

The Effect of Molecular Crowding with Nucleotide Length and Cosecutive Structure on DNA Duplex Stability

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Supporting Information

Table S1. Thermodynamic Parameters for the 8-mer DNA Duplex Formation in Various Solutions^a

Cosolute	ΔH° (kcal mol ⁻¹)	ΔS° (cal mol ⁻¹ K ⁻¹)	ΔG°_{25} (kcal mol ⁻¹)	T_m^b (°C)	T_m^c (°C)
1 M NaCl					
None	-62.2 ± 1.9	-171 ± 5.4	-11.2 ± 0.4	50.5	47.1
20 wt% EG	-50.9 ± 2.4	-140 ± 7.9	-9.3 ± 0.3	43.7	37.1
30 wt% EG	-47.3 ± 1.8	-131 ± 5.8	-8.3 ± 0.2	38.3	31.5
40 wt% EG	-49.6 ± 1.9	-141 ± 7.9	-7.4 ± 0.4	32.3	27.0
20 wt% 2-methoxyethanol	-53.2 ± 1.3	-150 ± 3.7	-8.5 ± 0.3	38.3	32.2
40 wt% 2-methoxyethanol	-47.5 ± 0.9	-138 ± 3.7	-6.3 ± 0.3	25.6	19.6
20 wt% 1,2-dimethoxyethane	-49.9 ± 2.0	-138 ± 5.7	-8.6 ± 0.4	39.9	34.4
30 wt% 1,2-dimethoxyethane	-49.1 ± 2.4	-138 ± 5.7	-7.8 ± 0.6	34.8	29.5
20 wt% 1,3-propanediol	-48.3 ± 1.1	-133 ± 3.6	-8.8 ± 0.5	41.8	34.0
40 wt% 1,3-propanediol	-48.8 ± 2.1	-138 ± 6.2	-7.6 ± 0.4	33.8	27.6
5 wt% glycerol	-59.6 ± 2.2	-163 ± 7.0	-10.9 ± 0.3	49.9	45.1
10 wt% glycerol	-58.5 ± 1.4	-162 ± 4.2	-10.2 ± 0.4	46.9	40.9
15 wt% glycerol	-53.9 ± 1.2	-147 ± 3.9	-9.9 ± 0.3	46.9	41.6
20 wt% glycerol	-53.9 ± 1.3	-148 ± 3.1	-9.6 ± 0.4	45.6	39.8
25 wt% glycerol	-50.4 ± 1.1	-138 ± 3.7	-9.3 ± 0.3	44.4	37.5
30 wt% glycerol	-48.3 ± 1.7	-132 ± 4.8	-9.0 ± 0.3	43.4	36.0
40 wt% glycerol	-49.4 ± 1.5	-137 ± 5.0	-8.7 ± 0.3	40.3	33.2
50 wt% glycerol	-50.8 ± 2.2	-143 ± 4.2	-8.3 ± 0.4	37.8	30.6
10 wt% PEG 200	-53.7 ± 1.7	-147 ± 5.6	-9.8 ± 0.2	46.4	40.4
20 wt% PEG 200	-51.4 ± 1.6	-143 ± 5.1	-8.7 ± 0.3	40.0	31.2
30 wt% PEG 200	-50.3 ± 1.8	-143 ± 5.6	-7.6 ± 0.2	33.2	27.6
40 wt% PEG 200	-47.8 ± 1.8	-138 ± 6.4	-6.8 ± 0.3	28.5	21.5
50 wt% PEG 200	-43.9 ± 2.3	-127 ± 8.2	-5.9 ± 0.4	22.8	17.1
20 wt% PEG 1000	-54.1 ± 2.1	-148 ± 6.9	-9.9 ± 0.3	46.8	41.1
40 wt% PEG 1000	-55.9 ± 3.0	-162 ± 9.6	-7.6 ± 0.3	32.7	27.0
20 wt% PEG 2000	-54.3 ± 2.7	-148 ± 8.7	-10.1 ± 0.4	47.9	42.1
40 wt% PEG 2000	-50.3 ± 1.5	-138 ± 5.8	-9.1 ± 0.4	42.8	36.9
20 wt% PEG 6000	-52.5 ± 3.7	-142 ± 12	-10.3 ± 0.6	49.5	42.6
10 wt% PEG 8000	-58.5 ± 3.2	-161 ± 10	-10.5 ± 0.4	48.2	42.6
15 wt% PEG 8000	-57.1 ± 2.7	-158 ± 8.4	-10.0 ± 0.5	45.9	40.2
20 wt% PEG 8000	-58.4 ± 3.5	-160 ± 6.3	-10.7 ± 0.4	49.7	42.0

