SUPPLEMENTARY MATERIALS

Tables

Table S1: Thermodynamic parameters associated with the thermal unfolding of α-LA in the absence and presence of varying concentrations of ficoll 70 at different pH values[a,b].

[Ficoll 70] (mg ml^{-1})	T_m (°C)	ΔH_m (kcal mol^{-1})	ΔG_D^o (kcal mol^{-1})
		pH 6.5	
0	50.8 ± 0.3	65 ± 3	3.52 ± 0.26
50	51.7 ± 0.2	65 ± 3	3.59 ± 0.28
100	52.3 ± 0.2	67 ± 2	3.76 ± 0.15
150	52.9 ± 0.1	68 ± 3	3.89 ± 0.29
200	53.6 ± 0.3	69 ± 3	4.04 ± 0.23
250	54.0 ± 0.3	70 ± 2	4.14 ± 0.16
300	54.3 ± 0.2	71 ± 3	4.23 ± 0.25
350	54.5 ± 0.3	72 ± 4	4.33 ± 0.31
		pH 6.0	
0	55.3 ± 0.2	72 ± 3	4.39 ± 0.31
50	55.5 ± 0.3	73 ± 3	4.51 ± 0.32
100	55.9 ± 0.3	74 ± 3	4.58 ± 0.30
150	56.5 ± 0.3	74 ± 4	4.63 ± 0.36
200	57.0 ± 0.2	75 ± 2	4.79 ± 0.17
250	57.4 ± 0.2	76 ± 3	4.89 ± 0.33
300	58.0 ± 0.3	77 ± 4	5.00 ± 0.37
350	58.2 ± 0.3	78 ± 3	5.11 ± 0.33
		pH 5.5	
0	59.7 ± 0.3 (59.6 ± 0.2)	76 ± 3 (76 ± 2)	4.99 ± 0.36 (4.99 ± 0.34)
50	59.8 ± 0.2	77 ± 2	5.13 ± 0.35
100	60.2 ± 0.2	78 ± 3	5.19 ± 0.46
150	60.6 ± 0.2	78 ± 2	5.23 ± 0.39
200	61.0 ± 0.3	79 ± 3	5.39 ± 0.36
250	61.4 ± 0.3	80 ± 3	5.50 ± 0.28
300	61.7 ± 0.3	81 ± 3	5.60 ± 0.38
350	61.9 ± 0.3 (61.8 ± 0.2)	82 ± 4 (82 ± 3)	5.72 ± 0.45 (5.71 ± 0.43)

[Ficoll 70], mg ml^{-1}	**ΔC_p, kcal mol^{-1} K^{-1}**
0	1.56 ± 0.09
50	1.55 ± 0.09
100	1.58 ± 0.06
150	1.57 ± 0.08
200	1.55 ± 0.07
250	1.56 ± 0.07
300	1.57 ± 0.06
350	1.57 ± 0.09

[a,b]Have the same meaning as in Table 1.

Table S2: Thermodynamic parameters associated with the thermal unfolding of α-LA in the absence and presence of varying concentrations of dextran 70 at different pH values[a,b].

[Dextran 70] (mg ml^{-1})	**T_m (°C)**	**ΔH_m (kcal mol^{-1})**	**ΔG_D^o (kcal mol^{-1})**
		pH 6.5	
0	50.8 ± 0.3	65 ± 3	3.52 ± 0.26
50	53.1 ± 0.3	66 ± 4	3.71 ± 0.30
100	54.4 ± 0.3	67 ± 3	3.87 ± 0.29
150	55.9 ± 0.1	68 ± 3	4.01 ± 0.31
200	56.9 ± 0.2	70 ± 2	4.27 ± 0.16
250	57.6 ± 0.2	71 ± 2	4.42 ± 0.16
300	57.8 ± 0.3	73 ± 3	4.62 ± 0.33
		pH 6.0	
0	55.3 ± 0.2	72 ± 3	4.39 ± 0.31
50	55.9 ± 0.2	73 ± 3	4.48 ± 0.32
100	57.4 ± 0.3	73 ± 2	4.57 ± 0.17
150	58.3 ± 0.3	74 ± 4	4.69 ± 0.37
200	59.4 ± 0.1	75 ± 2	4.88 ± 0.17
250	60.6 ± 0.3	76 ± 3	5.05 ± 0.35
300	60.8 ± 0.2	77 ± 3	5.16 ± 0.36
		pH 5.5	
0	59.7 ± 0.3 (59.6 ± 0.2)	76 ± 3 (76 ± 2)	4.99 ± 0.36 (4.99 ± 0.34)
50	59.9 ± 0.1	77 ± 3	5.07 ± 0.35
100	60.8 ± 0.2	78 ± 2	5.23 ± 0.40
150	61.6 ± 0.2	79 ± 2	5.35 ± 0.39
200	62.9 ± 0.3	79 ± 3	5.44 ± 0.37
250	63.6 ± 0.3	80 ± 3	5.60 ± 0.37
300	63.8 ± 0.3 (63.9 ± 0.2)	81 ± 2 (81 ± 3)	5.72 ± 0.41 (5.72 ± 0.41)

