannuru Venkatesu*

Department of Chemistry, University of Delhi, Delhi – 110 007.

Table 1S. Transition temperature (T_m), enthalpy change (ΔH) and heat capacity change (ΔC_p) determined by fluorescence spectroscopy and calculated Gibbs free energy changes (ΔG_u) in unfolding state at 25 ^{0}C for the α-chymotrypsin (CT) in various concentrations of ionic liquids (ILs)[a].

Sample	T_m ($^\circ$C)	ΔH kcalmol^{-1}	ΔG_u kcalmol^{-1}	ΔC_p kcalmol^{-1}K^{-1}
Pure CT in buffer	53.1	69.50	3.03	2.36
0.01 M [Bmim][SCN]	52.1	69.24	2.44	2.92
0.02 M [Bmim][SCN]	49.4	65.40	2.03	3.09
0.03 M [Bmim][SCN]	47.9	50.43	1.69	2.22
0.04 M [Bmim][SCN]	45.0	39.09	1.51	1.52
0.01 M [Bmim][HSO_4]	52.6	44.48	1.35	2.14
0.02 M [Bmim][HSO_4]	51.4	38.34	1.39	1.58
0.03 M [Bmim][HSO_4]	45.5	37.86	1.78	1.23
0.04 M [Bmim][HSO_4]	42.0	35.57	2.03	0.96
0.01 M [Bmim][Cl]	57.6	94.24	4.725	2.74
0.02 M [Bmim][Cl]	55.5	89.58	4.22	2.79
0.03 M [Bmim][Cl]	54.6	85.95	3.94	2.77
0.04 M [Bmim][Cl]	54.0	83.25	3.74	2.74
0.01 M [Bmim][Br]	58.3	71.10	3.63	2.02
0.02 M [Bmim][Br]	57.2	71.09	3.52	2.09
0.03 M [Bmim][Br]	56.9	69.40	3.41	2.10
0.04 M [Bmim][Br]	54.0	69.73	3.13	2.29
0.01 M [Bmim][CH_3COO]	59.6	83.74	4.43	2.29
0.02 M [Bmim][CH_3COO]	56.4	82.03	3.97	2.48
0.03 M [Bmim][CH_3COO]	55.8	79.03	3.76	2.44
0.04 M [Bmim][CH_3COO]	55.5	76.16	3.59	2.37
0.01 M [Bmim][I]	49.6	43.78	1.45	1.95
0.02 M [Bmim][I]	46.8	45.74	1.41	2.17
0.03 M [Bmim][I]	42.0	35.33	1.55	1.22
0.04 M [Bmim][I]	34.0	21.08	2.31	3.10

[a]Each Value is the average over three measurements. The error in T_m doesnot exceeds 0.1^{o}C. The estimated relative uncertainties in (ΔH), (ΔC_p) and (ΔG_u) are around 2% of the reported values.

Table 2S. Transition temperature (T_m), enthalpy change (ΔH) and heat capacity change (ΔC_p) determined by fluorescence spectroscopy and calculated Gibbs free energy changes (ΔG_u) in unfolding state at 25 ^{0}C for the α-chymotrypsin (CT) in various concentrations of ionic salts (Is)[a].

Sample	**T_m** ($^\circ$C)	**ΔH** kcalmol^{-1}	**ΔG_u** kcalmol^{-1}	**ΔC_p** kcalmol^{-1}K^{-1}
Pure CT in buffer	53.1	69.50	3.03	2.36
0. 01 M NaSCN	48.5	36.70	1.35	1.50
0. 02 M NaSCN	47.3	35.99	1.26	1.55
0. 03 M NaSCN	46.4	34.38	1.16	1.57
0. 04 M NaSCN	46.2	32.87	1.10	1.49
0. 01 M Na_2SO4	52.7	35.40	1.52	1.22
0. 02 M Na_2SO4	50.1	36.16	1.42	1.38
0. 03 M Na_2SO4	45.7	34.70	1.13	1.62
0. 04 M Na_2SO4	45.0	34.38	1.09	1.66
0.01 M NaCl	47.0	34.89	1.21	1.53
0.02 M NaCl	45.2	32.55	1.04	1.55
0.03 M NaCl	44.8	30.69	0.96	1.50
0.04 M NaCl	43.6	30.30	0.98	1.58
0.01 M NaBr	52.1	37.86	1.60	1.34
0.02 M NaBr	52.0	37.07	1.56	1.31
0.03 M NaBr	51.0	31.84	1.29	1.17
0.04 M NaBr	49.5	29.23	1.12	1.14
0. 01 M $NaCH_3COO$	48.0	35.54	1.28	1.48
0. 02 M $NaCH_3COO$	46.7	34.83	1.19	1.55
0. 03 M $NaCH_3COO$	46.7	33.97	1.16	1.51
0. 04 M $NaCH_3COO$	44.5	33.57	1.04	1.66
0.01 M NaI	51.6	38.36	1.60	1.37
0.02 M NaI	51.0	38.09	1.54	1.40
0.03 M NaI	48.4	38.64	1.35	1.51
0.04 M NaI	46.2	34.04	1.14	1.55

