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Capping Amyloid β-Sheets of the Tau-Amyloid Structure VQIVYK with Hexapeptides Designed To Arrest Growth. An ONIOM and Density Functional Theory Study

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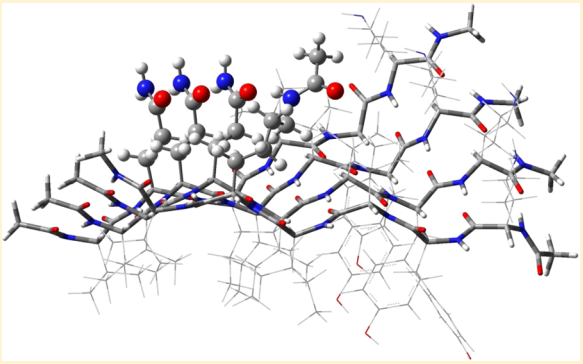
Supporting Information

ABSTRACT: We present ONIOM calculations using density functional theory (DFT) as the high and AM1 as the medium level that explore the abilities of different hexapeptide sequences to terminate the growth of a model for the tau-amyloid implicated in Alzheimer's disease. We delineate and explore several design principles (H-bonding in the side chains, using antiparallel interactions on the growing edge of a parallel sheet, using all-D residues to form rippled interactions at the edge of the sheet, and replacing the H-bond donor N−H's that inhibit further growth) that can be used individually and in combination to design such peptides that will have a greater affinity for binding to the parallel β-sheet of acetyl-VQIVYK-$NHCH_3$ than the natural sequence and will prevent another strand from binding to the sheet, thus providing a cap to the growing sheet that arrests further growth. We found peptides in which the Q is replaced by an acetyllysine (aK) residue to be particularly promising candidates, particularly if the reverse sequence (KYVIaKV) is used to form an antiparallel interaction with the sheet.



INTRODUCTION

Amyloid fibrils can be associated with several diseases, either as a cause or a symptom. In Alzheimer's disease, two different kinds of misfolding/aggregation occur: (1) plaques of aggregated β-sheet-like proteins form, and (2) the protein, tau, which normally promotes aggregation of tubulin to form the natural tubular material of neuron fibrils, instead forms other aggregates, which results in neurons' losing their neuron fibrils and, thus, their function.[1]

Amyloid fibrils result from the association of multiple individual peptide units into large clusters. The formation of these fibrils resembles a crystallization in one dimension. Short peptides containing the key sequences of 4−6 residues necessary for the formation of several amyloids have been crystallized and subsequently used to seed amyloid formation from the relevant proteins.[2] We have reported density functional theory (DFT) calculations that show how the glutamine (Q) residues in one of these peptide sequences ^{306}VQIVYK^{311}, essential for amyloid formation from the protein, tau, contribute to the tendency of a small peptide of this sequence to form crystals and the tendency of tau to form amyloids.[3,4] These calculations illustrate the importance of cooperative H-bonding, particularly between the amides contained in the side chains of the Q's, to the formation of stable amyloid structures.

In this paper, we explore methods of interrupting these cooperative interactions by designing peptides that can bind selectively to the growing β-sheet to prevent attachment of further VQIVYK segments, thus terminating the growth of the amyloid structure. To be effective, such peptides must bind more strongly to the growing amyloid sheet than to the native VQIVYK and disrupt continued growth by inhibiting the further binding of native VQIVYK. We evaluate several strategies that lead to design principles that enhance the interaction with the amyloid β-sheet then combine these to develop possible effective methods for disrupting further growth of the β-sheet structure: (1) removing the H-bonding donors on the amides by derivatizing with alkyl or other groups on these positions on either the backbone amido or the side chain of the Q or both and (2) changing Q to acetyllysine (aK), which will form an H-bond to Q that is sterically oriented to prevent the Q of another VQIVYK from continuing the stabilizing H-bonding chain.

We present ONIOM (B3LYP/AM1) calculations on the interaction energies and enthalpies of several hexapeptides (capped with acetyl and $NHCH_3$) with a sheet containing three strands of VQIVYK. We chose the three-stranded sheet as a practical balance between a sheet that is sufficiently large to exhibit H-bond cooperativity between the Q's yet sufficiently

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small to allow the multiple calculations with complete geometrical optimization (often of several different conformations of each species) that this work requires.

Previous experimental studies based upon sheet-forming propensities have suggested that peptides containing proline could inhibit growth of some β-sheets, including the Aβ amyloid that is also associated with Alzheimer's disease.[5−7] We have recently found that P residues when substituted for A's in certain positions of AAAAAA slightly enhance the interaction with the parallel (but not the antiparallel) all-AAAAAA sheet.[8] Additional bonding of another AAAAAA would be prevented by the lack of an H-bond donor on the P residue.

