Supplementary Information

Oligomeric Procyanidins Inhibit Insulin Fibrillation by Forming Unstructured and Off-Pathway Aggregates

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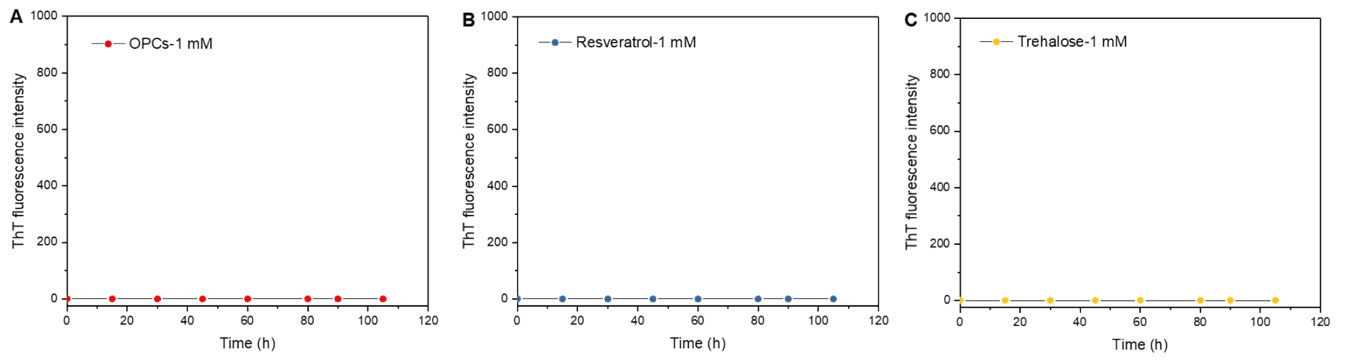
Fig. S1. ThT fluorescence intensity of (A) OPCs, (B) resveratrol, and (C) trehalose in the absence of NaCl.
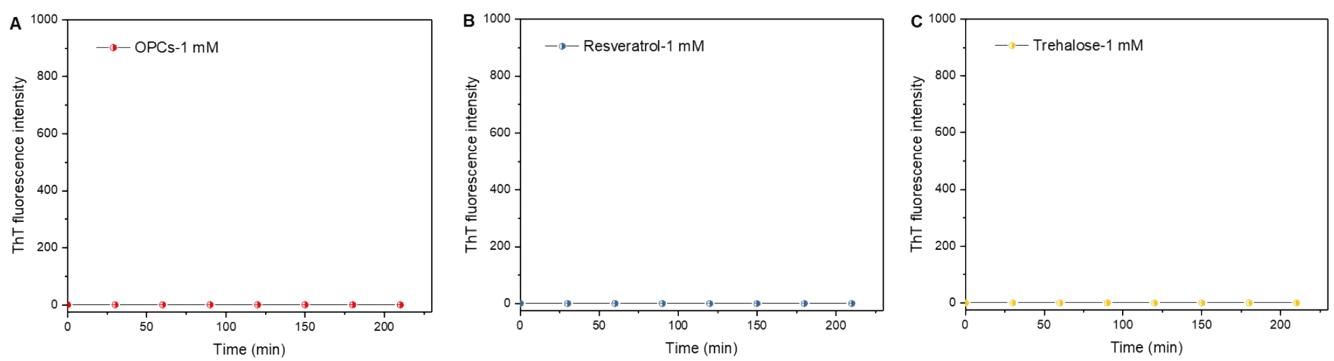

Fig. S2. ThT fluorescence intensity of (A) OPCs, (B) resveratrol, and (C) trehalose in the presence of 100 mM NaCl.

Table S1. Effects of OPCs, resveratrol, and trehalose on the kinetic parameters of insulin fibrillation in the absence of 100 mM NaCl.