[a]Each Value is the average over three measurements. The error in T_m doesnot exceeds 0.1^{o}C. The estimated relative uncertainties in (ΔH), (ΔC_p) and (ΔG_u) are around 2% of the reported values.

Fluorescence Spectroscopy

Cary Eclipse spectrofluorimeter from Varian optical spectroscopy instruments, Mulgrave, Victoria, Australia was used to monitor the fluorescence emission spectra of CT that was equipped with thermostated cell holders. The steady-state fluorescence measurements were conducted at a constant temperature using a circulating water bath controlled by a UV1007M192 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 (Trp) residues to the overall fluorescence emission. The experiments were carried out in the range between 20 to 90 ^{0}C by using a 1.0 cm sealed cell and both excitation and emission slit width were set at 5 nm. The fluorescence intensity at the emission maximum for the native enzyme (~332 nm) was continuously recorded as the temperature was decreased from 20 to 90 ^{0}C at an approximate rate of 1.0 ^{0}C/min. This allowed the samples to attain thermal equilibrium before the measurements.

Thermodynamic analysis of proteins from the fluorescence curves

The unfolding of proteins has been observed to approach closely a two-state folding mechanism experimentally such as that shown in equation 1.

$$\text{Folded} \leftrightarrow \text{Unfolded} \tag{1}$$

Experimentally, the fraction of unfolded molecules is measured by the intensity of the emission. The fraction unfolded is determined as

$$\alpha = \frac{[U]}{([F] + [U])} \tag{2}$$

$$\alpha = \frac{(I_f - I)}{(I_f - I_u)} \tag{3}$$

In an equation, α is the fraction of unfolded molecules, I is the measured intensity at a given temperature, I_f is the measured intensity of the folded state, and I_u is the intensity of the completely unfolded state. The transition temperature of the protein (T_m) is the temperature at which α = 0.5. The importance as well as the details of obtaining the thermodynamic parameters such as free energy change of unfolding (ΔG_u), enthalpy changes (ΔH), and heat capacity change (ΔC_p) through fluorescence thermal analysis is recently elucidated elsewhere.[1-4]

The equilibrium constant (K_{eq}) between the native and the denatured states at a given temperature was obtained using the following equation

$$K_{eq} = \frac{\alpha}{1-\alpha} \tag{4}$$

The difference in free energy between the denatured and the native conformations (ΔG) can then be calculated using the equation as below,

$$\Delta G = -RTln K_{eq} \tag{5}$$

Where, *R* is the gas constant (1.987 Cal mol^{-1}) and T is the absolute temperature.

The entropy changes (ΔS) were calculated using the laws of thermodynamics; we have a relation as shown through a relation as below,

$$\Delta S = \left[\frac{-(\partial \Delta G)}{\partial T}\right]_P \tag{6}$$

And therefore, ΔS (at T_m) = ΔS_m= the slope of ΔG vs T.

ΔH is needed at only a single temperature, and the best temperature to use is T_m, the midpoint of the thermal unfolding curve where K_{eq} = 1.Therefore, at T_m the free energy change can be termed as ($\Delta G(T_m)$) and can be expressed as below,

$$\Delta G(T_m) = 0 = \Delta H_c - T_c \Delta S_c \tag{7}$$

Now the ΔH_m= (T_m in K) × (slope at T_m).

Additionally, the heat capacity change was obtained as shown below,

$$\Delta S = \Delta C_P \ln (T_m/T_s) \tag{8}$$

Where, T_s is referred to as the standard temperature at which the stability of the protein is maximum.

The obtained values were used to calculate the ΔG of unfolding at any temperature T, using Gibbs Helmholtz equation as shown below,

$$\Delta G(T) = \Delta H_m\left[1 - \left(\frac{T}{T_m}\right)\right] - \Delta C_P\left[(T_m - T) + T ln(\frac{T}{T_m})\right] \tag{9}$$

References:

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2. P. L. Privalov, Y. V. Griko, S. Y. Venyaminov, *J. Mol. Biol*. 1986, **190**, 487-498.
3. S. N. Baker, T. M. McCleskey, S. Pandey, G. A. Baker, *Chem. Commun*. 2004, 940-941.