[Dextran 70], mg ml^{-1}	**ΔC_p, kcal mol^{-1} K^{-1}**
0	1.56 ± 0.09
50	1.58 ± 0.09
100	1.57 ± 0.07
150	1.58 ± 0.07
200	1.56 ± 0.08
250	1.55 ± 0.06
300	1.55 ± 0.09

[a,b]Have the same meaning as in Table 1.

Table S3: Thermodynamic parameters associated with the thermal unfolding of α-LA in the absence and presence of varying concentrations of dextran 40 at different pH values[a,b].

[Dextran 40] ($mg\ ml^{-1}$)	**T_m (°C)**	**ΔH_m ($kcal\ mol^{-1}$)**	**ΔG_D^o ($kcal\ mol^{-1}$)**
	pH 6.5		
0	50.8 ± 0.3	65 ± 3	3.52 ± 0.26
50	53.6 ± 0.2	66 ± 2	3.75 ± 0.14
100	55.1 ± 0.3	68 ± 3	3.97 ± 0.24
150	56.6 ± 0.3	69 ± 3	4.18 ± 0.32
200	58.1 ± 0.2	71 ± 2	4.39 ± 0.16
250	59.5 ± 0.1	72 ± 2	4.57 ± 0.24
300	60.0 ± 0.2	74 ± 3	4.81 ± 0.35
	pH 6.0		
0	55.3 ± 0.2	72 ± 3	4.39 ± 0.31
50	56.1 ± 0.2	72 ± 4	4.41 ± 0.35
100	57.8 ± 0.3	73 ± 3	4.56 ± 0.32
150	58.9 ± 0.3	74 ± 2	4.77 ± 0.20
200	60.7 ± 0.1	75 ± 3	4.89 ± 0.36
250	61.9 ± 0.2	76 ± 3	5.07 ± 0.37
300	62.1 ± 0.2	77 ± 3	5.21 ± 0.37
	pH 5.5		
0	59.7 ± 0.3 (59.6 ± 0.2)	76 ± 3 (76 ± 2)	4.99 ± 0.36 (4.99 ± 0.34)
50	60.1 ± 0.1	76 ± 3	5.00 ± 0.35
100	61.0 ± 0.2	77 ± 3	5.10 ± 0.36
150	61.9 ± 0.3	78 ± 4	5.31 ± 0.42
200	63.2 ± 0.1	79 ± 2	5.40 ± 0.19
250	64.3 ± 0.3	80 ± 3	5.60 ± 0.39
300	64.6 ± 0.3 (64.5 ± 0.2)	81 ± 3 (81 ± 2)	5.75 ± 0.38 (5.74 ± 0.37)

[Dextran 40], $mg\ ml^{-1}$	**ΔC_p, $kcal\ mol^{-1}\ K^{-1}$**
0	1.56 ± 0.09
50	1.57 ± 0.09
100	1.59 ± 0.07
150	1.55 ± 0.08
200	1.58 ± 0.08
250	1.56 ± 0.09
300	1.55 ± 0.09

[a,b]Have the same meaning as in Table 1.

Table S4: Thermodynamic parameters associated with the thermal unfolding of lysozyme in the absence and presence of varying concentrations of dextran 40 at different pH values[a,b].

