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Non-steroidal anti-inflammatory drug naproxen destabilizes A β amyloid fibrils: A molecular dynamics investigation

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Abstract

Using implicit solvent model and replica exchange molecular dynamics we examine the propensity of non-steroidal anti-inflammatory drug, naproxen, to interfere with A β fibril growth. We also compare the anti-aggregation propensity of naproxen with that of ibuprofen. Naproxen anti-aggregation effect is influenced by two factors. Similar to ibuprofen, naproxen destabilizes binding of incoming A β peptides to the fibril due to direct competition between the ligands and the peptides for the same binding location on the fibril surface (the edge). However, in contrast to ibuprofen naproxen binding also alters the conformational ensemble of A β monomers by promoting β -structure. The second factor weakens naproxen anti-aggregation effect. These findings appear to explain the experimental observations, according to which naproxen binds to A β fibril with higher affinity than ibuprofen, yet produces weaker anti-aggregation action.

Introduction

Aggregation of polypeptide chains and formation of cytotoxic amyloid fibrils are associated with a number of age-related diseases including Alzheimer's, Parkinson's, type II diabetes, and Creutzfeldt-Jakob disease.^{1,2} Among amyloidogenic sequences are A β peptides, 39 to 42 residue fragments of membrane precursor protein, produced in the course of cellular proteolysis³ (Fig. 1a). Genetic and clinical observations suggest that amyloidogenesis of A β peptides is a seminal event in Alzheimer's disease (AD).⁴ The most abundant A β alloform is 40-mer sequence, A β _{1–40}, which readily forms polymorphic amyloid fibrils depending on preparation conditions.⁵ According to solid-state NMR experiments under agitated conditions A β _{1–40} assembles into a two-fold symmetry fibril structure.⁶ Typical of many amyloid deposits A β peptides in this structure are organized into parallel in-register β -sheets. The individual β -sheets are laminated into four layers stabilized by extensive network of sidechain interactions.

Amyloid assembly involves multiple structural transitions initiated with the oligomerization of monomeric chains and followed by eventual appearance of amyloid fibrils.² Although fibrils reveal cytotoxic properties,⁷ recent experiments point to A β oligomers as primary cytotoxic species in AD.^{8,9} However, irrespective of their cytotoxicity fibrils are the reservoirs of A β monomers, which participate in the equilibrium “recycling” of polypeptides through different aggregated species.^{10–12} Because A β aggregation plays a central role in

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Supporting Information Available: Additional details concerning the model and methods used as well as simulation results are provided in Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AD, its inhibition or delay may represent a viable therapeutic approach. One of the potential anti-aggregation agents is a non-steroidal anti-inflammatory drug (NSAID) naproxen¹³ (Fig. 1b). It has been shown that long-term prophylactic use of naproxen reduces the risk of AD.^{14,15} Analysis of recent large-scale clinical trials revealed that when the patients with preexisting conditions are removed from consideration naproxen reduces the AD risk by 67%.¹³ This finding also suggests that naproxen might have no therapeutic effect in preexisting AD cases.¹⁶ In fact, mice models demonstrated that naproxen cannot reverse existing AD conditions in brain microglia.¹⁷ *In vitro* biophysical studies have implicated binding of naproxen to A β fibrils.¹⁸ Furthermore, upon coincubation naproxen reduces the amount of A β fibrils being formed from fresh A β monomers.¹⁸ Naproxen has also an ability to destabilize, but apparently not to dissociate, existing A β fibrils.¹⁹ At elevated concentrations naproxen was reported to inhibit A β fibril elongation.¹⁹

Despite experimental evidence supporting anti-aggregation effect of naproxen, the underlying molecular mechanisms remain unexplored. It is not clear if naproxen interferes with fibril formation because it competes with A β peptides for binding to A β fibrils. Furthermore, it is not known if naproxen changes the secondary structure in A β monomers, thus making them more or less amyloidogenic. Additionally, naproxen binding may change the A β aggregation interface involved in fibril growth. From the viewpoint of chemical physics, it is interesting to quantitatively evaluate the impact of naproxen on the thermodynamics of fibril elongation, e.g., to consider the changes in A β binding free energy landscape. These questions can be investigated by means of molecular dynamics (MD) simulations, which can map the interactions of A β species with the ligands at all-atom resolution.²⁰ In the past, MD probed the conformational ensembles of amyloidogenic monomers,^{21,22} the assembly of oligomers,^{23–27} and the fibril growth.^{28–31}