METHODS

We used the ONIOM[9,10] method as programmed in the Gaussian09[11] suite of computer programs. ONIOM divides the system into up to three segments, which can be treated at different levels of calculational complexity. Thus, one can treat the essential part of the system at the high level while the less critical parts of the system might be calculated at the medium or low level. For this study, we used only two levels (high and medium). We treated the backbones of the peptides (equivalent to a corresponding peptide containing only glycines) and the glutamine (Q) residues at the high level, with only the side chains that distinguish the other different residues from that of glycine at the medium level. The high level used hybrid DFT methods at the B3LYP/D95(d,p) level. This method combines Becke's 3-parameter functional[12] with the nonlocal correlation provided by the correlation functional of Lee, Yang, and Parr.[13] In the ONIOM method, there are unsatisfied valences in the high level at the interface between it and the medium level. These valences were satisfied by using the default method of capping them with a hydrogen atom in the direction of the connecting atom in the medium level with a C−H distance of 0.723 886 times the C−C distance. We used the AM1[14] semiempirical molecular orbital method for the ONIOM medium level. We have shown B3LYP to be preferable to several functionals, specifically parametrized to treat dispersion for calculations on peptides.[15]

We optimized all geometries in all internal degrees of freedom and performed vibrational calculations to ensure the geometries are true minima on the potential energy surfaces (PESs) for sheets containing up to five strands and to obtain the vibrational frequencies used to calculate the enthalpies at 298 K. We have found small imaginary frequencies for some of the calculations. We have previously encountered this problem, which appears to be related to the fineness of the grid used in the DFT procedure. As in the past, we have corrected the enthalpies by RT, which would be appropriate for a low frequency.[16]

In a previous study of five 17-amino-acid peptides,[17] we found little difference in relative energies between this procedure and another in which the side chains (in this case, the methyls) were subsequently optimized using DFT, with the (previously optimized) peptide chain held fixed. The current procedure also gave relative energies that agreed well with complete DFT optimizations for a series of five small 3_{10}-helical peptides.[18] We have used this procedure with success for several previous studies of peptide structures,[3,4,16,18−24] and have shown it to compare favorably with other functional/basis set combinations for calculations on the gas phase water dimer.[25]

The counterpoise correction (CP) for basis set superposition error (BSSE) has been applied to all interaction energies and enthalpies using the single point a posteriori procedure[26−29] because optimization of such large structures on a CP-corrected surface[30] would have been too computationally intensive, and the ONIOM and CP-optimization cannot be performed simultaneously using the Gaussian09 program. Balabin has recently emphasized the importance of BSSE correction for biochemical and other calculations.[31,32]

Because the model for these calculations is solid-state (the amyloids are not soluble), no solvation energies have been calculated, and the lysine residues are assumed to be neutral to avoid proximate positive charges.

RESULTS

We investigated several types of peptides to test their affinities for a three stranded VQIVYK parallel β-sheet, which we used as a model for the growing amyloid. Although the amyloid sheet consists of parallel strands, there is no reason to exclude antiparallel interactions of the capping strand with the proximate strand of the amyloid, so we considered both parallel and antiparallel interactions. In addition, we considered both pleated and rippled sheets where appropriate. Rippled sheets were proposed by Pauling and Corey for interactions between enantiomeric peptide strands.[33] Both of these conformations were found for polyglycine sheets because glycine (both from experiment[34] and theory[35]) is enantiomorphic, but only pleated structures were found for (all ^{L}polyalanine) polyalanine sheets.[4] The hexapeptides used can be categorized into several classes (see Table 1): (A) A simple hexaalanine that can interact with the amyloid model in four different combinations at each of the top and bottom positions (see Figure 1 for definitions of top and bottom), parallel and antiparallel combined with pleated and rippled sheets. (B) The reverse sequence of the amyloid hexapeptide, KYVIQV, again binding in both parallel and antiparallel forms and from both top and bottom. C) The hexapeptide (all-D) TLKIVW, one of several recently designed peptides and tested by Eisenberg et al. to disrupt the amyloid by impeding what they characterize as the "dry zippers" that they believe provide the favorable interactions between the β-sheets of the amyloids.[36] For this reason, we explored several all-D peptides that can form rippled sheets (including all-D VQIVYK). (D) Various VQIVYKs in which the Q's have one or two methyl or methoxy substituents in place of the N−H's on the side chain, which can bind only to the top of the amyloid model. All-D peptides have the additional advantage of increased stability to normal peptide degradation. (E) Two forms of VQIVYKs in which every second backbone N−H is methylated or otherwise substituted (in one, the first, third' and fifth; in the other, the second, fourth, and sixth). (F) Modifications of VQIVYK and KYVIQV, in which Q is replaced by acetyllysine (aK). Of those that have one or more

Table 1. Six Categories of Hexapeptides Considered As Caps for for a Three-Stranded VQIVYK Parallel β-Sheet

A	AAAAAA a useful standard
B	KYVIQV the reverse sequence of VQIVYK
C	(all-D) TLKIVW a peptide reported to inhibit amyloid growth
D	VQIVYKs where the Q have one or more N−H's derivatized
E	VQIVYKs where the backbone N−H's are derivatized
F	modifications of VQIVYK or KYVIQV with Q replaced by aK