[Dextran 40] (mg ml^{-1})	$T_{m\ (obs.)}$[c] (ºC)	$T_{m\ (corr.)}$[d] (ºC)	$\Delta H_{m\ (obs.)}$[c] (kcal mol^{-1})	$\Delta H_{m\ (corr.)}$[d] (kcal mol^{-1})	ΔG_D^o (kcal mol^{-1})
			pH 6.0		
0	57.9 ± 0.3	83.3 ± 0.3	86 ± 3	125 ± 3	12.41 ± 0.30
100	58.5 ± 0.2	83.9 ± 0.2	87 ± 3	126 ± 3	12.59 ± 0.44
150	59.2 ± 0.3	84.6 ± 0.3	88 ± 2	127 ± 2	12.83 ± 0.21
200	59.5 ± 0.3	84.9 ± 0.3	91 ± 4	130 ± 4	13.34 ± 0.59
250	60.3 ± 0.2	85.7 ± 0.2	93 ± 3	132 ± 3	13.66 ± 0.47
300	60.5 ± 0.3	85.9 ± 0.3	94 ± 2	133 ± 2	13.84 ± 0.34
			pH 5.0		
0	55.8 ± 0.3	80.2 ± 0.3	83 ± 3	121 ± 3	11.66 ± 0.24
100	57.6 ± 0.2	82.0 ± 0.2	84 ± 3	122 ± 3	11.88 ± 0.43
150	57.7 ± 0.3	82.1 ± 0.3	86 ± 2	124 ± 2	12.25 ± 0.20
200	58.1 ± 0.2	82.5 ± 0.2	88 ± 3	126 ± 3	12.59 ± 0.37
250	58.9 ± 0.3	83.3 ± 0.3	90 ± 3	128 ± 3	12.90 ± 0.44
300	59.1 ± 0.3	83.5 ± 0.3	92 ± 2	130 ± 2	13.23 ± 0.34
			pH 4.0		
0	52.2 ± 0.3	76.8 ± 0.3	79 ± 3	115 ± 3	10.56 ± 0.30
100	53.2 ± 0.3	77.8 ± 0.3	80 ± 3	116 ± 3	10.79 ± 0.40
150	53.5 ± 0.3	78.1 ± 0.3	81 ± 3	117 ± 3	10.99 ± 0.39
200	55.8 ± 0.2	80.4 ± 0.2	84 ± 2	120 ± 2	11.55 ± 0.27
250	56.9 ± 0.2	81.5 ± 0.2	86 ± 3	122 ± 3	11.86 ± 0.42
300	58.0 ± 0.1	82.6 ± 0.1	89 ± 3	125 ± 3	12.38 ± 0.43
			pH 3.0		
0	69.7 ± 0.2	-	103 ± 2	-	8.50 ± 0.23
100	70.8 ± 0.3	-	104 ± 2	-	8.77 ± 0.28
150	71.4 ± 0.3	-	106 ± 2	-	9.10 ± 0.26
200	73.2 ± 0.2	-	108 ± 3	-	9.46 ± 0.38
250	74.6 ± 0.2	-	111 ± 2	-	9.91 ± 0.28
300	75.8 ± 0.3	-	113 ± 2	-	10.26 ± 0.30

[Dextran 40], mg ml^{-1}	ΔC_p, kcal mol^{-1} K^{-1}
0	1.60 ± 0.09
100	1.59 ± 0.05
150	1.58 ± 0.07
200	1.58 ± 0.06
250	1.59 ± 0.08
300	1.59 ± 0.07

[a,b]Have the same meaning as in Table 1.
[c,d]Have the same meaning as in Table 2.

Table S5: Kinetic parameters of lysozyme in the absence and presence of different concentrations of dextran 40 at pH 7.0 and 25 °C[a].

[Dextran 40] (mg ml^{-1})	K_m (mg l^{-1})	k_{cat} x 10^{-5} (mg s^{-1} M^{-1})
0	83.75 ± 5.17	9.64 ± 0.09
100	77.45 ± 6.52	8.27 ± 0.09
150	71.97 ± 3.44	8.09 ± 0.06
200	67.13 ± 3.81	7.53 ± 0.09
250	63.24 ± 5.22	7.35 ± 0.06
300	56.05 ± 4.07	7.05 ± 0.05

[a]Have the same meaning as in Table 1.

