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Thermodynamic Analysis of the Molecular Interactions between Amyloid β-Protein Fragments and (−)-Epigallocatechin-3-gallate

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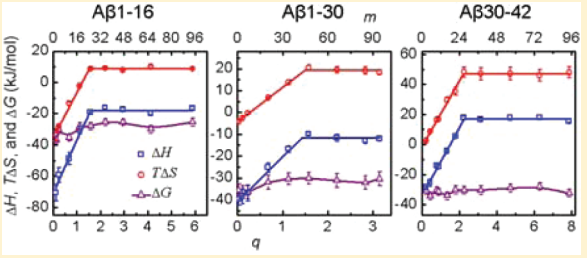
Supporting Information

ABSTRACT: (−)-Epigallocatechin-3-gallate (EGCG) has been proven effective in preventing the aggregation of amyloid β-protein 42 (Aβ42), and the thermodynamic interactions between Aβ42 and EGCG have been studied in our previous work (*J. Phys. Chem. B* **2010**, *114*, 11576). Herein, to further probe the interactions between different regions of Aβ42 and EGCG, three Aβ42 fragments (i.e., Aβ1−16, Aβ1−30, and Aβ31−42) were synthesized, and the thermodynamic interactions between each of the fragments and EGCG at different EGCG and salt concentrations were investigated by isothermal titration calorimetry. The results indicate that, although hydrogen bonding and hydrophobic interaction are both involved in the interactions between Aβ42 and EGCG, hydrogen bonding mainly happens in Aβ1−16 while hydrophobic interaction mainly happens in Aβ17−42. It is found that when Aβ42 and its fragments are saturated by EGCG, their thermodynamic parameters have linear relationships. The saturated binding stoichiometry (N_s) for Aβ42 is the sum of the N_s values for Aβ1−30 and Aβ31−42, while ΔH_s, ΔS_s, and ΔG_s for Aβ42 are half the sum of the values for Aβ1−30 and Aβ31−42. The result suggests that there are no specific interactions and binding sites in the Aβ42 and EGCG binding. The orders of ΔH_s and $T\Delta S_s$ values for the Aβ fragments are determined as Aβ17−42 > Aβ31−42 > Aβ1−30 > Aβ1−16. Moreover, there is significant enthalpy−entropy compensation in the binding of EGCG to Aβ42 and its fragments, resulting in insignificant change of ΔG with the change of the solution environment. The research has shed new light on the molecular mechanisms of the interactions between EGCG and Aβ42.



1. INTRODUCTION

One of the features of the Alzheimer's disease (AD) is the aggregation of amyloid β-protein (Aβ) from its soluble random coil form into β-sheet-rich fibrils.[1] Aβ is the proteolytic product of amyloid β-protein precursor and it contains 39−43 amino acid residues.[2] Among them, amyloid β-protein 42 (Aβ42) is considered to be the most vital factor to the onset of AD due to its strong neurotoxicity and aggregation capability.[3,4] Although the conformation of Aβ42 is variable and uncertain,[5,6] the secondary structure of Aβ42 monomer in fibrils is definite. Namely, it possesses a disordered hydrophilic N-terminal region in the residues 1−16,[7,8] which is also considered to be the minimal zinc-binding domain and contains two aspartates subject to protein aging,[9] a hydrophobic β-sheet-forming region in the residues 17−26, a turn region between the residues 27−30, and another β-sheet-forming region in the residues 31−42.[10,11] Based on these facts, many studies have concentrated on hunting for Aβ-aggregation inhibitors and investigating the interaction mechanisms between them.[12]

(−)-Epigallocatechin-3-gallate (EGCG) (Figure 1), a naturally occurring polyphenol derived from green tea, has been proved effective in reducing Aβ deposition[13] and Aβ-mediated cognitive impairment in vivo,[14,15] and preventing Aβ from aggregation in vitro.[16] Our previous work conducted by isothermal titration calorimetry (ITC) has investigated the thermodynamic interactions between Aβ42 and EGCG.[17] It was found that the binding stoichiometry (N), enthalpy change (ΔH), and entropy change (ΔS) are all linearly related to EGCG/Aβ42 ratio; the hydrophobic interaction increases while the hydrogen bonding decreases with the increase of EGCG/Aβ42 ratio. However, the features of the interactions between EGCG and the different regions of Aβ42 remain unclear. Therefore, in this work, different fragments of Aβ42, i.e., Aβ1−16, Aβ1−30, and Aβ31−42 (denoted as $A\beta_f$ in the following), were synthesized, and their thermodynamic interactions with

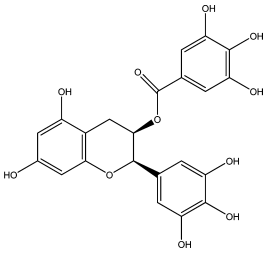


Figure 1. Chemical structure of EGCG.

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EGCG were investigated by ITC. From the experimental data, the thermodynamic parameters for the binding of EGCG to Aβ17−42 were calculated. The results are expected to shed new light on the molecular mechanisms for EGCG binding to Aβ42 and to facilitate the rational design of Aβ aggregation inhibitors.

2. EXPERIMENTAL METHODS

Materials. The amino acid sequence of Aβ42 is DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA. Aβ1−16, Aβ1−30, and Aβ31−42 are the fragments of Aβ42. Aβ42, together with its fragments, was obtained as lyophilized powder with a purity of >95% from GL Biochem (Shanghai, China). EGCG and hexafluoroisopropanol (HFIP) were purchased from Sigma (St. Louis, MO). Other chemicals were all of the highest purity available from local sources.