In recent studies we have explored the impact of ibuprofen on the mechanism of A β fibril growth.^{32,33} In this paper, we use united atom implicit solvent model and replica exchange molecular dynamics (REMD)³⁴ to examine the anti-aggregation effect of another NSAID ligand, naproxen. We show that naproxen anti-aggregation effect is influenced by two factors. Similar to ibuprofen, naproxen interactions destabilize binding of A β peptides to the fibril due to direct competition for the same binding location on the fibril surface between the ligands and the peptides. However, in contrast to ibuprofen naproxen binding also alters the conformational ensemble of A β monomers by increasing their β -structure content. This factor reduces naproxen anti-aggregation effect. We also investigated the structural changes in the aggregation interface involved in A β fibril growth occurring due to naproxen interference. We conclude the paper with the comparison of *in silico* and experimental data. Throughout the paper the anti-aggregation effect of naproxen is assessed by comparing with the A β fibril growth in water environment studied by us earlier.³¹ The results from those previous simulations are referred to as obtained in “water”.

Model and Simulation Methods

Molecular dynamics simulations

To simulate A β peptides coincubated with naproxen (Fig. 1c,d) we used CHARMM molecular dynamics (MD) program³⁵ and united atom force field CHARMM19 coupled with the SASA implicit solvent model.³⁶ The force field description, its applicability and testing can be found in our previous studies.^{25,37,38} In particular, we have shown that CHARMM19+SASA force field accurately reproduces the experimental distribution of chemical shifts for C $_{\alpha}$ and C $_{\beta}$ atoms in A β monomers.^{25,39} Parameterization of naproxen (Fig. 1b) in CHARMM19 force field has been developed and tested by us in the previous study.³⁸ The tests of the naproxen force field have indicated that the conformational

ensembles of naproxen computed in our simulations and determined by *ab initio* methods and NMR technique are consistent.^{38,40}

In this work we use the N-terminal truncated fragment of the full-length peptide, $A\beta_{10-40}$ (Fig. 1a).⁴¹ Similarities in the aggregation propensities of $A\beta_{1-40}$ and $A\beta_{10-40}$ follow from the following observations. First, solid-state NMR studies have shown that both peptides form similar two-fold symmetry fibril structures.^{6,42} It is also known that the first nine N-terminal residues in the $A\beta_{1-40}$ fibril are disordered.⁴³ Moreover, $A\beta_{1-40}$ fibrils were reported to seed the growth of $A\beta_{10-40}$. Second, according to the experiments⁴⁴ and simulations^{25,41} truncation of the first nine N-terminal amino acids results in minor changes in the conformational ensembles of $A\beta$ monomers and oligomers. Consequently, we use $A\beta_{10-40}$ as a model of the full-length $A\beta_{1-40}$ peptide.

The simulation system consists of six $A\beta_{10-40}$ peptides interacting with 60 naproxen molecules (Fig. 1c). Four peptides were constrained to form a fibril fragment, whereas two unconstrained peptides were free to associate or dissociate from the fibril. The fibril fragment was modeled using the coordinates of backbone atoms determined from the solid-state NMR measurements.⁶ The constraints linking the backbones of fibril peptides to their experimental positions were implemented using soft harmonic potentials with the constant $k_c = 0.6 \text{ kcal}/(\text{mol}\text{\AA}^2)$.³¹ The harmonic constraints permit backbone fluctuations with the amplitude of about 0.6 Å at 360K, which are comparable with the fluctuations of atoms on the surface of folded proteins.⁴⁵ Constraints were not applied to the side chains of fibril peptides. The constraints emulate the stability of amyloid fibrils known to be highly resistant to dissociation⁴⁶ and eliminate the necessity to simulate large fibril systems to achieve their stability. In addition, we considered a second simulation system, which includes a single unconstrained $A\beta_{10-40}$ monomer in the solution of 10 naproxen ligands (Fig. 1d). Both simulation systems were subject to spherical boundary condition with the radius $R_s = 90\text{\AA}$ and the force constant $k_s = 10 \text{ kcal}/(\text{mol}\text{\AA}^2)$. Consequently, the peptide concentration is on the order of *mM*. The concentration ratio of naproxen to $A\beta$ peptides, i.e., the ratio of the numbers of ligands and peptides, is 10:1, which is within the range used experimentally (from 1 to 22).^{19,47} Throughout the paper the peptides in orange in Fig. 1c are referred to as fibril and the red peptides are termed incoming.