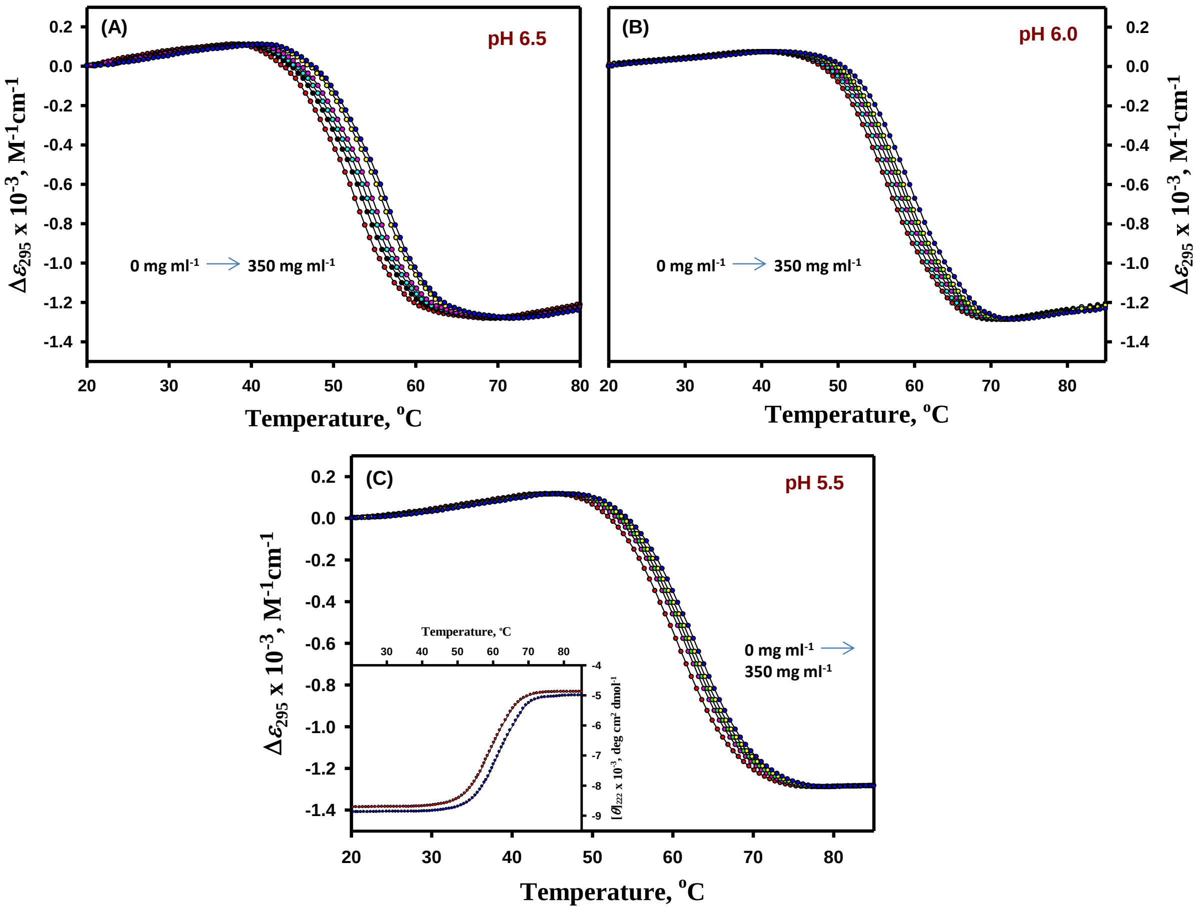

Figure S1. Thermal denaturation profiles of α-LA in the absence and presence of different concentrations of ficoll 70 at different pH values (panels A-C). Different concentrations of the crowding agent are shown by different colours: 0 mg ml^{-1} (red), 50 mg ml^{-1} (black), 100 mg ml^{-1} (cyan), 150 mg ml^{-1} (pink), 200 mg ml^{-1} (green), 250 mg ml^{-1} (yellow) and 350 mg ml^{-1} (blue). For the sake of clarity curves at all concentrations are not shown. Inset in panel C represents thermal denaturation profiles of α-LA measured by $[\theta]_{222}$ in the absence (red circle) and presence of the highest concentration of ficoll 70 (blue circle).