Pretreatment of Peptides. Lyophilized Aβ42 and Aβ_f's were stored at −80 °C. Before use, the vial containing peptide was allowed to equilibrate to room temperature for 30 min to avoid condensation upon opening.[18] Peptide stock solution was prepared by dissolving a peptide at 1 mg/mL in HFIP for 30 min in order to eliminate all the secondary structures. Thereafter, the solution was transferred to Eppendorf tube and the volatile solvent was removed by vacuum freeze-drying overnight. The peptide was then stored at −20 °C before use.[19]

Preparation of Sample Solutions. The buffer used for ITC experiments was 10 mmol/L sodium dihydrogen phosphate/disodium hydrogen phosphate buffer (PB) of pH 7.4. The required salt concentration was adjusted by adding definite amount of sodium chloride (NaCl).

The pretreated Aβ42 and Aβ_f's were dissolved in dimethyl sulfoxide (DMSO) and then diluted to 20 μmol/L with the PB buffer containing a definite NaCl concentration. The final content of DMSO was kept at 5% (v/v).[20] EGCG was dissolved in the same buffer solutions used for the Aβ_f with a final DMSO concentration of 5% (v/v).

Isothermal Titration Calorimetry. Isothermal calorimetric titrations were performed using a VP isothermal titration calorimeter (MicroCal, Northampton, MA). Experiments were carried out in a titration mode with a 1.425 mL sample cell containing an Aβ_f solution treated by 20 min degassing. A 10 μL EGCG solution in the same buffer as the Aβ_f solution was injected over 20 s for 25 times at a constant interval of 600 s via a 416 rpm rotating stirrer-syringe into the sample cell. In the control experiment, the titrant was injected to the buffer in the sample cell to obtain the heat of dilution. The value of the heat of dilution was subtracted from the experimental result in the final analysis. Each experiment was repeated three times, and the mean value with standard deviations was provided.

Titration data were analyzed by the evaluation software, MicroCal Origin, Version 7.0, provided by the manufacturer. Because there are no specific interactions between Aβ42 and EGCG, and they mainly interact through hydrogen bonding and hydrophobic interaction, it is assumed in this work that the binding sites on the Aβ_f's are identical and independent. Therefore, the binding energies of all possible binding sites (the binding sites on the peptides) are assumed the same.[16,17,21] Consequently, the integrated enthalpy was calculated from the single-site binding model by regressing the ITC results. This leads to the calculations of thermodynamics parameters, i.e., N, ΔH, $T\Delta S$, and Gibbs free energy (ΔG).

Analysis of Thermodynamic Parameters. The interacting forces involved in the binding of EGCG to Aβ are mainly hydrogen bonding, hydrophobic interaction, and van der Waals force.[16,17,21] van der Waals force is a universal molecular interaction; its magnitude is relatively small compared to hydrogen bonding, and the characteristic signs of the thermodynamic parameters related to van der Waals force are identical to hydrogen bonding. Hence, van der Waals force is integrated into hydrogen bonding in the following analysis. It is considered that the interacting forces between an Aβ_f and EGCG are mainly hydrogen bonding and hydrophobic interaction and the ΔH and $T\Delta S$ values of them can be expressed as follows.

$$\Delta H = \Delta H_{HB} + \Delta H_{HI} \quad (1)$$

$$T\Delta S = T\Delta S_{HB} + T\Delta S_{HI} \quad (2)$$

where the subscripts HB and HI represent the contributions of hydrogen bonding and hydrophobic interaction to the enthalpy and entropy changes, respectively. In the equations, ΔH_{HB} and ΔS_{HB} are negative while ΔH_{HI} and ΔS_{HI} are usually positive.[22,23]

In addition, two parameters are introduced to compare the thermodynamic parameters between different Aβ_f's, which are

$$m = [\text{EGCG}]/[\text{A}\beta_f] \quad (3)$$

$$q = [\text{EGCG}]/([\text{A}\beta_f] \times x) \quad (4)$$

where [EGCG] and [Aβ_f] are the final concentrations of EGCG and an Aβ_f in the sample cell, respectively, x is the number of the amino acid residues of the Aβ_f. Therefore, m stands for the molar ratio of EGCG to the peptide, and q represents the molar ratio of EGCG to amino acid residue.

Size-Exclusion Chromatography. To examine if significant oligomerization of Aβ42 and the Aβ_f's occurred during the 5 h ITC experiment, the fresh Aβ solutions and those incubated for 5 h with and without EGCG were analyzed by size-exclusion chromatography (SEC) with a Superdex 75 10/300 GL column (fractionation range, 3−70 kDa) equipped on the AKTA purifier 10 system (GE Healthcare, Uppsala, Sweden). The concentrations of peptides were all 20 μmol/L, and EGCG was 0 and 1 mmol/L. The peptide solutions for Aβ42 and Aβ1−30 were prepared with the typical solvents used in the ITC experiments (10 mmol/L PB with 100 mmol/L NaCl containing 5% DMSO (v/v)). Since the molecular weights of Aβ31−42 (1141) and Aβ1−16 (1954) are below the fractionation range of the Superdex 75 10/300 GL column, they cannot be separated with DMSO. Therefore, Aβ1−16, which is easy to be dissolved in water, was directly prepared with 10 mmol/L PB plus 100 mmol/L NaC1, while 1 mmol/L NaOH was used instead of 5% DMSO in the dissolution of Aβ31−42. Samples were filtered with a polyether sulfone membrane (nominal pore size, 0.45 μm) before injection. The eluant was 10 mmol/L PB with 100 mmol/L NaCl. The column effluent was monitored at 215 nm.

