

The Effects of Glycine, TMAO and Osmolyte Mixtures on the Pressure Dependent Enzymatic Activity of α -Chymotrypsin

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Supplementary Information

Table SI1: Calculated kinetic parameters (MICHAELIS constant (K_M), maximal velocity (v_{max}), turnover number (k_{cat}) and catalytic efficiency (k_{cat}/K_M)) of the α -CT reaction for all pressures and solution conditions ($T = 20^\circ\text{C}$).

	$p /$ bar	$K_M /$ 10^{-3} M	$v_{max} /$ $10^{-8} \text{ s}^{-1} \text{ M}^{-1}$	$k_{cat} /$ 10^{-3} s^{-1}	$k_{cat} K_M^{-1} /$ $\text{s}^{-1} \text{ M}^{-1}$
TRIS buffer	1	0.287 ± 0.055	1.82 ± 0.14	2.28 ± 0.18	7.94 ± 1.66
	500	0.223 ± 0.075	2.38 ± 0.22	2.98 ± 0.28	13.39 ± 4.68
	1000	0.244 ± 0.061	3.22 ± 0.26	4.02 ± 0.32	16.47 ± 4.33
	1500	0.212 ± 0.064	4.53 ± 0.41	5.66 ± 0.51	26.69 ± 8.41
	2000	0.135 ± 0.033	5.75 ± 0.39	7.19 ± 0.49	53.15 ± 13.48
Shrimp shallow buffer	1	0.389 ± 0.075	3.43 ± 0.20	4.29 ± 0.25	11.04 ± 2.23
	500	0.353 ± 0.112	4.06 ± 0.34	5.08 ± 0.43	14.40 ± 4.75
	1000	0.250 ± 0.068	4.67 ± 0.31	5.84 ± 0.39	23.36 ± 6.52
	1500	0.258 ± 0.058	6.09 ± 0.33	7.61 ± 0.42	29.51 ± 6.77
	2000	0.229 ± 0.056	7.78 ± 0.46	9.73 ± 0.57	42.53 ± 10.75
Deep sea buffer	1	0.387 ± 0.040	2.26 ± 0.06	2.82 ± 0.11	7.28 ± 0.78
	500	0.248 ± 0.029	2.70 ± 0.08	3.38 ± 0.11	13.62 ± 1.69
	1000	0.237 ± 0.044	3.55 ± 0.16	4.44 ± 0.21	18.72 ± 3.60
	1500	0.271 ± 0.085	5.09 ± 0.40	6.36 ± 0.40	23.49 ± 7.60
	2000	0.266 ± 0.077	7.04 ± 0.50	8.8 ± 0.64	33.10 ± 9.82
TRIS buffer + 1 M glycine	1	0.258 ± 0.079	3.27 ± 0.26	4.09 ± 0.32	15.85 ± 2.51
	500	0.239 ± 0.084	3.88 ± 0.34	4.85 ± 0.42	20.29 ± 3.66
	1000	0.181 ± 0.074	5.18 ± 0.49	6.48 ± 0.61	35.79 ± 7.49
	1500	0.205 ± 0.048	7.17 ± 0.40	8.96 ± 0.50	43.75 ± 10.47
	2000	0.199 ± 0.050	9.30 ± 0.56	11.62 ± 0.70	58.46 ± 16.17
TRIS buffer + 1 M TMAO	1	0.392 ± 0.105	2.28 ± 0.33	2.85 ± 0.42	7.26 ± 2.77
	500	0.287 ± 0.071	2.78 ± 0.35	3.48 ± 0.44	12.12 ± 6.33
	1000	0.245 ± 0.048	3.71 ± 0.35	4.64 ± 0.44	18.91 ± 4.16
	1500	0.274 ± 0.066	5.05 ± 0.60	6.31 ± 0.76	23.02 ± 6.62
	2000	0.173 ± 0.041	5.94 ± 0.63	7.43 ± 0.79	42.97 ± 10.56

Table SI2: Calculated activation volumes, $\Delta V^\ddagger$, of the α -CT reaction for each substrate concentration at different solution conditions.