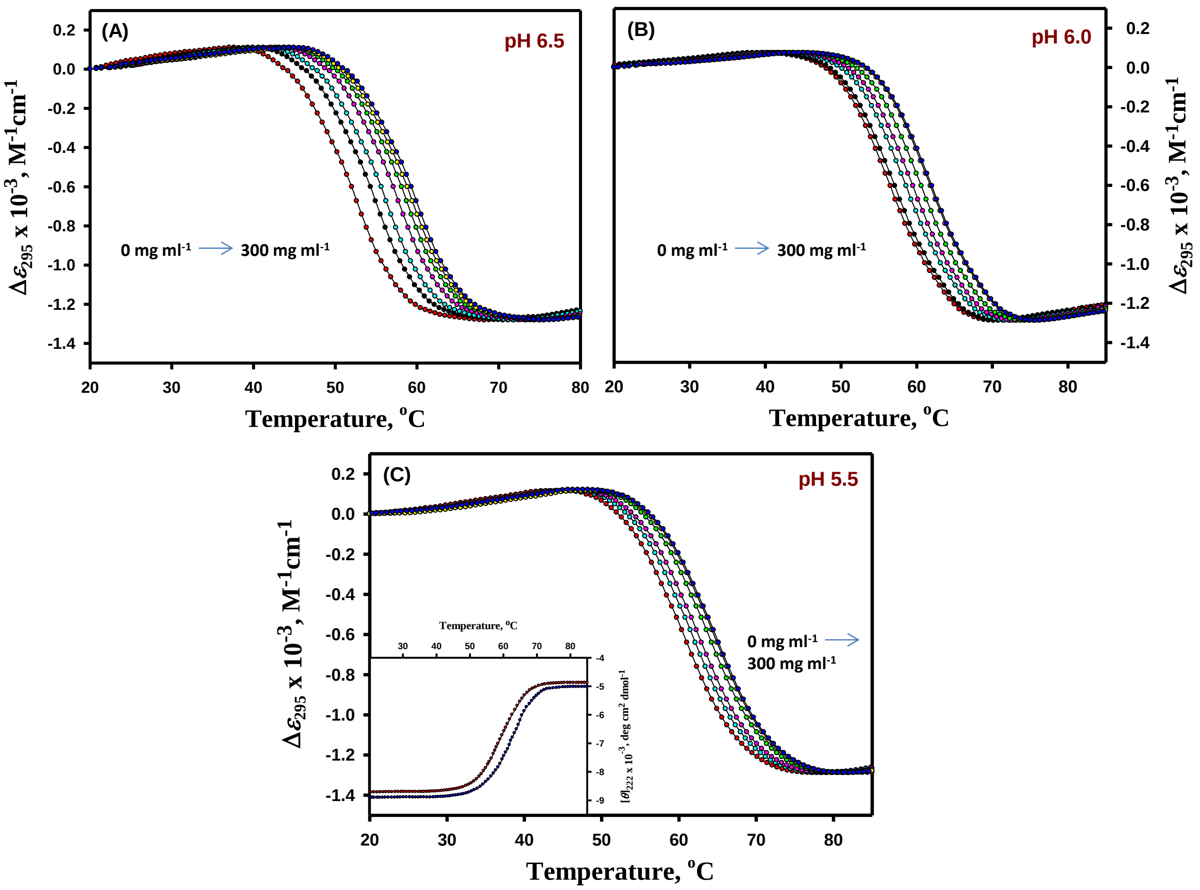

Figure S2. Thermal denaturation profiles of α-LA in the absence and presence of different concentrations of dextran 70 at different pH values (panels A-C). Different concentrations of the crowding agent are shown by different colours: 0 mg ml^{-1} (red), 50 mg ml^{-1} (black), 100 mg ml^{-1} (cyan), 150 mg ml^{-1} (pink), 200 mg ml^{-1} (green), 250 mg ml^{-1} (yellow) and 300 mg ml^{-1} (blue). For the sake of clarity curves at all concentrations are not shown. Inset in panel C represents thermal denaturation profiles of α-LA measured by $[\theta]_{222}$ in the absence (red circle) and presence of highest concentration of dextran 70 (blue circle).

