

Supporting Information

Physicochemical Insights into Stabilisation of Stressed Lysozyme and Glycine Homopeptides by Sorbitol

Ritutama Ghosh, Nand Kishore*

*Corresponding author (Email: nandk@chem.iitb.ac.in)

Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai – 400 076, India.

Table S1

Values of density (ρ), apparent molar volume ($V_{2,\emptyset}$), speed of sound (u), adiabatic compressibility (k_s) and apparent molar compressibility ($K_{S,2,\emptyset}$) of glycine and its peptides in aqueous mixture of (0.100 mol kg⁻¹ DTAB + 0.100 mol kg⁻¹ sorbitol) at $T = 298.15$ K and $p = 1.0 \times 10^5$ Pa.

$m/(\text{mol kg}^{-1})$	$\rho/(\text{g cm}^{-3})$	$V_{2,\emptyset}/(\text{cm}^3 \text{mol}^{-1})$	$u/(\text{m s}^{-1})$	$10^{13} k_s/(\text{Pa}^{-1})$	$10^{12} K_{S,2,\emptyset}/(\text{cm}^3 \text{mol}^{-1} \text{Pa}^{-1})$
Glycine					
0	1.00491		1508.80	43.71	
0.1018	1.00810	43.50	1514.10	43.27	-24.47
0.2021	1.01131	43.69	1519.42	42.83	-24.14
0.2312	1.01216	43.63	1520.86	42.71	-24.07
0.3072	1.01450	43.79	1524.72	42.40	-23.58
0.4033	1.01736	43.74	1529.51	42.02	-23.32
0.4410	1.01849	43.78	1531.41	41.87	-23.20
0.4832	1.02015	43.09	1534.35	41.64	-24.55
0.5872	1.02283	43.93	1538.56	41.30	-22.51
Diglycine					
0	1.00491		1508.73	43.72	
0.0299	1.00656	76.60	1511.24	43.50	-39.04
0.0357	1.00688	76.58	1511.70	43.46	-38.35
0.0384	1.00705	76.65	1511.96	43.44	-38.05
0.0436	1.00739	76.60	1512.49	43.39	-40.24
0.0491	1.00767	76.09	1512.91	43.36	-39.22

0.0542	1.00795	76.19	1513.31	43.32	-38.82
0.0649	1.00852	76.59	1514.22	43.24	-38.51
Triglycine					
0	1.00490		1508.75	43.72	
0.0300	1.00721	111.59	1511.83	43.44	-43.79
0.0406	1.00802	111.84	1512.87	43.34	-42.78
0.0495	1.00869	111.93	1513.77	43.26	-42.61
0.0500	1.00875	111.49	1514.05	43.24	-45.65
0.0550	1.00911	111.97	1514.34	43.21	-42.64
0.0600	1.00950	111.74	1514.82	43.17	-42.55
0.0649	1.00986	111.99	1515.30	43.13	-42.20
Pentaglycine					
0	1.00496		1508.80	43.71	
0.0020	1.00519	186.91	1509.11	43.68	-57.88
0.0025	1.00525	186.42	1509.16	43.68	-51.75
0.0032	1.00533	186.35	1509.24	43.67	-48.86
0.0035	1.00536	186.68	1509.25	43.67	-42.75
0.0041	1.00544	186.36	1509.36	43.66	-46.75
0.0045	1.00548	186.30	1509.41	43.65	-47.53
0.0050	1.00555	186.51	1509.46	43.65	-44.68
Standard uncertainties (u): $u(T) = \pm 0.01$ K, $u(p) = \pm 1$ kPa, $u(\rho) = \pm 0.15 \times 10^{-3}$ g cm ⁻³ , $u(u) = 0.5$ m s ⁻¹ , $u_r(m) = 0.02$. Molalities of glycine and its peptides are given per kg of (DTAB + sorbitol).					

Table S2

Molar enthalpies of interaction ΔH_m of glycine and homopeptides are reported at different molalities (m) in (0.100 mol kg⁻¹ DTAB + 0.100 mol kg⁻¹ sorbitol) at $T = 298.15$ K and $p = 1.0 \times 10^5$ Pa. The molalities of glycine, diglycine, triglycine and pentaglycine in the syringe were 0.200, 0.025, 0.025, 0.003 mol kg⁻¹, respectively. The volume of the ITC cell is 0.94 cm³ and the injection volume is 9.96 μ l. All the values of ΔH_m in this table are per mol of the total amino acid added from the syringe upon corresponding injection step. The first two columns give final molalities of glycine and its peptides and (DTAB + sorbitol) in the cell of the ITC after each injection, respectively.