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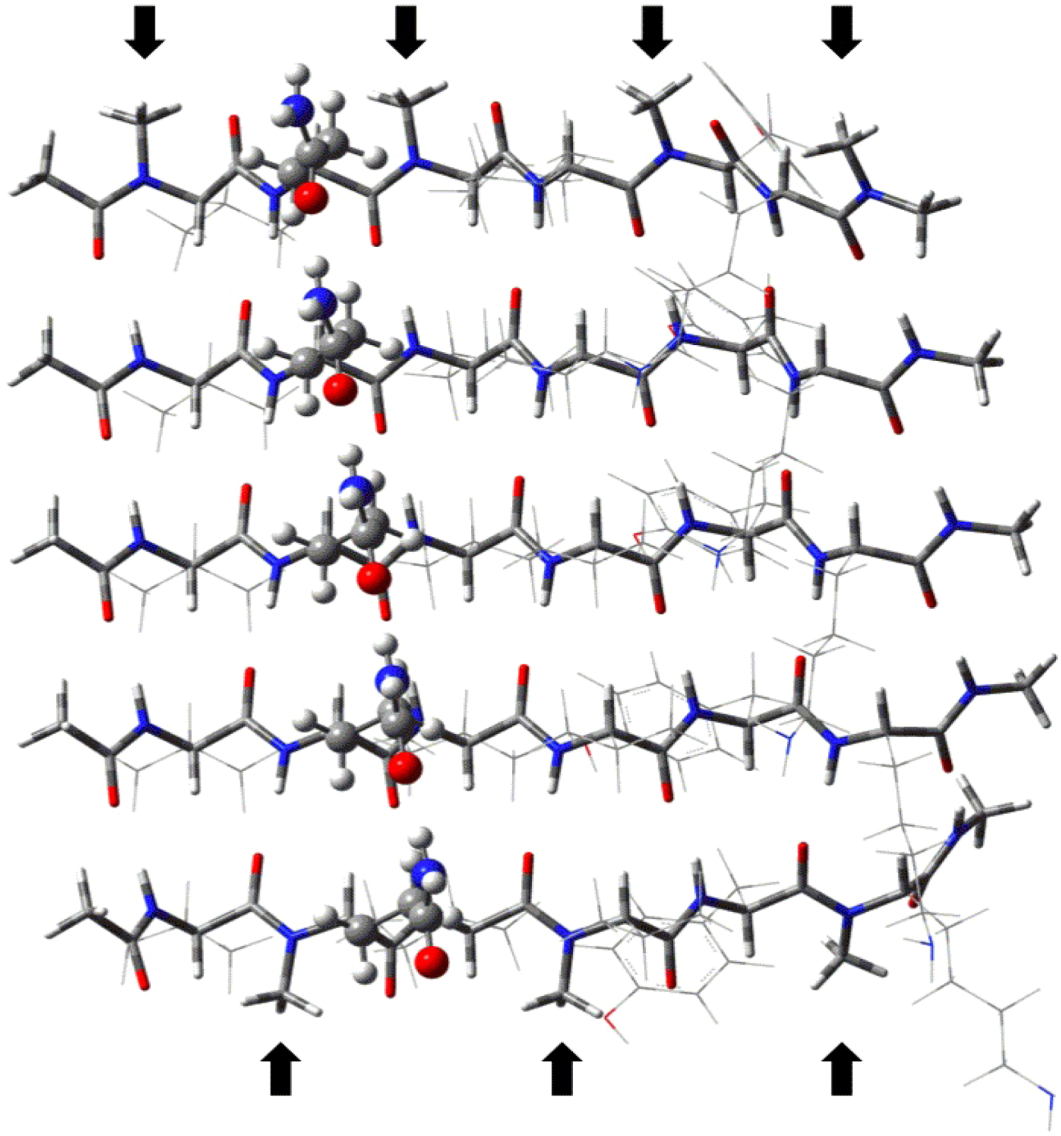

Figure 1. Structure of a three-stranded β-sheet of acetyl-VQIVYK−$NHCH_3$ with capping strands of two modified peptides that are appropriately methylated to prevent further growth of the β-sheet form at the top and bottom. The arrows indicate the methyls. The peptide backbones are depicted as tubes; the Q-side chains, as ball and stick; and the other side chains, as wire-frame. Note that the methylated peptides are methylated in different positions.

N−H's replaced by N−CH_3, some can only bind to the top; others, the bottom of the amyloid model. We compared these with the normal VQIVYK peptide attached in the normal parallel-pleated manner and in three alternate conformations (parallel-rippled and antiparallel-pleated and -rippled). We considered 33 different peptide interactions at the top and 28 at the bottom, in total, in addition to the "normal" VQIVYK sequence.

In addition to determining the ΔH_{int}'s, we decompose the energetic interactions of the capping peptide with the sheets into the H-bonding and distortion energies, as we have recently done in our studies of polyglycine[35] and polyalanine[4] β-sheets. We define the H-bonding energies (ΔE_{HB}) as the interactions between the capping strand and the sheet frozen in the geometries of the optimized capped sheet, and the distortion energies (ΔE_{dist}), as the energies required to change the geometry of the relaxed extended capping strand and the relaxed three-stranded sheet to those assumed in the optimized capped sheet. In the cases that we treat here, the capping peptide can be different from the others, so the optimized geometry of this peptide (hence, its distortion energy) might be substantially different for each case. The structures of the extended strands contain slightly cooperative C_5-*intra*strand cyclic H-bonds, as previously reported.[37−41] The interaction energy of the capping peptide with the sheet will be a combination of ΔE_{HB} and ΔE_{dist}, whose sum equals the energy of interaction with respect to the optimized cap and sheet (ΔE_{int}) with respect to the optimized capping strand. We also include the enthalpies of interaction (ΔH_{int}) of the optimized capping peptide with the optimized sheets in the tables. Since ΔE_{HB} and ΔE_{dist}, involve differences in energies of species that are not minima on the potential energy surfaces, frequencies and ΔH_{HB} and ΔH_{dist}'s would not be meaningful.