3. RESULTS AND DISCUSSION

Oligomeric States of Aβ42 and Aβ_f's. Aβ42 was reported to exist as a mixture of monomer and small oligomers in rapid equilibrium.[24] Aβ31−42 was also reported to exist as assemblies in PB.[25] There are no reports available in the literature on the states of Aβ1−30 and Aβ1−16. So, in this work the oligomeric states of Aβ42 and its fragments with and without EGCG during the 5 h incubation of ITC experiments were first estimated and compared by SEC. The SEC results and corresponding analyses are shown in Figure S1 and Table S1,

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respectively, in the Supporting Information. It is observed that the SEC of Aβ42 shows two peaks and the retention volume of the first small peak (peak 1 in Table S1) is 8.3−8.4 mL, corresponding to a molecular weight of 114−119 kDa (the molecular weight of Aβ42 monomer is 4514 Da). According to Bitan et al.,[24] this peak stands for protofibrils. Similarly, the SECs of Aβ1−30 and Aβ31−42 also show small peaks, which respectively stand for oligomers of 28−30 and 5.7−7.5 kDa (the molecular weights of Aβ1−30 and Aβ31−42 are 3389 and 1141 Da, respectively). The main peak (peak 2 in Table S1) of Aβ42 stands for a mixture of low molecular weight oligomers as reported by Bitan et al.[24] Herein, since the apparent molecular weight for the main peak is between the monomer and dimer of each peptide, to simplify calculations, the percentage of monomer is estimated by assuming that the four peptides all exist as monomers and dimers in the solution. The results in Table S1 show that only 19%−27% of Aβ42 is monomeric, slightly higher than that reported by Bitan et al.[24] (about 20%). Nonetheless, the association extents of the four peptides can be estimated and compared by this way. The percentages of monomers are in the order of Aβ1−16 > Aβ1−30 > Aβ42 > Aβ31−42 (Table S1), indicating the association extents of the four peptides increase in the opposite order.

Table S1 also shows that the percentages of monomers decrease after 5 h incubation (except for Aβ1−30, which shows a slight increase), indicating that Aβ_f's self-associate to some extent during the 5 h ITC experiments. To evaluate the effects of self-association on the ITC results of EGCG binding to Aβ_f's, a buffer solution (PB with a NaCl concentration range of 0−200 mmol/L) without EGCG was used for the titration of each Aβ_f solution in the same buffer. The results show that the enthalpies were all zero or only slightly exothermic. Since the data at different conditions almost overlap with each other, only the enthalpies at a physiological condition (pH 7.4, 100 mmol/L NaCl, and 37 °C) are selected and shown in Figure S2 (in the Supporting Information) to make the figure concise and readable. These results are similar to those of Aβ42 conducted in our earlier work.[17] This phenomenon mainly stems from the fact that the interactions involved in the self-association of Aβ42 are mainly hydrophobic and electrostatic interactions.[18,26,27] Hydrophobic interaction introduces relatively small positive ΔH, and electrostatic interaction introduces relatively small negative ΔH,[22,23] leading to the small overall enthalpies. Anyway, the above results indicate that the self-associations of Aβ_f's do not significantly affect the studies on the binding of EGCG to Aβ_f's by ITC.

It should be noted that the thermodynamic parameters obtained by ITC are apparent values representing the interactions between EGCG and the mixture of monomer and oligomers of each Aβ_f. Therefore, the parameters may be somewhat different from those for pure monomers. However, since the oligomer forms within 1−2 min and is in rapid equilibrium with monomers, it is considered that the obtained parameters reflect the real situations that happen for Aβ42 and Aβ_f's in PB.

Effect of Salt Concentration. In this section, the q value was set fixed as 0.22 in order to compare the thermodynamic parameters between the Aβ_f's as well as Aβ42 obtained in our previous work, in which the q value was also 0.22.[17] The ITC results for the titration of EGCG to the three Aβ_f's at different salt concentrations are shown in Figures S3−S5 (in the Supporting Information) and the thermodynamic parameters derived from the model fitting are summarized in Figure 2.

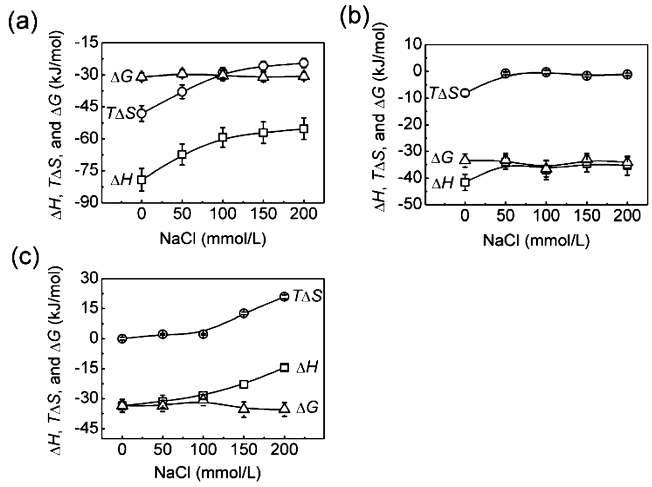

Figure 2. Thermodynamic parameters for the interactions between EGCG and Aβ1−16 (a), EGCG and Aβ1−30 (b), and EGCG and Aβ31−42 (c) at different salt concentrations. The initial and final concentrations of Aβ_f's were 20 and 16.81 μmol/L, respectively. The initial and final concentrations of EGCG were 0.38 and 0.06 mmol/L (a), 0.71 and 0.11 mmol/L (b), and 0.29 and 0.05 mmol/L (c), respectively. q is set constant at 0.22 in panels a−c. Experiments were carried out in 10 mmol/L PB (pH 7.4) at 37 °C. The solid lines are drawn to guide the eye.

It is well-known that hydrogen bonding becomes weaker and leads to less negative ΔH and $T\Delta S$ with increasing salt concentration while hydrophobic interaction takes the opposite trend. Both the ΔH and $T\Delta S$ values for Aβ1−16 are minus and become less negative with the increase of salt concentration (Figure 2a), suggesting that the interactions between EGCG and Aβ1−16 are mainly hydrogen bonding. Figure 2b shows that the ΔH and $T\Delta S$ values for Aβ1−30 are almost independent of salt concentration, indicating that hydrogen bonding and hydrophobic interaction are both involved and they nearly make a balance at the salt concentration range. Namely, the decrease of hydrogen bonding with increasing salt concentration is compensated by the increase of hydrophobic interaction. From Figure 2c for Aβ31−42, however, we can see that the ΔH value is negative while the $T\Delta S$ value is positive. Since hydrophobic interaction contributes to relatively small positive ΔH and relatively large positive $T\Delta S$ while hydrogen bonding contributes to relatively large negative ΔH and relatively small negative $T\Delta S$,[22,23] the results in Figure 2c indicate that both hydrogen bonding and hydrophobic interaction are involved in the interactions between EGCG and Aβ31−42.

