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Supplementary Information

The Effects of Chondroitin Sulfate and Serum Albumin on the Fibrillation of Human Islet Amyloid Polypeptide at Phospholipid Membranes

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1. Methods

1.1 Simulation of Thioflavin T Fluorescence Kinetics

Time-dependent ThT fluorescence curves were fitted using a sigmoidal growth model and the data of the lag time (T_{lag}), the time required to reach half of the fluorescence intensity (T_{50}) and the apparent first-order constant (k) were calculated using eqs 1 and 2.^{1,2}

$$Y = Y_0 + (Y_{max} - Y_0) / (1 + \exp - (T - T_{50}) / k) \quad (1)$$

$$T_{lag} = T_{50} - 2/k \quad (2)$$

Where Y_{max} and Y_0 are the maximum and initial fluorescence intensity, respectively.

1.2 Calculation of the Secondary Structure Contents

The secondary structure contents were calculated by the CDPro software package using the program CONTIN. A reference set of SMP56 including 56 proteins was used in the analyses of CD data.³

2. Supplementary Figures and Tables

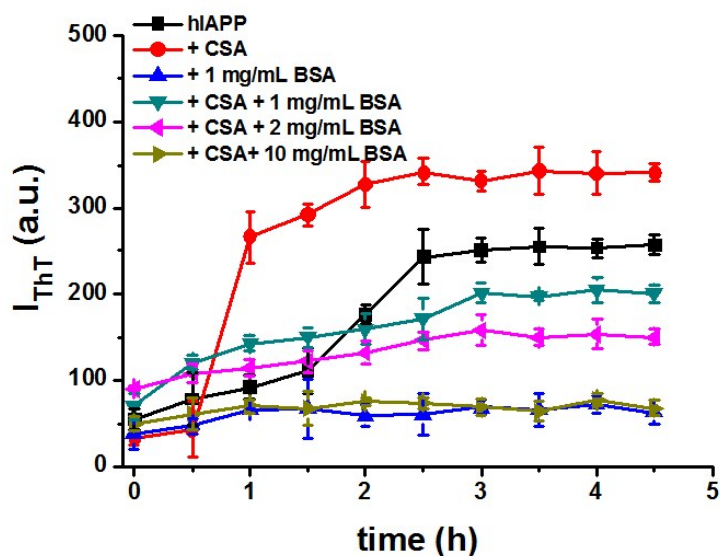


Figure S1 The effects of CSA (0.06 mg/mL) and varying concentrations of BSA on the amyloid fibrillation of hIAPP (15 μ M or 0.06 mg/mL) incubated with POPC LUVs at a P:L of 1:200, monitored by the ThT binding assays.

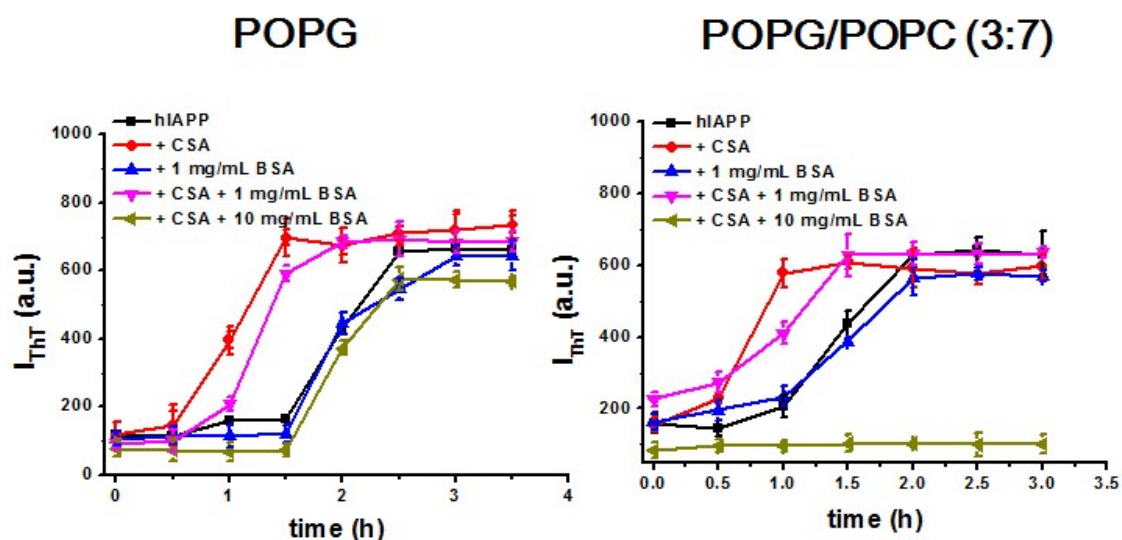


Figure S2 The effects of CSA (0.06 mg/mL) and varying concentrations of BSA on the amyloid fibrillation of hIAPP (15 μ M or 0.06 mg/mL) incubated with POPG LUVs and POPG/POPC 3:7 LUVs at a P:L of 1:200, monitored by the ThT binding assays.

Table S1 The data obtained from the ThT binding assays of 0.06 mg/mL hIAPP in

PBS (pH 7.4) containing various concentrations of CSA and CSA+BSA.

Peptide	T _{lag} (h)	T ₅₀ (h)	Y _{max}
hIAPP	3.75	4.40	78.78
+ 0.012 mg/mL CSA	2.38	3.03	507.07
+ 0.06 mg/mL CSA	2.2	2.6	375.98
+ 0.3 mg/mL CSA	0.97	1.32	241.41
+ 0.3 mg/mL CSA + 1 mg/mL BSA	0.62	2.96	72.09

Table S2 The data obtained from the ThT binding assays of 0.06 mg/mL hIAPP in POPC LUV solutions containing various concentrations of CSA and CSA+BSA at pH 7.4.