Because ΔE_{int} and ΔH_{int} depend upon both ΔE_{HB} and ΔE_{dist}, a favorable ΔH_{int} could be due to a small ΔE_{dist}, a large negative ΔE_{HB}, or both. Because each capping peptide has a different optimized structure, which may change in a different way upon substitution, we combine two criteria to ascertain which

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Table 2. Relevant Energies and Enthalpies for the Most Effective Capping Peptides[a]

capping peptides			ΔH_{int}	ΔE_{HB}	ΔE_{dist}		ΔE_{int}
					cap	sheet	
			Top				
KYVIacKV MMMM	S2	AP	−38.5	−54.8	5.9	7.2	−41.7
VQIVYK Q_{2M} 1c7	S5	P	−32.8	−53.3	10.9	5.3	−37.2
KYVIacKV	S7	AP	−32.3	−55.3	13.0	6.6	−35.7
KYVIQV	S4	AP	−30.4	−55.8	16.1	5.6	−34.0
AQAAAA MMMM	S13	P	−29.6	−54.1	15.2	5.3	−33.6
VQIVYK HMHH 2	S15	P	−29.5	−53.5	14.8	5.3	−33.3
VQIVYK Q_M trans 1c7	S19	P	−28.5	−53.1	15.2	5.3	−32.6
			Natural Amyloid				
VQIVYK		P	−28.1	−53.0	15.6	5.3	−32.0
			Bottom				
KYVIacKV MMM	S36	AP	−37.5	−56.2	10.4	5.4	−40.4
VQIVYK HMM	S39	P	−33.7	−53.2	9.9	5.8	−37.6
VQIVYK HMH	S43	P	−32.2	−53.6	12.0	5.5	−36.1
KYVIacKV	S44	AP	−31.7	−55.0	14.7	5.1	−35.2
KYVIQV	S40	AP	−29.2	−53.8	15.7	5.0	−33.1
			Natural Amyloid				
VQIVYK		P	−28.1	−53.2	16.1	5.1	−32.0

[a]Those with more favorable ΔH_{int} and ΔE_{HB} than the natural amyloid sequence (in kcal/mol). The following abbreviations are used: AP (antiparallel), P (parallel); Q_M, Q_{2M} (one or 2 methyls in the Q side chain); sequences of Ms and H's indicate methylated backbone N−H's starting from the N-terminus. All structures form pleated sheets. The designation of the capping peptide is followed by the figure number in the SI that corresponds to the capped sheet.

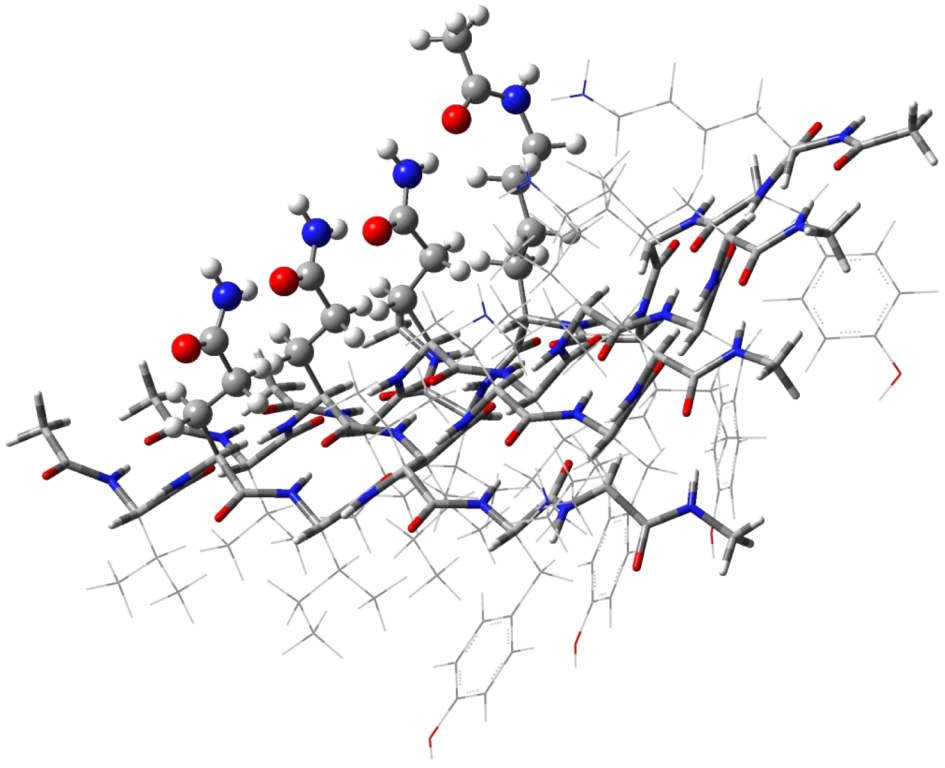

Figure 2. Structure of a three-stranded β-sheet of acetyl-VQIVYK-$NHCH_3$ capped with KYVIaKV (AP) at the top. The peptide backbones are depicted as tubes; the Q and aK side chains, as ball and stick; and the other side chains, as wire-frame.