Effect of *q*. The ITC results for the titration of EGCG to Aβ_f's as a function of EGCG concentration are shown in Figures S6−S8 in the Supporting Information for the three fragments. The ΔH, $T\Delta S$, and ΔG values derived from the model calculations are shown in Figure 3 as functions of q and m.

As shown in Figure 3, the ΔH and $T\Delta S$ values for the three fragments are all linearly related to q at low q values (q less than about 1.5−2.2) as described by eqs 5−10.

Aβ1−16:

$$\Delta H = 33.79q - 70.73 \quad (0 < q < 1.57)\ (R^2 = 0.9779) \tag{5}$$

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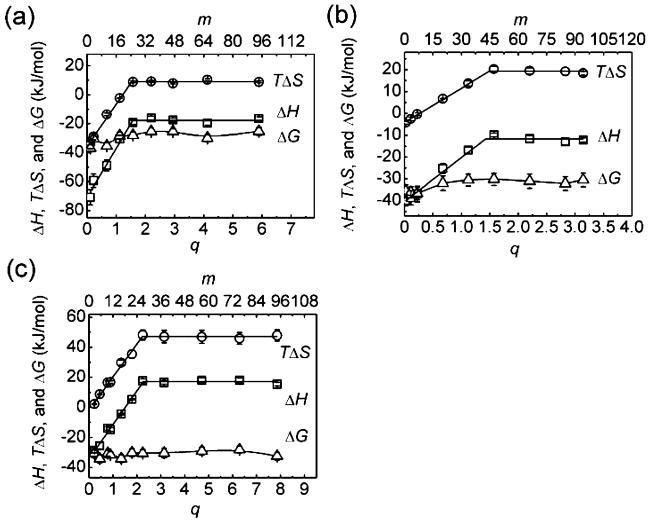

Figure 3. Thermodynamic parameters for the interactions between EGCG and Aβ1−16 (a), EGCG and Aβ1−30 (b), and EGCG and Aβ31−42 (c) as a function of EGCG concentration. The initial and final concentrations of Aβ_f's were 20 and 16.81 μmol/L, respectively. The initial EGCG concentrations were in the range of 0−10 mmol/L, and the final ones were 15.85% of the initial values. q and m values are calculated from eqs 3 and 4, respectively. Experiments were carried out in 10 mmol/L PB containing 100 mmol/L NaCl (pH 7.4) at 37 °C. The solid lines of ΔH and $T\Delta S$ are calculated according to eqs 5 and 6 (a), eqs 7 and 8 (b), and eqs 9 and 10 (c). The solid lines of ΔG are drawn to guide the eye.

$$T\Delta S = 29.02q - 35.46 \quad (0 < q < 1.53)\ (R^2 = 0.9914) \tag{6}$$

Aβ1−30:

$$\Delta H = 20.18q - 40.42 \quad (0 < q < 1.43)\ (R^2 = 0.9923) \tag{7}$$

$$T\Delta S = 15.76q - 4.19 (0 < q < 1.49) (R^2 = 0.9990) \tag{8}$$

Aβ31−42:

$$\Delta H = 22.65q - 34.24 \quad (0 < q < 2.27)\ (R^2 = 0.9926) \tag{9}$$

$$T\Delta S = 21.94q - 1.76 \quad (0 < q < 2.23)\ (R^2 = 0.9918) \tag{10}$$

Since the decrease of hydrogen bonding leads to the increase of ΔH_{HB} (<0) and ΔS_{HB} (<0), and the increase of hydrophobic interaction leads to the increase of ΔH_{HI} (>0) and ΔS_{HI} (>0), it can be deduced from Figure 3 that the relative importance of hydrogen bonding gradually decreases with the increase of q at low q values while that of hydrophobic interaction gradually increases. The reason for this interesting behavior is that the formation of hydrogen bonding needs specific steric orientations of the donor and acceptor and the increase of EGCG concentration leads to a significant steric hindrance effect precluding the molecule alignments.

With the further increase of q, the ΔH and $T\Delta S$ values reach plateaus at q larger than about 1.5−2.2, indicating that the Aβ_f's have been saturated by EGCG and the excess EGCG molecules cannot bind to the peptides due to steric hindrance. This phenomenon is consistent with that observed for Aβ42 in our earlier work (see Figure 3, and eqs 6 and 7 therein).[17] Herein, the q value at which the thermodynamic parameter becomes unchanged with increasing EGCG concentration, i.e., an Aβ_f becomes saturated by EGCG, is termed as a saturated q value (sq). Thus, the mean values of the thermodynamic parameters for an Aβ_f at $q \geq$ sq are calculated for comparisons. These parameters are characterized by a subscript "s" as listed in Table 1. It is observed from the table that the ΔH_s, $T\Delta S_s$, and ΔG_s values for Aβ42, Aβ1−30, and Aβ31−42 fit the following equation:

$$Y_s(\text{A}\beta 42) = [Y_s(\text{A}\beta 1{-}30) + Y_s(\text{A}\beta 31{-}42)]/2 \tag{11}$$

where Y_s is ΔH_s, $T\Delta S_s$, or ΔG_s. It can be seen that the right-hand side of the equation is divided by a coefficient 2. This coefficient rises from the fact that ΔH, $T\Delta S$, and ΔG are intensive quantities that respectively mean the enthalpy change, entropy change, and Gibbs free energy change introduced by per mole injectant (EGCG in this work). Namely, each Y_s in the equation stands for the thermodynamic parameter contributed by one mole of EGCG binding to a certain peptide, and therefore the right-hand side should be divided by the number of fragments (it is 2 herein). By comparing the calculated Y_s values for Aβ42 and those from the ITC measurement, it is seen that eq 11 holds well since the differences between the calculated and measured values all fall in the deviation ranges. As mentioned above, Aβ42 and Aβ_f's exist as a mixture of monomers and oligomers in solution and the association extents also differ from each other. Therefore, the standard errors for the thermodynamic parameters are relatively large. However, since these monomers and oligomers are in rapid equilibrium, the interactions between EGCG and Aβ42 are nonspecific, and eq 11 holds, it is considered that this phenomenon does not have significant influence on the ITC results for the binding of EGCG to Aβ_f's.