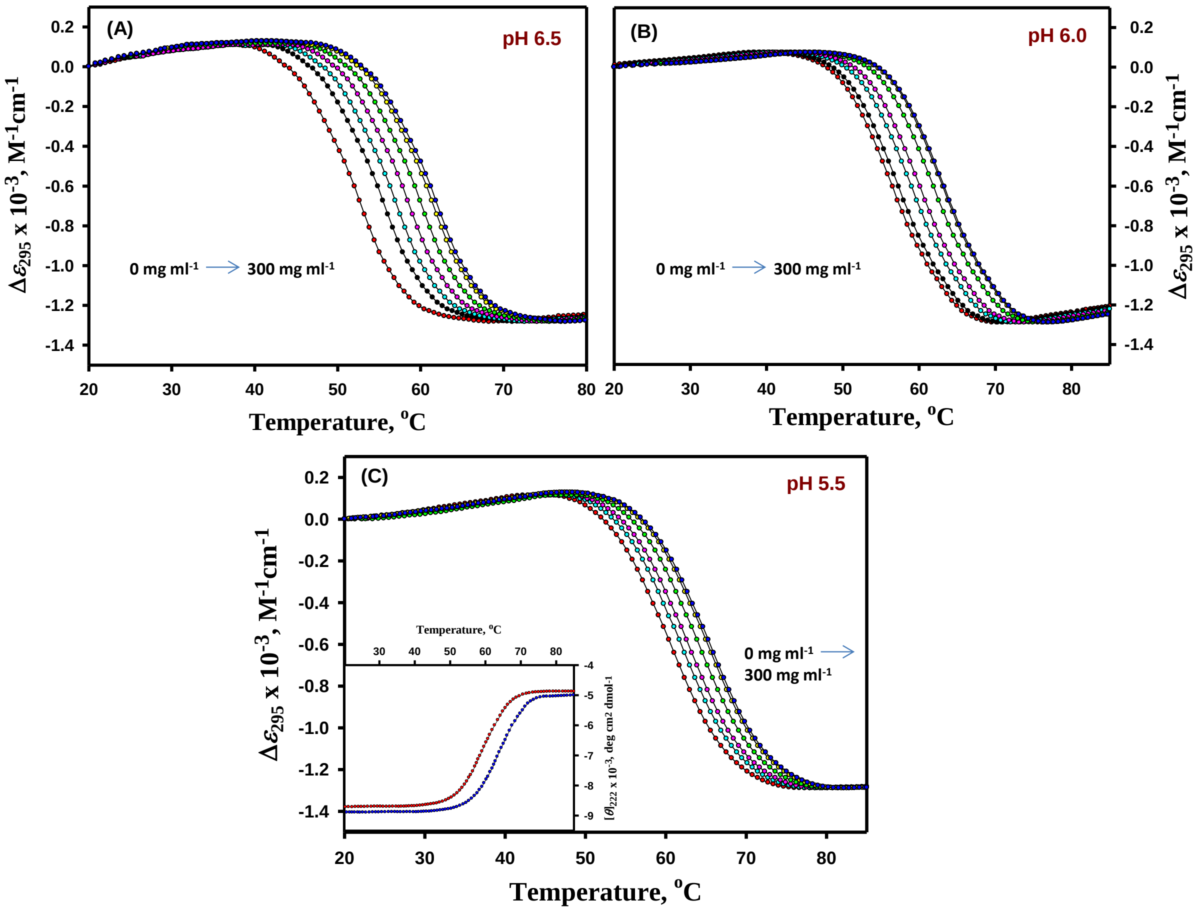

Figure S3. Thermal denaturation profiles of α-LA in the absence and presence of different concentrations of dextran 40 at different pH values (panels A-C). Different concentrations of the crowding agents are shown by different colours: 0 mg ml^{-1} (red), 50 mg ml^{-1} (black), 100 mg ml^{-1} (cyan), 150 mg ml^{-1} (pink), 200 mg ml^{-1} (green), 250 mg ml^{-1} (yellow) and 300 mg ml^{-1} (blue). For the sake of clarity curves at all concentrations are not shown. Inset in panel C represents thermal denaturation profiles of α-LA measured by $[\theta]_{222}$ in the absence (red circle) and presence of highest concentration of dextran 40 (blue circle).