capping peptides bind preferentially to the three-stranded sheet: ΔH_{int} and ΔE_{HB}. If both of these indicate a preference, the preferences indicated by our calculations become more secure. Table 2 contains those peptide caps that meet both criteria. This table provides data for seven peptides that bind to the top and five that bind to the bottom better than the natural amyloid sequence VQIVYK. Most contain at least one N−H that is methylated to prevent further H-bonding. These can form only H-bonds, to either the top or the bottom. Two of these peptides, AP KYVIQV and AP KYVIaKV, can form H-bonds to either the top or bottom of the sheet. The former should not be useful to block continued amyloid formation because a normal VQIVYK could simply bind to it to continue the parallel sheet of VQIVYKs with one antiparallel

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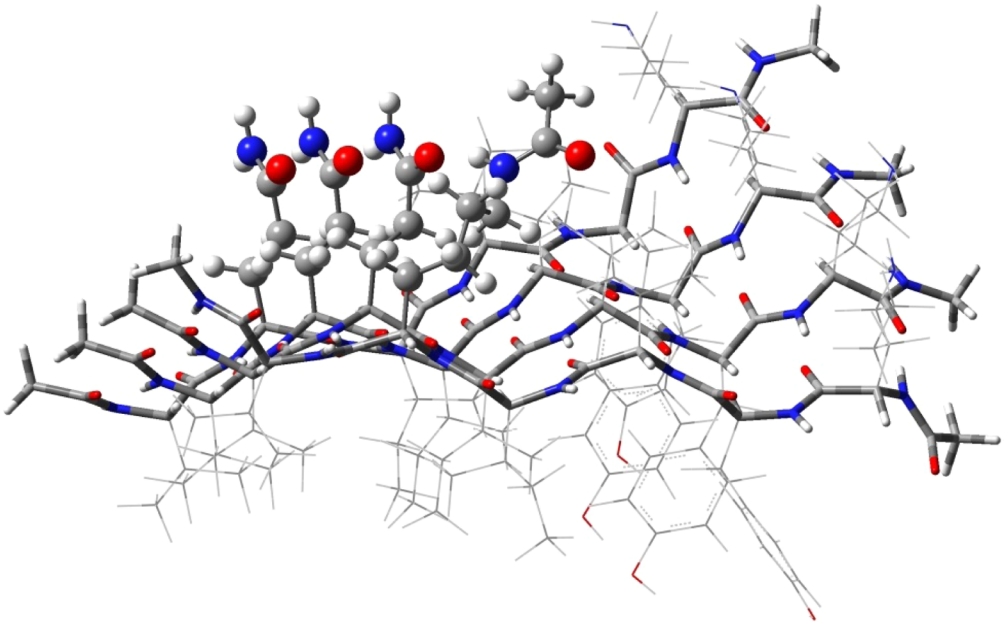

Figure 3. Structure of a three-stranded β-sheet of acetyl-VQIVYK-NHCH$_3$ capped with KYVIaKV (AP) at the bottom. The peptide backbones are depicted as tubes; the Q and aK side chains, as ball and stick; and the other side chains, as wire-frame.

interruption. However, AP KYVIaKV assumes a geometry that would prevent the continued cooperative H-bonding chain of Q side chains essential to the stability of the amyloid. From the geometry of the capped sheet in Figures 2 and 3, one sees the inaccessibility of the H-bonding C═O or N−H of the aK amide group to further H-bonding from the Q's. The peptides in Table 2 with the strongest interactions at both the top and bottom interact in the AP fashion. Our previous reports have shown that AP interactions are more energetically favorable than P interactions for β-sheets of both hexaalanines[4] and hexaglycines.[35]

Table 3 contains capping peptides with ΔH_{int}'s that are more favorable than that for VQIVYK but ΔE_{HB}'s that are less favorable. For these, the lower ΔE_{dist}'s compensate for their unfavorable ΔE_{HB}'s. This table contains several peptides that form rippled sheets. These contain all-D residues, as rippled interactions occur between strands containing residues of opposite chirality. Table 4 lists those peptides for which both ΔH_{int} and ΔE_{HB} are less favorable than those of VQIVYK. As in previous optimizations of alanine β-sheets, we have often found C_7 cyclic H-bonding near the termini of the sheets.[4,16] We have indicated structures containing a C_7 interaction in the relevant entries of the tables.

DISCUSSION

The principal goal of this work is to provide a rational approach to arresting the growth of tau amyloid fibrils and to extend the principle to amyloid formation in general. As mentioned above, we see this as a problem that involves primarily one-dimensional crystal growth. We designed the capping peptides that we have considered for two purposes: (1) to find peptides that would bind more tightly than VQIVYK and (2) to test the principles that could be used for such a design. The peptides with the highest affinities for the VQIVYK sheet demonstrate these principles separately and in combination with each other.

We begin by discussing these principles:

(A) Cooperative H-bonding between the Q side chains provides an important stabilization to parallel VQIVYK β-sheets. We have previously shown this in two earlier reports on these sheets[3,4] and another on α-helices where every fourth residue is Q and the others are all A.[23] Several experimental reports support this view. A capped QQQ tripeptide shows C═O stretches of the side chains consistent with H-bonding.[42] Several short peptide sequences that are essential to amyloids and have been crystallized involve Q's whose side chains H-bond to each other (as well as, N's which also do this).[2] Long sequences of Q's in peptides lead to amyloid-like structures.[43,44] Thus, we consider H-bonding to Q by a peptide side chain to be an important design principle. We have considered peptides in which the Q is replaced by aK because this residue can provide a similar cooperative H-bond.