Since the interactions between Aβ and EGCG are nonspecific, it can be deduced from eq 11 that the Y_s values for Aβ42, Aβ1−16, and Aβ17−42 also have the following relationship:

Table 1. N_s, ΔH_s, $T\Delta S_s$, and ΔG_s Values for the Binding of EGCG to Aβ42 and Its Fragments

	N_s	ΔH_s (kJ/mol)	$T\Delta S_s$ (kJ/mol)	ΔG_s (kJ/mol)
Aβ42	22.35 ± 1.79	2.09 ± 0.20	35.12 ± 1.84	−33.01 ± 1.89
calcd for Aβ42[a]	21.44 ± 2.05	2.73 ± 1.19	33.27 ± 0.84	−30.54 ± 1.28
Aβ1−16	10.06 ± 0.52	−17.80 ± 1.59	8.98 ± 0.85	−26.78 ± 2.12
calcd for Aβ17−42[b]	12.29 ± 2.05	21.97 ± 1.18	61.25 ± 0.84	−39.24 ± 1.27
Aβ1−30	10.90 ± 1.01	−11.66 ± 1.31	19.32 ± 0.80	−30.98 ± 0.88
Aβ31−42	10.54 ± 1.04	17.11 ± 1.06	47.22 ± 0.88	−30.10 ± 1.67

[a]Data were calculated from eq 11 for ΔH_s, $T\Delta S_s$, and ΔG_s and eq 16 for N_s. [b]Data were calculated from eq 12 for ΔH_s, $T\Delta S_s$, and ΔG_s and eq 17 for N_s.

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$$Y_s(\text{A}\beta42) = [Y_s(\text{A}\beta1{-}16) + Y_s(\text{A}\beta17{-}42)]/2 \quad (12)$$

Thus, the Y_s values for Aβ17−42 can be calculated from eq 12 with the values for Aβ42 and Aβ1−16. The calculated results are also provided in Table 1.

It can also be found from the table that the orders of ΔH_s and $T\Delta S_s$ values for the Aβ_f's are Aβ17−42 > Aβ31−42 > Aβ1−30 > Aβ1−16. Thus, hydrogen bonding becomes more important in the binding of EGCG to Aβ_f's in the sequence of Aβ17−42, Aβ31−42, Aβ1−30, and Aβ1−16, while the importance of hydrophobic interaction decreases. The hydrogen bonding between EGCG and Aβ42 mainly happens in Aβ1−16, the random-coil region having 13 hydrophilic amino acid residues (see Table S2), while the hydrophobic interaction mainly happens in Aβ17−42, the β-sheet-forming region containing 84% of the hydrophobic residues of Aβ42. It can also be seen that the increasing order of ΔH_s and $T\Delta S_s$ values for Aβ42 and Aβ_f's are the same as the association extents of them (Table S1), indicating that hydrophobic interaction is essential for the self-association of Aβ42.

Binding Stoichiometry. The binding stoichiometry (N) for the interactions between EGCG and Aβ_f's at different salt concentrations can also be derived from the model fitting in Figures S3−S5 in the Supporting Information and the results are summarized in Figure 4. Statistical analysis shows that the

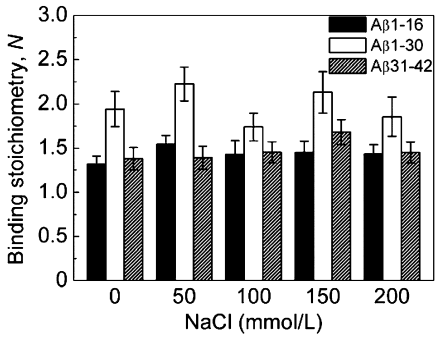

Figure 4. Binding stoichiometry N for the interactions between EGCG and Aβ1−16, Aβ1−30, and Aβ31−42 at different salt concentrations. The N values were calculated by fitting the single-site binding model to the experimental data.

mean values of N are 1.98 ± 0.2 for Aβ1−16, 1.43 ± 0.08 for Aβ1−30, and 1.47 ± 0.21 for Aβ31−42, implying that N is essentially independent of salt concentration for the Aβ_f's.

Figure 5 shows the N values for the Aβ_f's as a function of q derived from the model fitting in Figures S6−S8 in the Supporting Information. It is observed that the changing trends of N are similar to those of ΔH and $T\Delta S$ discussed above. Namely, N is linearly proportional to q at q < sq as depicted by eqs 13−15.

$$\text{A}\beta1{-}16: \quad N = 6.18q \quad (0 < q < 1.63)\ (R^2 = 0.9959) \quad (13)$$

$$\text{A}\beta1{-}30: \quad N = 7.11q \quad (0 < q < 1.55)\ (R^2 = 0.9930) \quad (14)$$

$$\text{A}\beta31{-}42: \quad N = 4.18q \quad (0 < q < 2.52)\ (R^2 = 0.9813) \quad (15)$$

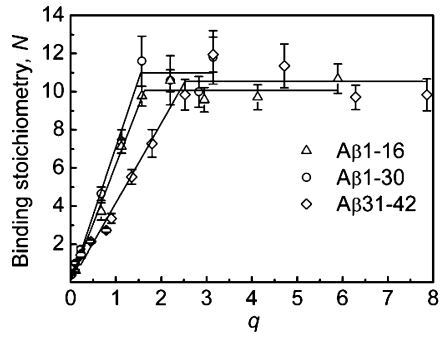

Figure 5. Binding stoichiometry N for the interactions between EGCG and Aβ1−16, Aβ1−30, and Aβ31−42 as a function of EGCG concentration. The N values were calculated by fitting the single-site binding model to the experimental data. The solid lines are calculated from eqs 13−15.