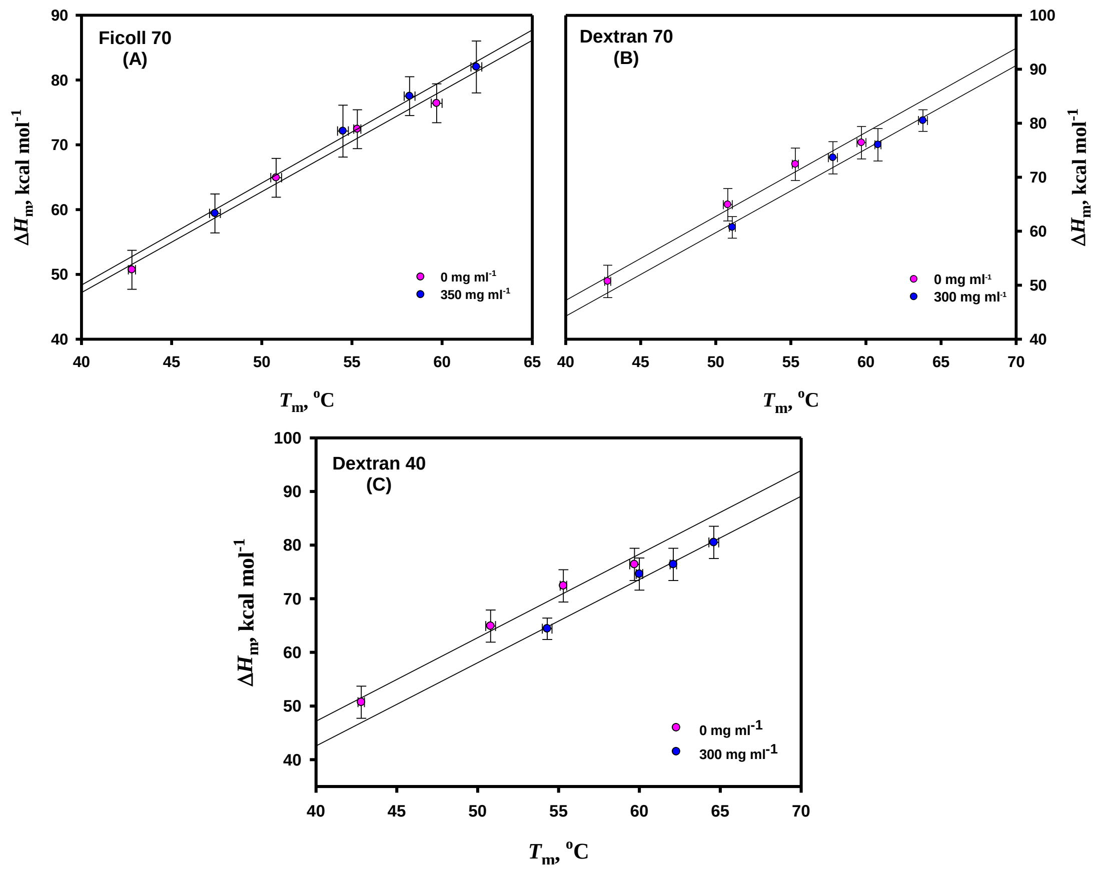

Figure S4. Plots of ΔH_m versus T_m of α-LA in the absence (pink) and presence of highest concentration (blue) of all the crowders at different pH values. ΔC_p is estimated from the slope of each plot.