100 mM NaCl

None	-58.8±3.2	-161±9.9	-10.2±0.6	46.2	44.3
10 wt% PEG 200	-60.2±3.5	-169±2.5	-9.6±0.2	42.5	38.4
20 wt% PEG 200	-56.4±1.7	-160±6.0	-8.8±0.3	39.1	33.0
30 wt% PEG 200	-56.6±3.3	-163±9.0	-7.8±0.3	33.8	29.1
40 wt% PEG 200	-51.2±2.7	-148±7.9	-7.2±0.5	30.7	24.1
50 wt% PEG 200	-59.9±1.8	-183±5.7	-5.3±0.4	20.5	15.9

^aAll experiments were done in a buffer containing NaCl, 10 mM Na₂HPO₄ (pH 7.0), and 1 mM Na₂EDTA, and cosolute indicated. The thermodynamic parameters were evaluated from the average values obtained from the curve fitting and T_m^{-1} versus log(C_i) plots in addition to the data of 50wt % PEG200 at 100 mM NaCl determined only from T_m^{-1} versus log(C_i) plots due to the low T_m . The examined DNA concentration was no less than the range from 150 to 1.5 μ M (more than 100-fold) besides the data of 40wt % 2-methoxyethanol (180~3 μ M), 40wt % PEG200 (175~2 μ M at 1 M NaCl, and 200~9 μ M at 100 mM NaCl), and 50wt % PEG200 (160~3 μ M at 1 M NaCl, and 200~8 μ M at 100 mM NaCl) due to the low T_m . ^bMelting temperature was calculated at a total strand concentration of 100 μ M. ^cMelting temperature was calculated (or measured) at a total strand concentration of 5 μ M.

Table S2. Osmolality (mmol/kg) and Logarithms of Water and Solute Activities in the Absence or Presence of the Cosolute^a

Cosolute	Osmolality	$\ln a_w (\times 10^{-2})$
1 M NaCl		
None	1856±4	-3.37
20 wt% EG	5332±12	-9.68
30 wt% EG	7952±7.5	-14.4
40 wt% EG	9640±29	-17.5
20 wt% 2-methoxyethanol	4902±10	-8.90
40 wt% 2-methoxyethanol	7173±24	-13.0
20 wt% 1,2-dimethoxyethane	3796±3.8	-6.89
30 wt% 1,2-dimethoxyethane	4800±13	-8.72
20 wt% 1,3-propanediol	4730±16	-8.59
40 wt% 1,3-propanediol	6810±24	-12.4
5 wt % glycerol	2616±7	-4.75
10 wt% glycerol	3572±3	-6.49
15 wt% glycerol	3997±6	-7.26
20 wt% glycerol	4781±3	-8.68
25 wt% glycerol	5798±6	-10.5
30 wt% glycerol	6235±3	-11.3
40 wt% glycerol	7689±15	-14.0
50 wt% glycerol	8999±30	-16.3
10 wt% PEG 200	2670±2	-4.85
20 wt% PEG 200	3490±4	-6.34
30 wt% PEG 200	4419±4	-8.03
40 wt% PEG 200	5117±3	-9.29
50 wt% PEG 200	5936±6	-10.8
20 wt% PEG 1000	2921±6	-5.31
40 wt% PEG 1000	3666±5	-6.66
20 wt% PEG 2000	2496±7	-4.53
40 wt% PEG 2000	2975±6	-5.40
20 wt% PEG 6000	2784±6	-5.06
10 wt% PEG 8000	2345±1	-4.26
15 wt% PEG 8000	2598±4	-4.71
20 wt% PEG 8000	2639±10	-4.79
100 mM NaCl		
None	206±0.8	-0.37
10 wt% PEG 200	865±2	-1.57
20 wt% PEG 200	1752±3	-3.18
30 wt% PEG 200	2522±2	-4.58
40 wt% PEG 200	3010±7	-5.47
50 wt% PEG 200	4108±9	-7.46

^aAll experiments were done in a buffer containing NaCl 10 mM Na₂HPO₄ (pH 7.0), 1 mM Na₂EDTA, and cosolute indicated.

The data for EG, 2-methoxyethanol, 1,2-dimethoxyethane, and 1,3-propanediol were obtained with the freezing point depression osmometry, and the data for other cosolutes were obtained with the vapor phase osmometry.