(B) Intermolecular antiparallel β-sheets are more stable than their parallel counterparts for simple structures such as acetylA_6NH_2 and acetylG_6NH_2, according to our previous calculations.[4,35] Hence, we would expect AP interaction between the sheet and the cap to probably enhance the affinity of the cap for the sheet.

(C) Rippled sheets can form only from alternating strands containing residues of opposite chiralities. Because we deal only with capping peptides, the rippled interaction would occur only between the cap and the proximate strand. One of the polymorphs of polyglycine I (which is actually two polymporphs) forms rippled sheets.[34] Glycine is the only natural residue that can do this because it is enantiomorphic. Our calculations on polyglycine suggest the rippled and pleated sheets have similar energies but that the vibrational contribution to the enthalpy is lower for the rippled sheet.[35] Because all-D TLKIVW has been reported to be useful for arresting amyloid growth,[36] we considered rippled interactions to see if they provided better binding.

(D) Derivitizing one or more N−H's to methyls or other groups (here, methoxyl) will prevent H-bonding by the donor (as already noted), but it can also affect (1) the

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Table 3. Relevant Energies and Enthalpies for the Possibly Effective Capping Peptides[a]

capping peptides			ΔH_{int}	ΔE_{HB}	ΔE_{dist} cap	ΔE_{dist} sheet	ΔE_{int}
			Top				
VacKIVYK MMMM	S1	P	−38.9	−50.1	5.0	3.1	−42.0
TLKIVW 1c7	S3	AP	−34.0	−48.1	4.7	5.4	−38.0
R TLKIVW	S6	AP	−33.4	−46.1	5.0	4.4	−36.6
VQIVYK MMMM	S9	P	−29.6	−51.3	11.8	4.9	−34.5
VQIVYK Q_{2OM}	S8	P	−30.9	−51.7	11.3	5.4	−35.1
R TLKIVW1c7	S12	P	−30.0	−46.7	6.2	6.8	−33.6
Pl VQIVYK HMMM	S11	P	−29.9	−52.2	13.4	5.1	−33.7
VacKIVYK	S10	P	−29.8	−52.2	12.6	5.9	−33.7
Pl AAAAAA	S14	AP	−29.8	−43.6	6.3	4.0	−33.4
R AAAAAA 1c7	S16	AP	−29.7	−43.1	6.0	3.8	−33.3
VQIVYK HHMH	S17	P	−28.8	−53.0	14.9	5.3	−32.8
VQIVYK MHHH	S20	P	−28.8	−52.1	14.6	5.0	−32.5
TLKIVW	S24	P	−28.3	−45.0	7.1	6.1	−31.8
VQIVYK MHHM	S21	P	−28.3	−51.4	14.4	5.0	−32.0
amyloid VQIVYK		P	−28.1	−53.0	15.6	5.3	−32.0
			Bottom				
VQIVYK MMM	S35	P	−37.1	−52.6	6.2	5.5	−40.9
VacKIVYK MMM	S37	P	−36.0	−51.6	6.7	5.4	−39.5
VQIVYK Tbut Tbut Tbut	S38	P	−35.2	−51.0	5.8	6.0	−39.2
R TLKIVW	S41	AP	−33.2	−44.2	3.9	3.7	−36.6
TLKIVW	S42	AP	−32.6	−45.2	4.9	3.9	−36.3
VQIVYK MHH	S45	P	−31.2	−52.5	12.4	5.0	−35.1
VQIVYK FFF	S47	P	−30.5	−51.9	13.2	4.4	−34.4
AQAAAA MMM	S46	P	−30.5	−52.0	12.7	4.7	−34.6
VQIVYK HHM	S49	P	−30.0	−52.6	13.6	5.2	−33.8
TLKIVW	S50	P	−29.9	−43.7	5.8	4.3	−33.6
R AAAAAA	S51	AP	−29.8	−43.1	5.7	4.0	−33.4
VacKIVYK	S48	P	−29.4	−52.7	13.6	5.3	−33.8
AAAAAA	S52	AP	−29.4	−44.9	7.3	4.5	−33.1
R VQIVYK	S55	AP	−28.7	−52.4	14.0	6.2	−32.2
amyloid VQIVYK		P	−28.1	−53.2	16.1	5.1	−32.0

[a]Those with more favorable ΔH_{int} but not ΔE_{HB} than the natural amyloid sequence (in kcal/mol). The following abbreviations are used: AP (antiparallel), P (parallel); Q_M, Q_{2M} (one or 2 methyls in the Q side chain); Q_{OM}, Q_{2OM} (one or two methoxyls on the Q side chain) sequences of Ms and H's indicate methylated backbone N−H's starting from the N-terminus. All structures form pleated sheets except those marked with an "R", which are rippled sheets. The capping peptides of the rippled sheets contain all-D amino acid residues. The designation of the capping peptide is followed by the figure number in the SI that corresponds to the capped sheet.

strengths of the H-bonds to the acceptor (C═O) and (2) the relaxed geometry (thus, the ΔE_{dist}) of the strand.

All of those peptides that bind better than VQIVYK by both the ΔH_{int} and the ΔE_{HB} criteria (those of Table 2) have either a Q or an aK that can H-bond to the chain of H-bonding Q's in the amyloid. On the other hand, all but three of the peptides that have the least favorable interactions (those listed in Table 4) lack the H-bond to the side chain of the Q. Parallel VQIVYK Q_{OM} (both cis trans) and VQIVYK HHHM are the exceptions. When the VQIVYK fragment forms an AP pleated or a P rippled interaction (with all-D residues), the Q on the capping peptide faces the opposite side of the sheet from the others. The three exceptions suggest that the substituent effects of methoxyl on Q and one methyl on the last backbone N−H from the N-terminus weaken the H-bonds to the C═O's of their amide groups.