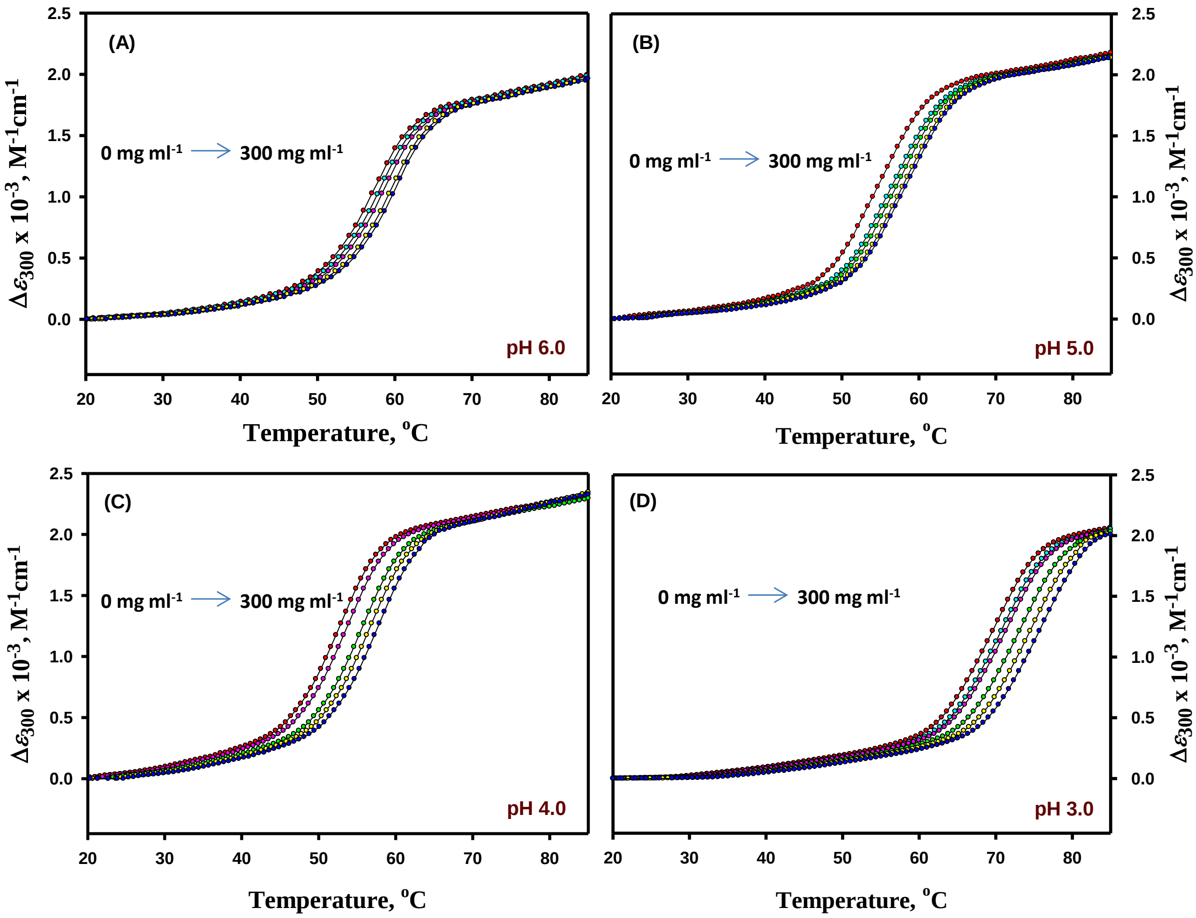

Figure S5. Thermal denaturation profiles of lysozyme in the absence and presence of different concentrations of dextran 40 at different pH values (panels A-D). Different concentrations of dextran 40 are shown by different colours: 0 mg ml^{-1} (red), 100 mg ml^{-1} (cyan), 150 mg ml^{-1} (pink), 200 mg ml^{-1} (green), 250 mg ml^{-1} (yellow) and 300 mg ml^{-1} (blue). For the sake of clarity curves at all concentrations are not shown.

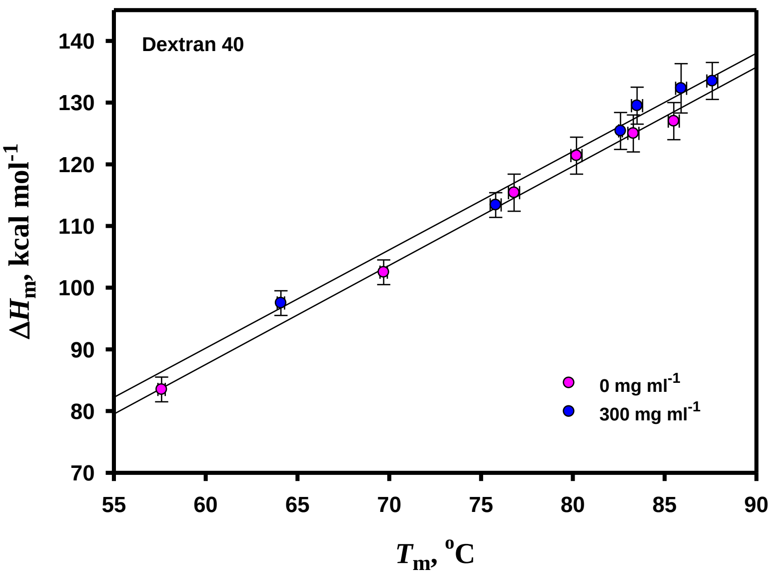

Figure S6. Plots of ΔH_{m} versus T_{m} of lysozyme in the absence (pink) and presence of the highest concentration (blue) of dextran 40 at different pH values. ΔC_{p} is estimated from the slope of each plot.

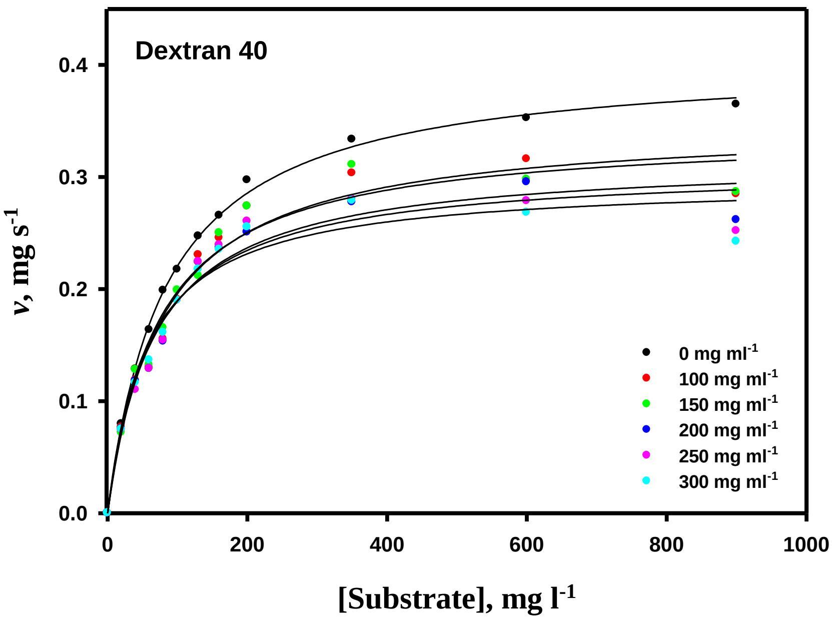

Figure S7. Plots of Initial velocity (v) versus [substrate], the substrate concentration for lysozyme in the absence and presence of different concentrations of dextran 40 at pH 7.0 and 25 °C.