At $q \geq$ sq, N becomes almost unchanged and is denoted as a saturated binding stoichiometry (N_s). The mean values of N_s calculated from Figure 5 are listed in Table 1. The result is similar to that of Aβ42 reported earlier (see Figure 2 therein),[17] and the N_s for which is also given in Table 1.

Similar to eq 11, it is observed from the table that the N_s values for Aβ42, Aβ1−30, and Aβ31−42 follow eq 16:

$$N_s(\text{A}\beta42) = N_s(\text{A}\beta1{-}30) + N_s(\text{A}\beta31{-}42) \quad (16)$$

It can be seen that there is no coefficient 2 in the right-hand side of eq 16 as compared to eqs 11 and 12. This difference comes from the different physical meanings of N_s and Y_s. N_s represents the maximal number of EGCG molecules that can bind to one Aβ42 molecule or its fragments, and therefore it can be directly summed up. It is obvious that eq 16 holds well since the difference between the calculated and measured N_s values for Aβ42 is less than 5% (Table 1). This is another piece of evidence that the oligomeric states of Aβ42 and Aβ_f's do not significantly affect the ITC results for the binding of EGCG to these peptides.

Accordingly, it can be deduced that the N_s value for Aβ42 is the sum of those for Aβ1−16 and Aβ17−42 as depicted by eq 17.

$$N_s(\text{A}\beta42) = N_s(\text{A}\beta1{-}16) + N_s(\text{A}\beta17{-}42) \quad (17)$$

Thus, N_s(Aβ17−42) can be calculated from the equation with the N_s values for Aβ42 and Aβ1−16, and the result is also given in Table 1.

Because Aβ1−16 has four more amino acid residues than Aβ31−42, the N(Aβ1−16) value is higher than N(Aβ31−42) at low EGCG concentrations (q < 1.5) (see Figure 5). However, when the peptides become saturated by EGCG (q > 1.5), the N_s(Aβ1−16) and N_s(Aβ31−42) values (see Figure 5 and Table 1) are similar to each other. It is considered that this phenomenon mainly stems from the different properties of hydrogen bonding and hydrophobic interaction. As discussed in the section on Effect of q, the formation of hydrogen bonds needs specific steric orientations of the donor and acceptor, while hydrophobic interaction has no specificity and directivity. Due to the increased steric hindrance, the relative importance of hydrogen bonding gradually decreases while that of hydrophobic interaction gradually increases with the increase of EGCG concentration. Since the interaction between Aβ1−16 and EGCG is mainly hydrogen bonding, it becomes saturated by EGCG at a smaller q value (about 1.5, see Figure

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5). As for Aβ31−42, its interaction with EGCG is mainly hydrophobic, so it becomes saturated by EGCG at a larger q value (about 2.2, see Figure 5). Therefore, the opposite effects of EGCG concentration (i.e., q value) on hydrogen bonding and hydrophobic interaction result in the similar N_s(Aβ1−16) and N_s(Aβ31−42) values.

It can also be observed from Table 1 that, although Aβ42 has 3.5 times the amino acid residues as Aβ31−42, the N_s(Aβ42) value is only about 2 times the N_s(Aβ31−42) value. A similar phenomenon was observed for Aβ1−30 and Aβ31−42, which shows similar N_s values despite over 2-fold difference in peptide size. This result is considered mainly due to the steric hindrance effect resulting from the different conformations of Aβ42, Aβ1−30, and other small Aβ_f's. Although the conformation of Aβ42 in aqueous solution is variable,[28] it does fold in certain secondary structures (including random coil, α-helix, β-sheet, and/or β-turn) rather than adopt a fully extended state.[29] Hence, the steric hindrance effects arising from the folded conformations of Aβ42 reduce the EGCG-accessible surface on Aβ42, leading to the relatively small N_s(Aβ42) value in comparison to its large peptide size. Moreover, it has been reported that Aβ13−22 monomers exist as β-strands and other conformations in solution,[30] and Aβ1−28 forms β-sheet in solution.[31] Since Aβ1−30 contains Aβ13−22 and Aβ1−28, it is considered that Aβ1−30 also adopts similar secondary structures in solution, which, similar to that of Aβ42, leads to smaller EGCG-accessible surface and then smaller value of N_s(Aβ1−30) in comparison to its large peptide size. By contrast, Aβ1−16 was reported to be disordered in the solution.[10] As for Aβ31−42, since the time in which half-maximal β-sheet conformation formed is 36 h in phosphorus buffer (pH 7.4),[25] it is expected that the peptide mainly exists in unfolded state in solution within the 5 h ITC experiment. Therefore, the extended conformations of Aβ31−42 and Aβ1−16 have little steric hindrance effect on the binding stoichiometry, leading to their N_s values comparable to that of Aβ1−30 (Table 1). In summary, the steric hindrance effects increase with the increase of residue numbers, leading to the reduction of N_s values in the same way. Interestingly, because eq 16 holds, it is likely that the steric hindrance effects for Aβ42 and Aβ1−30 are similar.