$10^3 \times m /$ (mol kg ⁻¹)		$\Delta H_m / (\text{J mol}^{-1})$	$10^3 \times m /$ (mol kg ⁻¹)		$\Delta H_m / (\text{J mol}^{-1})$
Glycine	(DTAB + sorbitol)		Diglycine	(DTAB + sorbitol)	
4.21	97.9	23.7	0.52	97.9	22.8
6.21	96.9	23.8	0.77	96.9	22.6
8.16	95.9	23.7	1.02	95.9	10.8
10.04	95.0	23.7	1.25	95.0	5.0
11.86	94.0	23.7	1.48	94.0	-3.1
13.63	93.1	21.3	1.70	93.1	3.1
15.35	92.2	21.1	1.91	92.2	3.1
17.01	91.3	21.3	2.12	91.3	27.0
18.62	90.4	21.5	2.32	90.4	27.9
20.18	89.6	21.0	2.52	89.6	46.4
21.70	88.7	22.2	2.70	88.7	-0.1
23.17	87.9	21.2	2.89	87.9	-2.1
24.59	87.1	20.5	3.06	87.1	70.6
25.97	86.3	15.9	3.24	86.3	44.0
27.31	85.5	16.9	3.40	85.5	-9.9
28.61	84.7	15.4	3.57	84.7	-9.9
29.87	84.0	12.5	3.72	84.0	34.6
31.09	83.2	14.5	3.87	83.2	64.1
32.27	82.5	16.6	4.02	82.5	11.5
33.42	81.8	15.8	4.17	81.8	-0.0

34.53	81.1	14.4	4.30	81.1	50.8
35.61	80.4	21.6	4.44	80.4	52.0
36.66	79.7	18.6	4.57	79.7	54.3
37.67	79.0	19.3	4.70	79.0	19.0
Triglycine			Pentaglycine		
0.54	97.9	121.7	0.06	97.9	43.6
0.79	96.9	129.8	0.09	96.9	-217.7
1.04	95.9	140.0	0.12	95.9	-217.7
1.28	95.0	181.0	0.15	95.0	-196.7
1.51	94.0	153.3	0.17	94.0	-25.2
1.74	93.1	104.8	0.20	93.1	124.5
1.96	92.2	135.5	0.23	92.2	-84.4
2.17	91.3	145.6	0.25	91.3	20.0
2.37	90.4	161.0	0.27	90.4	217.8
2.57	89.6	168.0	0.30	89.6	208.4
2.77	88.7	166.5	0.32	88.7	210.5
2.95	87.9	166.3	0.34	87.9	218.9
3.14	87.1	166.5	0.36	87.1	154.1
3.31	86.3	166.3	0.38	86.3	1.5
3.48	85.5	119.8	0.40	85.5	-136.9
3.65	84.7	108.9	0.42	84.7	-264.0
3.81	84.0	112.9	0.44	84.0	-264.3
3.97	83.2	127.3	0.46	83.2	-264.1
4.12	82.5	139.7	0.48	82.5	-163.2
4.26	81.8	132.3	0.49	81.8	-232.5
4.40	81.1	116.9	0.51	81.1	-232.4
4.54	80.4	160.3	0.53	80.4	71.6
4.68	79.7	150.6	0.54	79.7	-55.7
4.81	79.0	150.0	0.56	79.0	7.5

Table S3

Enthalpies of dilution $\Delta_{dil}H_m$ of pentaglycine and (0.100 mol kg⁻¹ DTAB + 0.100 mol kg⁻¹ sorbitol) at different molalities (m) in water at $T = 298.15$ K and $p = 1.0 \times 10^5$ Pa. The molality of pentaglycine in the syringe was 0.003 mol kg⁻¹. All the values of $\Delta_{dil}H_m$ in this table are per mol of the total pentaglycine added from the syringe upon corresponding injection step. The first column gives the final molalities of pentaglycine in the ITC cell subsequent to each injection. (0.100 mol kg⁻¹ DTAB + 0.100 mol kg⁻¹ sorbitol) taken in the cell of ITC with water. The final molalities of aqueous (DTAB + sorbitol) in the cell of ITC are given in the third column. The volume of the ITC cell is 0.94 cm³ and the injection volume of water is 9.96 μ l each.