Interestingly, the methyl substituent effects upon ΔE_{HB} for parallel VQIVYK caps vary between enhancement by 0.5 to reduction of 1.2 kcal/mol per methyl, which suggests them to be minor and difficult to predict. Although we have not examined the substituent effects of fluoro, methoxyl, and tertiary butyl (*t*-bu) in as much detail as those of methyl, they all reduce the magnitude of ΔE_{HB}. All substituents on the backbone N−H's tend to reduce the ΔE_{dist} of the cap, sometimes quite significantly. For example, three methyls on the bottom cap reduce ΔE_{dist} by 9.9, whereas 3 *t*-bu's reduces it by 10.8 kcal/mol. Thus, the effects of substituting the N−H's with other entities does not appear to be primarily electronic. Rather, they sterically induce the strand into a conformation more favorable for binding to the sheet. The only exception to this trend, a trans methoxyl substitution on the Q side chain (rather than the backbone), increases ΔE_{dist} by only 0.2 kcal/mol. We note that the TLKIVW and AAAAAA caps have some of the lowest ΔE_{dist} values.

The ΔH_{int}'s with AAAAAA at both the top and bottom are more favorable than VQIVYK for the AP, but less favorable for the P interaction. The ΔE_{HB}'s are less favorable because of the lack of the H-bond between the side chains, so the lower ΔE_{dist}'s cause the favorable enthalpy, which is consistent with the earlier finding that the AP AAAAAA sheets are more favorable than the P sheets.[4] The rippled APs with the all-D version of this peptide are not significantly different from the

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Table 4. Relevant Energies and Enthalpies for the Least Effective Capping Peptides[a]

capping peptides			ΔH_{int}	ΔE_{HB}	ΔE_{dist}		ΔE_{int}
					cap	sheet	
			Top				
AAAAAA MMMM	S23	P	−27.9	−45.5	8.9	4.6	−31.9
VQIVYK HHHM	S25	P	−27.6	−52.1	15.4	5.2	−31.5
VQIVYK Q_{OM} trans 1c7	S27	P	−27.0	−52.7	16.3	5.3	−31.1
VQIVYK Q_{OM} 1c7	S26	P	−26.9	−52.4	15.8	5.3	−31.2
AAAAAA 2c7	S28	P	−26.8	−45.7	9.6	5.3	−30.9
VQIVYK FFFF	S29	P	−26.8	−48.8	14.1	4.4	−30.3
VQIVYK	S30	AP	−26.6	−48.4	13.3	5.2	−29.9
R VQIVYK 1c7	S32	AP	−26.2	−46.7	13.3	4.3	−29.1
KYVIQV 2c7	S18	P	−25.3	−48.0	13.6	5.3	−29.1
R KYVIQV 2c7	S22	AP	−25.2	−46.2	13.8	4.1	−28.3
R AAAAAA 1c7	S33	P	−24.9	−44.1	9.8	5.5	−28.9
R KYVIQV 2c7	S31	P	−22.4	−46.2	14.3	6.4	−25.5
R VQIVYK	S34	P	−21.1	−45.5	15.0	5.9	−24.5
			Bottom				
R TLKIVW	S57	P	−27.6	−42.6	6.2	5.1	−31.3
VQIVYK	S59	AP	−26.8	−50.4	14.8	5.3	−30.3
AAAAAA MMM	S58	P	−26.8	−44.5	9.5	4.4	−30.6
AAAAAA	S60	P	−25.9	−44.1	9.9	4.2	−30.0
R KYVIQV	S56	AP	−25.5	−45.2	12.7	4.1	−28.4
R KYVIQV	S53	P	−25.2	−49.8	15.0	5.7	−29.1
KYVIQV	S54	P	−25.0	−50.2	16.2	5.0	−28.9
R AAAAAA 1c7	S61	P	−24.8	−43.1	9.2	5.2	−28.7
R VQIVYK	S62	P	−20.2	−44.7	16.0	5.2	−23.6

[a]Those with less favorable ΔH_{int} and ΔE_{HB} than the natural amyloid sequence (in kcal/mol). The following abbreviations are used: AP (antiparallel), P (parallel); Q_{OM}, Q_{2OM} (one or two methoxyls on the Q side chain) sequences of Ms and H's indicate methylated backbone N−H's starting from the N-terminus. All structures form pleated sheets except those marked with an "R", which are rippled sheets. The capping peptides of the rippled sheets contain all-D amino acid residues. The designation of the capping peptide is followed by the figure number in the SI that corresponds to the capped sheet.

pleated interactions. Of the peptides that lack an H-bond to the Q's, TLKIVW has stronger ΔE_{HB}'s than AAAAAA, with a maximum of −48.1 for the AP interaction at the top and an overall average 1.2 kcal/mol greater than AAAAAA for all eight (four each with the normal and all-D residues) possible interactions. The (all-D) TLKIVW reported by Eisenberg[36] has rather favorable ΔH_{int}'s (but not ΔE_{HB}'s) for AP interactions at both top and bottom. These can be found in Table 3 with the other peptide caps that have favorable ΔH_{int}'s but not ΔE_{HB}'s. They derive much of their binding affinity from the relatively low ΔE_{dist}'s for the cap.