	$c_{\text{Substrate}} /$ mM	$\Delta V^\ddagger /$ cm³ mol⁻¹
TRIS buffer	0.1	-17.76 $\pm$ 1.19
	0.25	-19.12 $\pm$ 1.69
	0.75	-16.10 $\pm$ 1.28
	2	-15.50 $\pm$ 0.39
	4	-15.31 $\pm$ 0.48
Shrimp shallow buffer	0.1	-15.53 $\pm$ 0.65
	0.25	-13.63 $\pm$ 1.04
	0.75	-11.80 $\pm$ 0.48
	2	-11.94 $\pm$ 0.94
	4	-10.11 $\pm$ 0.71
Deep sea buffer	0.1	-17.35 $\pm$ 0.61
	0.25	-17.43 $\pm$ 0.43
	0.75	-14.28 $\pm$ 0.36
	2	-14.15 $\pm$ 0.88
	4	-15.70 $\pm$ 1.00
TRIS buffer + 1 M glycine	0.1	-15.62 $\pm$ 0.46
	0.25	-15.51 $\pm$ 1.47
	0.75	-14.60 $\pm$ 1.12
	1.5	-13.31 $\pm$ 0.63
	3	-14.16 $\pm$ 0.87
TRIS buffer + 1 M TMAO	0.1	-16.01 $\pm$ 0.71
	0.25	-15.40 $\pm$ 0.86
	0.75	-15.68 $\pm$ 0.71
	2	-13.41 $\pm$ 0.86
	4	-13.22 $\pm$ 0.42

Table SI3: *p*-Values calculated using the hypothesis testing: two-sample t-test, with significance level fixed at 0.05 for the comparison of selected kinetic data.

MICHAELIS constant, K_M		Catalytic efficiency, $k_{cat} K_M^{-1}$	
compared data sets	<i>p</i> -value	compared data sets	<i>p</i> -value
1 bar, TRIS buffer x 1 bar, Shrimp shallow buffer	0.13290	1 bar, TRIS buffer x 1 bar, Shrimp shallow buffer	0.04981
1 bar, TRIS buffer x 1 bar, Deep sea buffer	0.06396	1 bar, TRIS buffer x 1 bar, Deep sea buffer	0.28977
1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M Glycine	0.63195	1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M Glycine	0.02342
1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M TMAO	0.20081	1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M TMAO	0.46893
2000 bar, TRIS buffer x 2000 bar, Shrimp shallow buffer	0.06812	2000 bar, TRIS buffer x 2000 bar, Shrimp shallow buffer	0.23731
2000 bar, TRIS buffer x 2000 bar, Deep sea buffer	0.05348	2000 bar, TRIS buffer x 2000 bar, Deep sea buffer	0.05231
2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M Glycine	0.14120	2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M Glycine	0.56563
2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M TMAO	0.28967	2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M TMAO	0.18713
1 bar, TRIS buffer x 2000 bar, TRIS buffer	0.01534	1 bar, TRIS buffer x 2000 bar, TRIS buffer	0.00132
1 bar, Shrimp shallow buffer x 2000 bar, Shrimp shallow buffer	0.04205	1 bar, Shrimp shallow buffer x 2000 bar, Shrimp shallow buffer	0.00341
1 bar, Deep sea buffer x 2000 bar, Deep sea buffer	0.07142	1 bar, Deep sea buffer x 2000 bar, Deep sea buffer	0.00395
1 bar, TRIS buffer + 1 M Glycine x 2000 bar, TRIS buffer + 1 M Glycine	0.33503	1 bar, TRIS buffer + 1 M Glycine x 2000 bar, TRIS buffer + 1 M Glycine	0.00407
1 bar, TRIS buffer + 1 M TMAO x 2000 bar, TRIS buffer + 1 M TMAO	0.02848	1 bar, TRIS buffer + 1 M TMAO x 2000 bar, TRIS buffer + 1 M TMAO	0.000532
Turnover number, k_{cat}		Maximum velocity, v_{max}	
compared data sets	<i>p</i> -value	compared data sets	<i>p</i> -value
1 bar, TRIS buffer x 1 bar, Shrimp shallow buffer	0.00015	1 bar, TRIS buffer x 1 bar, Shrimp shallow buffer	0.00015
1 bar, TRIS buffer x 1 bar, Deep sea buffer	0.01095	1 bar, TRIS buffer x 1 bar, Deep sea buffer	0.01095
1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M Glycine	0.00103	1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M Glycine	0.00103
1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M TMAO	0.09477	1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M TMAO	0.09477
2000 bar, TRIS buffer x 2000 bar, Shrimp shallow buffer	0.00315	2000 bar, TRIS buffer x 2000 bar, Shrimp shallow buffer	0.00315
2000 bar, TRIS buffer x 2000 bar, Deep sea buffer	0.02548	2000 bar, TRIS buffer x 2000 bar, Deep sea buffer	0.02548
2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M Glycine	0.00084	2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M Glycine	0.00084
2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M TMAO	0.67744	2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M TMAO	0.67744
1 bar, TRIS buffer x 2000 bar, TRIS buffer	0.000082	1 bar, TRIS buffer x 2000 bar, TRIS buffer	0.000082
1 bar, Shrimp shallow buffer x 2000 bar, Shrimp shallow buffer	0.000055	1 bar, Shrimp shallow buffer x 2000 bar, Shrimp shallow buffer	0.000055
1 bar, Deep sea buffer x 2000 bar, Deep sea buffer	0.000088	1 bar, Deep sea buffer x 2000 bar, Deep sea buffer	0.000088
1 bar, TRIS buffer + 1 M Glycine x 2000 bar, TRIS buffer + 1 M Glycine	0.000069	1 bar, TRIS buffer + 1 M Glycine x 2000 bar, TRIS buffer + 1 M Glycine	0.000069
1 bar, TRIS buffer + 1 M TMAO x 2000 bar, TRIS buffer + 1 M TMAO	0.000882	1 bar, TRIS buffer + 1 M TMAO x 2000 bar, TRIS buffer + 1 M TMAO	0.000882
1 bar, Shrimp shallow buffer x 1 bar, Deep sea buffer	0.000258	1 bar, Shrimp shallow buffer x 1 bar, Deep sea buffer	0.000258