$10^3 \times m /$ (mol kg ⁻¹)	$\Delta_{dil}H_m /$ (J mol ⁻¹)	$10^3 \times m /$ (mol kg ⁻¹)	$\Delta_{dil}H_m /$ (μ J mol ⁻¹)
Pentaglycine		(0.100 mol kg ⁻¹ DTAB + 0.100 mol kg ⁻¹ sorbitol)	
0.06	-660.3	97.9	779
0.09	-666.5	96.9	777
0.12	-606.7	95.9	772
0.15	-553.7	95.0	774
0.17	-505.5	94.0	766
0.20	-515.9	93.1	766
0.23	-480.5	92.2	772
0.25	-487.1	91.3	771
0.27	-485.3	90.4	757
0.30	-481.0	89.6	751
0.32	-476.8	88.7	754
0.34	-450.3	87.9	752
0.36	-469.7	87.1	739
0.38	-458.2	86.3	734
0.40	-437.7	85.5	739
0.42	-459.5	84.7	737
0.44	-445.6	84.0	727
0.46	-415.8	83.2	716
0.48	-434.8	82.5	714

0.49	-449.1	81.8	711
0.51	-400.2	81.1	701
0.53	-369.5	80.4	681
0.54	-394.0	79.7	686
0.56	-404.5	79.0	680

Standard uncertainties (u): $u(T) = \pm 0.01$ K, $u(P) = \pm 1$ kPa, $u_r(m) = 0.02$, $u_r(\Delta_{dil}H_m) = 0.02$. Molality of amino acids is given per kg of water.

Table S4

Enthalpy of dilution $\Delta_{dil}H^o$ of 0.3×10^{-3} mol dm⁻³ lysozyme (pH = 2.1) in 0.010, 0.025 and 0.100 mol kg⁻¹ aqueous DTAB taken in the cell of the ITC at $T = 298.15$ K and $p = 1.0 \times 10^5$ Pa. The volume of the ITC cell is 0.94 cm³ and the injection volume is 9.96×10^{-6} dm³ each. All the values of $\Delta_{dil}H^o$ in this table are per mol of the total protein added from the syringe upon corresponding injection step. The first and the second column gives final concentration of the protein and molality of DTAB in the cell of the ITC after each injection, respectively.

$10^6 \times c /$	$10^3 \times m /$	$\Delta_{dil}H^o /$	$10^6 \times c /$	$10^3 \times m /$	$\Delta_{dil}H^o /$
(mol dm ⁻³)	(mol kg ⁻¹)	(J mol ⁻¹)	(mol dm ⁻³)	(mol kg ⁻¹)	(J mol ⁻¹)
Lysozyme	DTAB		Lysozyme	DTAB	
7.2	9.79	2.44	7.2	24.5	13.19
10.7	9.69	1.63	10.7	24.2	11.19
14.1	9.59	1.12	14.1	24.0	8.53
17.3	9.50	0.80	17.3	23.7	5.51
20.4	9.40	0.35	20.4	23.5	-0.50
23.5	9.31	0.60	23.5	23.3	-9.47
26.5	9.22	0.46	26.5	23.0	-16.48
29.3	9.13	-0.08	29.3	22.8	-19.17
32.1	9.04	-0.23	32.1	22.6	-17.69
34.8	8.96	0.82	34.8	22.4	-14.39
37.4	8.87	1.64	37.4	22.2	-12.13
39.9	8.79	2.06	39.9	22.0	-9.69
42.4	8.71	2.58	42.4	21.8	-7.46
44.8	8.63	3.27	44.8	21.6	-6.33
47.1	8.55	2.19	47.1	21.4	-4.96
49.3	8.47	0.77	49.3	21.2	-3.95
51.5	8.40	0.28	51.5	21.0	-2.93

53.6	8.32	0.42	53.6	20.8	-2.45
55.6	8.25	0.57	55.6	20.6	-2.15
57.6	8.18	0.05	57.6	20.4	-1.54
59.5	8.11	-0.02	59.5	20.3	-0.90
61.4	8.04	-0.43	61.4	20.1	-0.55
63.2	7.97	0.12	63.2	19.9	-0.13
64.9	7.90	-0.17	64.9	19.8	0.13

$10^6 \times c /$	$10^3 \times m /$	$\Delta_{dil} H^\circ /$
(mol dm ⁻³)	(mol kg ⁻¹)	(J mol ⁻¹)
Lysozyme	DTAB	
7.2	97.9	-73.27
10.7	96.9	-72.31
14.1	95.9	-81.27
17.3	95.0	-87.18
20.4	94.0	-79.22
23.5	93.1	-82.75
26.5	92.2	-93.18
29.3	91.3	-108.38
32.1	90.4	-113.30
34.8	89.6	-118.08
37.4	88.7	-140.70
39.9	87.9	-149.94
42.4	87.1	-139.71
44.8	86.3	-140.79

47.1	85.5	-149.03
49.3	84.7	-168.32
51.5	84.0	-182.75
53.6	83.2	-189.65
55.6	82.5	-210.33
57.6	81.8	-215.15
59.5	81.1	-217.55
61.4	80.4	-225.44
63.2	79.7	-232.60
64.9	79.0	-232.21