Enthalpy−Entropy Compensation. To analyze the phenomenon of enthalpy−entropy compensation (EEC) in the binding of EGCG to Aβ42 and its fragments, the ΔH and $T\Delta S$ data displayed in Tables 2 and 4 in ref 17 and Figures 2 and 3 in this work are compared in Figure 6. It is seen that a straight line described by eq 18 can well express the plot:

$$T\Delta S = \alpha\Delta H + T\Delta S_0 = 0.97\Delta H + 30.0 \quad (R^2 = 0.9709) \tag{18}$$

where α and $T\Delta S_0$ are the slope and intercept of the straight line, respectively. In the equation, the value of α is 0.97. It means that 97% of the favorably negative ΔH is offset by the unfavorably negative $T\Delta S$ or the favorably positive $T\Delta S$ has to be used to compensate 97% of the unfavorably positive ΔH. For this reason, it is considered that despite the intensities of hydrogen bonding and hydrophobic interaction being different for the different regions of Aβ42 and varying with salt concentration, the ΔG values are only slightly affected by these factors due to the large EEC. Similar phenomenon was observed in the study of protein−ligand interactions,[32] in which the authors stated that in many interactions between protein and small molecules the favorable enthalpy or entropy changes are disappointingly attenuated by EEC.

The term $T\Delta S_0$ in eq 18 is independent of ΔH. It is an index of the quantitative measurement of dehydration during the binding, which stands for the dehydration of proteins and ligands during the binding process.[33] Herein, the positive value of $T\Delta S_0$ is mainly due to the dehydration accompanied by the binding of EGCG to Aβ_f, i.e., hydrophobic interaction.

Further Discussion. Harvey et al.[34] have reported that EGCG inhibits Aβ-evoked fibril formation and neuronal cell death. Ehrnhoefer et al.[16] have found that EGCG redirects Aβ into unstructured, off-pathway oligomers and the interactions between EGCG and Aβ are mainly hydrogen bonding and hydrophobic interaction. Recently, Liu et al.[35] found by molecular simulations that EGCG directly interacts with Aβ and expels water from the surface of the peptide, which is essential for its inhibition effects. They also reported that both polar and nonpolar interactions are involved in the binding. Our thermodynamic studies conducted previously[17] and herein have further supported the above findings that both hydrogen bonding and hydrophobic interaction (i.e., polar and nonpolar interactions) are involved in the binding of EGCG to Aβ. More specifically, this research has found that hydrogen bonding mainly happens in Aβ1−16 while hydrophobic interaction mainly happens in Aβ17−42. Moreover, the results show that, although ΔH and $T\Delta S$ change with solution environment, the ΔG values for EGCG binding to Aβ42 and Aβ_f's are essentially invariable. This result indicates that the hydrogen bonding and hydrophobic interaction change oppositely with the change of solution environment and compensate each other. Namely, the delicate balance of hydrogen bonding and hydrophobic interaction ensures the binding of EGCG to Aβ over a broad region of solution conditions.

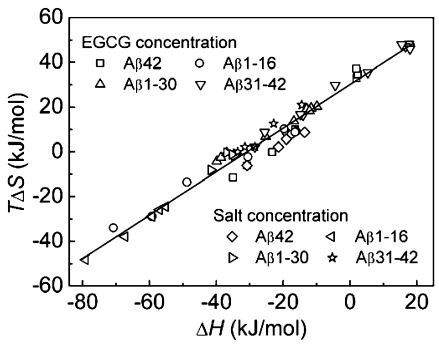

Figure 6. ΔH vs TΔS plot for the binding of EGCG to Aβ42, Aβ1−16, Aβ1−30, and Aβ31−42 at different EGCG and salt concentrations. The solid line is calculated from eq 18.

4. CONCLUSIONS

To probe the molecular interactions between different regions of Aβ42 and EGCG, three fragments of Aβ42, i.e., Aβ1−16, Aβ1−30, and Aβ31−42, were synthesized, and the thermodynamic parameters (N, ΔH, ΔS, and ΔG) between each of the fragments and EGCG were determined by ITC. The results indicated that, although both hydrogen bonding and hydrophobic interaction are involved in the interactions between Aβ42 and EGCG, they mainly act at different regions of Aβ42. Importantly, hydrogen bonding mainly happens in Aβ1−16 (rich in hydrophilic residues) while hydrophobic interaction mainly happens in Aβ17−42 (rich in hydrophobic residues).

The results also indicate that, when Aβ42 and its fragments are saturated by EGCG, the thermodynamic parameters for them have linear relationships. The N_s for Aβ42 is the sum of the N_s values for Aβ1−30 and Aβ31−42, while ΔH_s, ΔS_s, and ΔG_s for Aβ42 are half the sum of the values for Aβ1−30 and Aβ31−42. It is thus deduced from the results that there are no specific interactions and binding sites in the Aβ42 and EGCG binding. The saturated q values slightly decrease with the increase of the residue number of Aβ_f's. There is a significant EEC in the binding of EGCG to Aβ42 and its fragments, which results in insignificant change of ΔG with the change of the solution environment. The research is expected to facilitate the fundamental research on the inhibition of Aβ42 aggregation.