P:L	Peptide	T _{lag} (h)	T ₅₀ (h)	Y _{max}
1:25	hIAPP	1.13	1.49	167.08
	+ 0.06 mg/mL CSA	0.28	0.36	255.67
	+ 0.06 mg/mL CSA + 1 mg/mL BSA	0	1.72	143.53
	+ 0.06 mg/mL CSA + 2 mg/mL BSA	0	2.11	94.98
1:200	hIAPP	1.16	1.82	258.92
	+ 0.06 mg/mL CSA	0.62	0.84	328.7
	+ 0.06 mg/mL CSA + 1 mg/mL BSA	0	1.51	213.3
	+ 0.06 mg/mL CSA + 2 mg/mL BSA	0	1.60	145.68

Table S3 The data obtained from the ThT binding assays of 0.06 mg/mL hIAPP in POPG LUV solutions containing various concentrations of CSA, BSA and CSA+BSA at pH 7.4.

P:L	Peptide	T _{lag} (min)	T ₅₀ (min)	Y _{max}
1:25	hIAPP	18.04	22.05	345.07
	+ 0.06 mg/mL CSA	15.08	22.98	568.29
	+ 0.3 mg/mL CSA	13.08	18.78	885.72
	+ 1 mg/mL BSA	14.34	21.2	384.5
	+ 10 mg/mL BSA	12.71	18.53	316.84
	+ 0.06 mg/mL CSA + 1 mg/mL BSA	14.08	25.27	336.16
1:200	hIAPP	96	116.4	547.58
	+ 0.06 mg/mL CSA	42.6	60.6	585.15
	+ 1 mg/mL BSA	99	114.6	614.72
	+ 0.06 mg/mL CSA + 1 mg/mL BSA	114.6	73.8	688.92
	+ 0.06 mg/mL CSA + 10 mg/mL BSA	117.6	88.8	571.26

Table S4 The data obtained from the ThT binding assays of 0.06 mg/mL hIAPP in POPG/POPC 3:7 LUV solutions containing various concentrations of CSA, BSA

and CSA + BSA at pH 7.4.

P:L	Peptide	T _{lag} (min)	T ₅₀ (min)	Y _{max}
1:25	hIAPP	16.74	20.10	662.9
	+ 0.06 mg/mL CSA	14.12	17.92	550.7
	+ 0.3 mg/mL CSA	11.15	14.59	663.1
	+ 1 mg/mL BSA	13.24	16.6	499.93
	+ 0.06 mg/mL CSA + 1 mg/mL BSA	15.9	20.33	783.29
1:200	hIAPP	61.2	85.2	646.92
	+ 0.06 mg/mL CSA	27.6	39.6	608.61
	+ 1 mg/mL BSA	65.4	88.2	577.09
	+ 0.06 mg/mL CSA + 1 mg/mL BSA	94.8	61.2	636.48

Table S5 The secondary structure data for 0.2 mg/mL hIAPP in PBS at pH 7.4 in the absence and presence of 0.2 mg/mL CSA obtained by the CD measurements.

Peptide	Time (h)	Secondary structure (%)			
		Helix	Strand	Turn	Unordered
hIAPP	0	6	28	55.3	11.7
	1	5.2	27.8	55	12
	2	5.3	28.1	54.6	12
	3	5	50.3	32.9	11.7
	4	5.4	49.5	33.4	11.8
	5	4.6	50.6	32.9	11.9
hIAPP + CSA	0	35.9	20.8	33.2	10.1
	1	5.1	48.5	34.2	12.2
	2	4.4	50.3	33.1	12.2
	3	4.3	50.6	32.9	12.3
	4	4.6	51	32.6	11.8
	5	4.5	50.3	33.3	11.9

Table S6 The secondary structure data for 0.2 mg/mL hIAPP incubated with POPC LUVs at a P:L of 1:25 at pH 7.4 in the absence and presence of 0.2 mg/mL CSA obtained by the CD measurements.

Peptide	Time (h)	Secondary structure (%)			
		Helix	Strand	Turn	Unordered
hIAPP	0	5.4	27.7	54.8	12.2
	1	5.2	27.6	54.9	12.3
	2	5.6	28.6	53.3	12.5
	3	4.5	49.8	34.2	11.5
hIAPP + CSA	0	43.8	9.9	33	13.3
	1	15.7	38.4	34	12
	2	11.4	38.2	28.4	10.9
	3	6.8	42.6	37.4	13.3

Table S7 The secondary structure data for 0.2 mg/mL hIAPP incubated with POPG LUVs at a P:L of 1:25 at pH 7.4 in the absence and presence of 0.2 mg/mL CSA obtained by the CD measurements.

Peptide	Time (min)	Secondary structure (%)			
		Helix	Strand	Turn	Unordered
hIAPP	0	45.1	14.9	29.8	10.3
	15	48.3	9.8	30.3	11.6
	30	43.8	15.2	25.7	15.1
	45	5.8	55.4	24.3	14.6
	60	8.6	58.7	26.8	15.9
hIAPP + CSA	0	43.5	15.5	28.8	12.2
	15	46.4	9.8	31.4	12.4
	30	46.1	14.2	28.4	11.3
	45	5.6	59.4	24.3	10.7
	60	5.3	59.7	23.2	11.7

References

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