Several of the peptides with the most favorable interactions (table 2) combine the H-bonding to the Q's with an AP interaction. Because the AP interaction with VQIVYK cannot accommodate an H-bond between the Q's, we accomplished this by using KYVIQV, the reverse order of the amyloid sequence. This peptide has significantly stronger ΔH_{int}'s and ΔE_{HB}'s for interactions with both the top and bottom. There is no reason to expect these to arrest amyloid growth because a normal VQIVYK should be capable of binding to the other side of this peptide to extend the growth. Nevertheless, we found that substituting an aK for Q resulted in larger ΔH_{ini}'s and ΔE_{HB}'s only slightly smaller. However, because the geometries of the resulting sheets make further H-bonding by VQIVYK extremely unfavorable, whether bound to the top or bottom of the sheet, these might be useful for capping amyloid growth. Replacing one or more of the H-bond acceptor N−H's of the backbone amides or that of the Q with a methyl (or other substituent) would inhibit growth from one side.

As seen from tables 2−4, replacing the N−H's of the backbone and the Q with one or more methyls enhances the ΔH_{int}'s vs the same peptides without the methyls. However, most of this enhancement comes from lower ΔE_{dist}'s, rather than better ΔE_{HB}'s. Thus, like TLKIVW, they derive significant stabilization from their relatively low ΔE_{HB}'s. Replacing the backbone N−H's with methyls on KYVIacKV for binding at the bottom provides the exception. On the other hand, methylating the N−H(s) on the Q-side chain for binding at the top enhances both ΔH_{int} and ΔE_{HB}.

As already noted, many of the best interactions combine the advantages of the H-bond to the Q's with an AP interaction between the cap and the sheet. These interactions can be combined by reversing the order of the residues in the capping peptide (VQIVYK−>KYVIQV) so that the Q's remain adjacent to each other on the interacting strands. Replacing the Q with an aK on the capping peptide does not provide a large change in the interactions. The ΔH_{int}'s become slightly enhanced, and the ΔE_{HB}'s, slightly reduced for the P interactions; the reverse occurs for AP from the bottom, and both are slightly reduced for binding from the bottom. Nevertheless, the AP interactions with KYVIaKV remain quite favorable for interaction at both ends of the sheet (ΔH_{int} by 3.6−4.2, and ΔE_{HB}, by 1.8−2.3 kcal/mol). Solely on the basis of these ΔH_{int}'s, at physiological temperature, these should bind 20−1000 times more favorably than the natural sequence. Except for KYVIaKV, all the promising candidates listed in Table 2 can block the growth of the VQIVYK amyloid from only one direction. Thus, one would need a combination of two of these to effectively stop

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the growth. This renders KYVIaKV the most attractive candidate for a model drug. Adding another VQIVYK to a trimer capped with KYVIaKV at either end (illustrated in Supporting Information Figures S63 and S64) is less favorable than adding VQIVYK to a tetramer by 5.1 (top) and 2.9 (bottom) for ΔH_{int} and 6.2 (top) and 3.9 (bottom) kcal/mol for ΔE_{HB}, which should be sufficient to prevent further growth of the amyloid sheet.

The equilibrium between a growing amyloid and a peptide-based therapeutic agent depend upon the free energy difference between the solid amyloid and the dissolved agent. Furthermore, the kinetic of addition of the agent to the amyloid will depend upon the concentration of the agent in solution. To be effective, both the thermodynamics and the kinetics must be favorable for the process. If one were to design a peptide to be used as a practical anti-Alzheimer's medication based upon the results of these calculations, one would need to control the solubility of the peptide. To be effective, the peptide would have to be sufficiently soluble to diffuse to the growing tau amyloid and leave the solution, but not so soluble that it would energetically prefer the solvated environment to binding at the edge of the amyloid. A recent report that increasing the histidine (H) content of peptides rich in Q reduces the extent of aggregation of these peptides by increasing their solubility[45] illustrates this effect. Another common method of modulating the solubilities of the peptide would be attaching the polyethyleneglycol (PEG), which is not generally toxic. Because the electronic substituent effects are small and those on ΔE_{dist} are generally favorable, one might expect replacing one or more N−H's with some form of PEG would not adversely affect the binding interaction of the capping peptide.

We have not considered the possibility that the K's might be protonated in the amyloid because they would be adjacent to each other and, thus, highly repulsive if completely protonated in a low dielectric medium (the solid amyloid). The possibility exists that the K's might be protonated (or partially so), which would require the presence of counterions (OH^-, for example). The stability of OH^- in a solid would be less than in aqueous solution and depends upon the proximity to the positive charges. At this time, we do not have the means to evaluate this possibility properly; however, we note that as a limitation of this study.

Future work in this area might focus on combining the affinity of the all-D TLKIVW cap developed by Eisenberg[36] with a side chain that can increase the stability of the sheet by H-bonding to the Q side chains. This might be accomplished by substituting Q or aK for L in that structure because L occupies the position in register with the Q in the amyloid that would allow for H-bond cooperativity to the cap.

■ ASSOCIATED CONTENT

Supporting Information

Supporting Information consisting of the full reference for Gaussian 09, figures depicting each of the 62 capped amyloids and the amyloid capped with AP KYVIaKV at both top and bottom with another VQIVYK, and Cartesian coordinates for all structures reported. This information 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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