■ ASSOCIATED CONTENT

Supporting Information

Additional information (Tables S1 and S2, Figures S1 to S8) are provided. Table S1 shows the SEC analyses for Aβ42 and Aβ_f's in the absence and presence of EGCG at different incubation times derived from Figure S1. Table S2 lists the numbers of hydrophilic and hydrophobic amino acid residues of Aβ42 and Aβ_f's. Figure S1 shows the SEC results for Aβ42 and Aβ_f's in the absence and presence of EGCG at different incubation times. Figure S2 shows the ITC results for the self-association of Aβ_f's. Figures S3−S5 show the ITC results of effects of salt concentration on the integrated enthalpy of the binding of EGCG to Aβ1−16, Aβ1−30, and Aβ31−42, respectively. Figures S6−S8 show the ITC results of effects of EGCG concentration on the integrated enthalpy of the binding of EGCG to Aβ1−16, Aβ1−30, and Aβ31−42, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Goedert, M.; Spillantini, M. G. *Science* **2006**, *314*, 777.
(2) Kang, J.; Lemaire, H.-G.; Unterbeck, A.; Salbaum, J. M.; Masters, C. L.; Grzeschik, K.-H.; Multhaup, G.; Beyreuther, K.; Muller-Hill, B. *Nature* **1987**, *325*, 733.
(3) Harper, J. D.; Lansbury, P. T., Jr. *Annu. Rev. Biochem.* **1997**, *66*, 385.
(4) Shen, L.; Ji, H.-F.; Zhang, H.-Y. *J. Phys. Chem. B* **2008**, *112*, 3164.
(5) Yang, M.; Teplow, D. B. *J. Mol. Biol.* **2008**, *384*, 450.
(6) Westermark, P.; Benson, M. D.; Buxbaum, J. N.; Cohen, A. S.; Frangione, B.; Ikeda, S.; Masters, C. L.; Merlini, G.; Saraiva, M. J.; Sipe, J. D. *Amyloid* **2005**, *12*, 1.
(7) Triguero, L.; Singh, R.; Prabhakar, R. *J. Phys. Chem. B* **2008**, *112*, 2159.
(8) Zheng, J.; Jang, H.; Ma, B.; Nussinov, R. *J. Phys. Chem. B* **2008**, *112*, 6856.
(9) Zirah, S.; Kozin, S. A.; Mazur, A. K.; Blond, A.; Cheminant, M.; Segalas-Milazzo, I.; Debey, P.; Rebuffat, S. *J. Biol. Chem.* **2006**, *281*, 2151.
(10) Luhrs, T.; Ritter, C.; Adrian, M.; Riek-Loher, D.; Bohrmann, B.; Doeli, H.; Schubert, D.; Riek, R. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17342.
(11) Masman, M. F.; Eisel, U. L.; Csizmadia, I. G.; Penke, B.; Enriz, R. D.; Marrink, S. J.; Luiten, P. G. *J. Phys. Chem. B* **2009**, *113*, 11710.
(12) Liu, R.; Barkhordarian, H.; Emadi, S.; Park, C. B.; Sierks, M. R. *Neurobiol. Dis.* **2005**, *20*, 74.
(13) Li, Q.; Gordon, M.; Tan, J.; Morgan, D. *Exp. Neurol.* **2006**, *198*, 576.
(14) Rezai-Zadeh, K.; Arendash, G. W.; Hou, H. Y.; Fernandez, F.; Jensen, M.; Runfeldt, M.; Shytle, R. D.; Tan, J. *Brain Res.* **2008**, *1214*, 177.
(15) Rezai-Zadeh, K.; Shytle, D.; Arendash, G.; Sun, N.; Hou, H.; Zeng, J.; Mori, T.; Morgan, D.; Tan, J. *Cell Transplant* **2007**, *16*, 342.
(16) Ehrnhoefer, D. E.; Bieschke, J.; Boeddrich, A.; Herbst, M.; Masino, L.; Lurz, R.; Engemann, S.; Pastore, A.; Wanker, E. E. *Nat. Struct. Mol. Biol.* **2008**, *15*, 558.
(17) Wang, S. H.; Liu, F. F.; Dong, X. Y.; Sun, Y. *J. Phys. Chem. B* **2010**, *114*, 11576.
(18) Stine, W. B., Jr.; Dahlgren, K. N.; Krafft, G. A.; LaDu, M. J. *J. Biol. Chem.* **2003**, *278*, 11612.
(19) Lin, M. S.; Chiu, H. M.; Fan, F. J.; Tsai, H. T.; Wang, S. S.; Chang, Y.; Chen, W. Y. *Colloids Surf. B* **2007**, *58*, 231.
(20) Liu, R.; Barkhordarian, H.; Emadi, S.; Park, C. B.; Sierks, M. R. *Neurobiol. Dis.* **2005**, *20*, 74.
(21) Lemkul, J. A.; Bevan, D. R. *Biochemistry* **2010**, *49*, 3935.
(22) Ross, P. D.; Subramanian, S. *Biochemistry* **1981**, *20*, 3096.
(23) Sturtevant, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2236.
(24) Bitan, G.; Kirkitadze, M. D.; Lomakin, A.; Vollers, S. S.; Benedek, G. B.; Teplow, D. B. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 330.
(25) Li, H.; Monien, B. H.; Fradinger, E. A.; Urbanc, B.; Bitan, G. *Biochemistry* **2010**, *49*, 1259.
(26) Lin, M. S.; Chen, L. Y.; Tsai, H. T.; Wang, S. S.; Chang, Y.; Higuchi, A.; Chen, W. Y. *Langmuir* **2008**, *24*, 5802.
(27) Banerjee, A.; Maji, S. K.; Drew, M. G. B.; Haldar, D.; Das, A. K. *Tetrahedron* **2004**, *60*, 5935.
(28) Baumann, M. H.; Kallijarvi, J.; Haltia, M. *Biochemistry* **2001**, *40*, 10032.
(29) Urbanc, B.; Cruz, L.; Ding, F.; Sammond, D.; Khare, S.; Buldyrev, S. V.; Stanley, H. E.; Dokholyan, N. V. *Biophys. J.* **2004**, *87*, 2310.
(30) Mothana, B.; Roy, S.; Rauk, A. *Arkivoc* **2009**, 116.
(31) Fraser, P. E.; Nguyen, J. T.; Surewicz, W. K.; Kirschner, D. A. *Biophys. J.* **1991**, *60*, 1190.
(32) DeLorbe, J. E.; Clements, J. H.; Teresk, M. G.; Benfield, A. P.; Plake, H. R.; Millspaugh, L. E.; Martin, S. F. *J. Am. Chem. Soc.* **2009**, *131*, 16758.
(33) Leung, D. H.; Bergman, R. G.; Raymond, K. N. *J. Am. Chem. Soc.* **2008**, *130*, 2798.
(34) Harvey, B. S.; Musgrave, I. F.; Ohlsson, K. S.; Fransson, A.; Smid, S. D. *Food Chem.* **2011**, *129*, 1729.
(35) Liu, F. F.; Dong, X. Y.; He, L.; Middelberg, A. P.; Sun, Y. *J. Phys. Chem. B* **2011**, *115*, 11879.