	Fibril Formation[b]			
	Lag time (h)	Growth rate	Intensity[c]	Amyloidogenic[d]
Insulin	43.2 ± 7.9	34.8 ± 0.6	701.8 ± 127.3	+++
Insulin + OPCs				
100 μM	67.0 ± 2.0	3.9 ± 0.1	155.2 ± 5.7	+
500 μM	NA[e]	NA	NA	--
1 mM	NA	NA	NA	--
Insulin + resveratrol				
100 μM	43.2 ± 9.6	30.0 ± 0.4	634.2 ± 149.1	+++
500 μM	48.5 ± 10.9	16.0 ± 0.2	356.1 ± 69.3	++
1 mM	45.3 ± 2.8	15.2 ± 0.2	368.3 ± 21.9	++
Insulin + trehalose				
100 μM	38.6 ±8.9	37.5 ± 0.9	635.9 ± 44.5	+++
1 mM	39.9 ± 2.0	25.0 ± 0.3	576.2 ± 25.0	+++

[a]All assays were repeated 10 times. [b]The concentration of bovine insulin was 2 mg/mL. [c]The fluorescence intensity of insulin alone was set as reference value. [d]Semi-quantitative analysis of ThT fluorescence-based amyloid formation: +++ designates samples with fluorescence intensity >75% of insulin intensity; ++, values between 50% and 75% insulin intensity; +, values between 20% and 50% insulin intensity; and --, values <20% of insulin intensity. Note: ThT fluoresence data were obtained strictly according to the experimental protocol in this work. [e]Data are not available, in that the weak ThT intensity data cannot get a good fit with the kinetic equation.

Table S2. Effects of OPCs, resveratrol, and trehalose on the kinetic parameters of insulin fibrillation in the presence of 100 mM NaCl.

	Fibril Formation[b]			
	Lag time (min)	Growth rate	Intensity[c]	Amyloidogenic[d]
Insulin	59.1 ± 5.7	27.6± 0.3	797.0 ± 81.4	+++
Insulin + OPCs				
100 μM	62.9 ± 10.6	11.4 ± 0.1	400.7 ± 58.4	++
500 μM	95.0 ± 8.3	3.7 ± 0.1	128.5 ± 13.3	+
1 mM	119.0 ± 1.1	2.7 ± 0.1	100.7± 1.3	+
Insulin + resveratrol				
100 μM	61.1 ± 4.3	17.5 ± 0.3	427.6 ± 32.9	++
500 μM	53.2 ± 5.9	8.4 ± 0.1	303.9 ± 25.9	+
1 mM	53.6 ± 7.8	5.27 ± 0.1	325.1 ± 28.3	+
Insulin + trehalose				
100 μM	33.3 ± 8.4	17.3 ± 0.2	472.9 ± 85.6	++
1 mM	41.9 ± 8.6	17.3 ± 0.1	609.6 ± 84.9	+++

[a]All assays were repeated 10 times. [b]The concentration of bovine insulin was 2 mg/mL. [c]The fluorescence intensity of insulin alone was set as reference value. [d]Semi-quantitative analysis of ThT fluorescence-based amyloid formation: +++ designates samples with fluorescence intensity >75% of insulin intensity; ++, values between 50% and 75% insulin intensity; +, values between 20% and 50% insulin intensity; and --, values <20% of insulin intensity. Note: ThT fluoresence data were obtained strictly according to the experimental protocol in this work.

Table S3. Secondary structure contents of insulin incubated with different compounds in the presence of 100 mM NaCl.

Samples	Time (min)	Secondary structure			
		α-helix	β-strand	β-turn	Others
Insulin	**0**	60.2%	11.3%	9.1%	19.3%
	60	28.8%	11.6%	14%	45.6%
	150	0	37.9%	23.3%	38.9%
Insulin + OPCs	**0**	60.2%	11.3%	9.1%	19.3%
	60	16.8%	8.8%	7.5%	67.1%
	150	0	30.2%	20.6%	49.2%
Insulin + resveratrol	**0**	60.2%	11.3%	9.1%	19.3%
	60	11.2%	23%	14.4%	51.3%
	150	0	24.8%	18.3%	56.9%
Insulin + trehalose	**0**	60.2%	11.3%	9.1%	19.3%
	150	0	36.9%	18.4%	44.7%

Table S4. Secondary structure contents of insulin in the absence or presence of 100 mM NaCl.

Samples	Secondary structure			
	α-helix	β-strand	β-turn	Others
Insulin (45 h, without NaCl) [a]	35.7%	13.8%	12.2%	38.3%
Insulin (60 min, with NaCl)	28.8%	11.6%	14.0%	45.6%

[a] Data obtained from our previous work[1] is re-calculated using the BestSel server.

Reference

1   R. Liu, R. X. Su, W. Qi and Z. M. He, *Biochem. Biophys. Res. Commun.*, 2011, **409**, 229-234.