Table SI4: *p*-Values calculated using the hypothesis testing: two-sample t-test, with significance level fixed at 0.05 for selected volumetric data.

Activation volume, $\Delta V^\ddagger$		Activation volume, $\Delta V^\ddagger$	
compared data sets	<i>p</i> -value	compared data sets	<i>p</i> -value
0.1 mM SPpNA, TRIS buffer x 0.1 mM SPpNA, Shrimp shallow buffer	0.04774	0.1 mM SPpNA, Shrimp shallow buffer x 0.1 mM TRIS buffer SPpNA,	0.04774
0.1 mM SPpNA, TRIS buffer x 0.1 mM SPpNA, Deep sea buffer	0.62505	0.1 mM SPpNA, Shrimp shallow buffer x 0.1 mM SPpNA, Deep sea buffer	0.01885
0.1 mM SPpNA, TRIS buffer x 0.1 mM SPpNA, TRIS buffer + 1 M Glycine	0.04523	0.1 mM SPpNA, Shrimp shallow buffer x 0.1 mM SPpNA, TRIS buffer + 1M Glycine	0.85434
0.1 mM SPpNA, TRIS buffer x 0.1 mM SPpNA, TRIS buffer + 1 M TMAO	0.09628	0.1 mM SPpNA, Shrimp shallow buffer x 0.1 mM SPpNA, TRIS buffer +1 M TMAO	0.00026
4 mM SPpNA, TRIS buffer x 4 mM SPpNA, Shrimp shallow buffer	0.00046	4 mM SPpNA, Shrimp shallow buffer x 4 mM SPpNA, TRIS buffer	0.00046
4 mM SPpNA, TRIS buffer x 4 mM SPpNA, Deep sea buffer	0.57543	4 mM SPpNA, Shrimp shallow buffer x 4 mM SPpNA, Deep sea buffer	0.00139
4 mM SPpNA, TRIS buffer x 3 mM SPpNA, TRIS buffer + 1 M Glycine	0.1155	4 mM SPpNA, Shrimp shallow buffer x 3 mM SPpNA, TRIS buffer + 1 M Glycine	0.00335
4 mM SPpNA, TRIS buffer x 4 mM SPpNA, TRIS buffer + 1 M TMAO	0.00476	4 mM SPpNA, Shrimp shallow buffer x 4 mM SPpNA, TRIS buffer + 1 M TMAO	0.00284