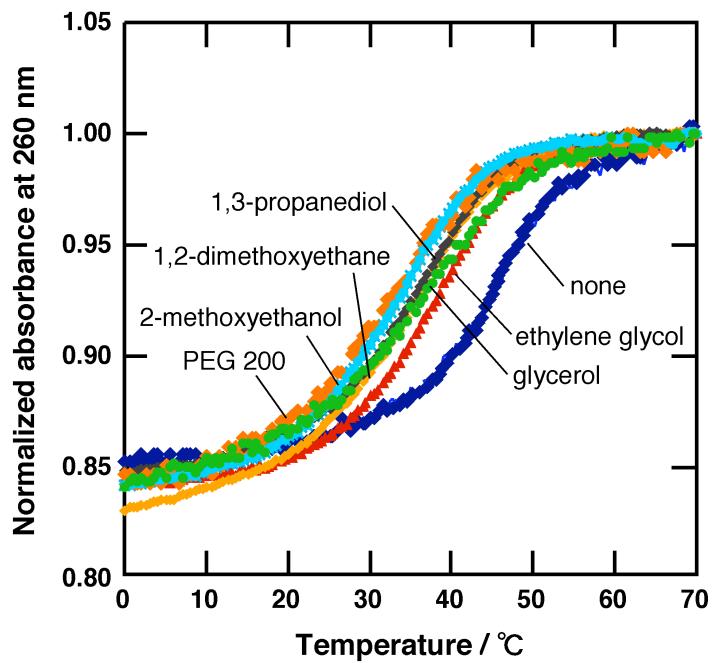


Figure S1. UV melting curves of 5 μ M ATGCGCAT in a buffer containing 1 M NaCl, 10 mM Na₂HPO₄ (pH 7.0), and 1 mM Na₂EDTA in the absence of and in the presence of 20 wt% cosolute.

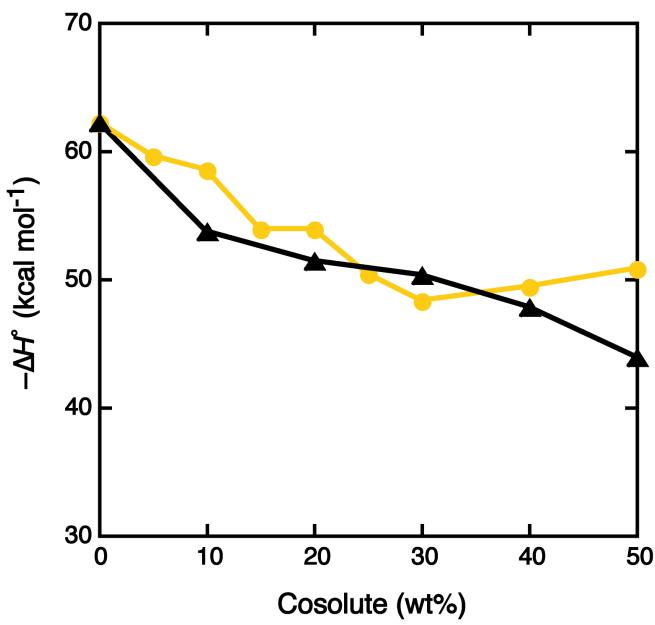


Figure S2. Plots of ΔH° as a function of PEG 200 (black triangles) or glycerol (yellow circles) wt%.

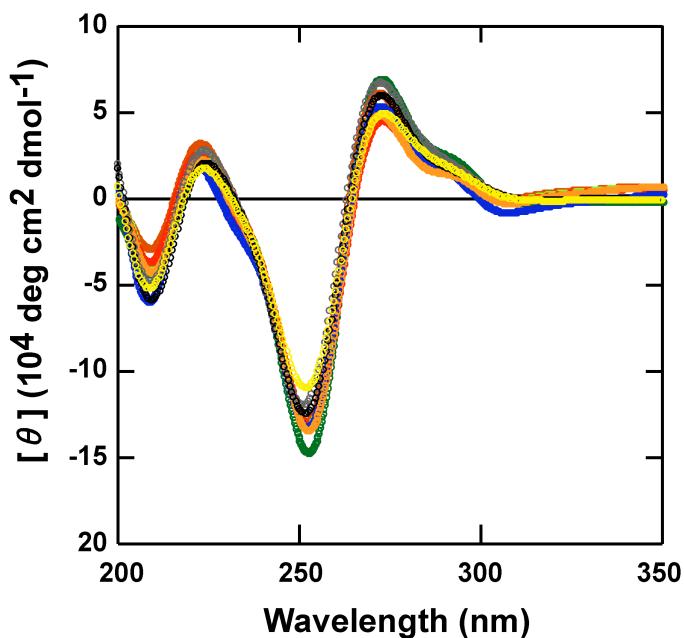


Figure S3. CD spectra of 70 μM ATGCGCAT at 4 °C in a buffer containing 1 M NaCl, 10 mM Na_2HPO_4 (pH 7.0), and 1 mM Na_2EDTA in the absence of (gray), and in the presence of 40 wt% PEG 200 (black), 40 wt% PEG 1000 (dark green), 20 wt% PEG 8000 (dark red), 40 wt% glycerol (yellow), 40 wt% ethylene glycol (green), 40 wt% 1,3-propanediol (orange), 40 wt% 2-methoxyethanol (red), or 40 wt% 1,2-dimethoxyethane (blue).