Replica exchange simulations

To perform conformational sampling we used replica exchange molecular dynamics (REMD).³⁴ In all, 24 replicas were distributed linearly in the temperature range from 330 to 560K (for the fibril system) or from 300 to 530K (for the monomer system) with the increment of 10K. The exchanges were attempted every 20ps (fibril) or 80ps (monomer) between all neighboring replicas with the average acceptance rates of 22 or 57%, respectively. Fourteen (fibril) and four (monomer) REMD trajectories were produced resulting in the cumulative simulation times of 67μs and 77 μs, respectively. Between replica exchanges the system evolved using NVT underdamped Langevin dynamics with the damping coefficient $\gamma = 0.15 \text{ ps}^{-1}$ and the integration step of 2 fs. Because the initial parts of REMD trajectories are not equilibrated and must be excluded from thermodynamic analysis, the cumulative equilibrium simulation times were reduced to $\tau_{sim} \approx 47\mu\text{s}$ (fibril) and 67μs (monomer). The REMD trajectories were started with random distributions of (incoming) peptides and ligands. The convergence of REMD simulations and error analysis are presented in Supporting Information.

Computation of structural probes

Interactions formed by $A\beta$ peptides and naproxen were probed by computing the number of side chain contacts and hydrogen bonds (HBs). A side chain contact occurs, if the distance

between the centers of mass of side chains is less than 6.5 Å. This cut-off approximately corresponds to the onset of hydration of side chains as the separation distance between them increases. A contact is assumed hydrophobic, if it involves two apolar amino acids. An incoming peptide is bound, if it forms at least one side chain contact with the fibril. Computation of contacts between naproxen and A β is described in Supporting Information.

The backbone HBs between peptide NH and CO groups were assigned according to Kabsch and Sander.⁴⁸ The same definition was applied to detect HBs between naproxen and peptide backbone NH groups.³⁸ Following our previous studies we distinguished three classes of peptide-fibril HBs.³¹ The first includes any peptide-fibril HB, whereas the second and third classes are restricted to the HBs involved in the formation of parallel (antiparallel) β -sheets by incoming peptides on the fibril edge. These HBs referred to as parallel (pHB) and anti-parallel (aHB) are defined in Supporting Information.

Secondary structure in A β peptides was computed using the distribution of (ϕ, ψ) backbone dihedral angles. Specific definitions of β -strand and helix states can be found in our earlier studies.²⁵ Definitions of radial distribution functions $g_p(r)$ and $g_l(r)$ are given in Supporting Information. The distributions of states produced by REMD were analyzed using multiple histogram method,⁴⁹ which allowed us to compute thermodynamic averages of various structural quantities as well as free energy landscapes. Throughout the paper angular brackets $\langle \dots \rangle$ imply thermodynamic averages. The growth of A β fibril in naproxen solution is studied at the temperature 360K, at which A β peptide locks into fibril-like state during fibril growth in aqueous environment.^{31,37}

Results

Naproxen destabilizes the interactions between incoming A β peptides and the fibril

To study the growth of A β fibril in naproxen solution we analyzed the interactions formed between incoming A β peptides and the fibril (Fig. 1c). To this end, we used REMD to compute the thermal averages of the numbers of peptide-fibril side chain contacts $\langle C(T) \rangle$ and parallel HBs $\langle N_{phb}(T) \rangle$ as a function of temperature (Fig. 2). Side-chain interactions describe nonspecific binding of incoming peptide to the fibril, whereas the formation of parallel HBs reflects the emergence of fibril-like state in the bound peptide. Fig. 2 demonstrates that the decrease in temperature results in binding of incoming A β peptides to the fibril. At 360K the numbers of side chain contacts and parallel HBs are $\langle C \rangle \approx 27.8$ and $\langle N_{phb} \rangle \approx 3.8$, respectively. To determine the impact of naproxen on peptide-fibril interactions we plot in Fig. 2 the same probes computed for the system free of ligands.³¹ It is clear that naproxen considerably weakens the peptide-fibril interactions. For example, at 360K in water there are $\langle C(w) \rangle \approx 42.7$ contacts and $\langle N_{phb(w)} \rangle \approx 6.0$ parallel HBs formed between incoming peptide and the fibril.³¹ Qualitatively similar results are obtained if one considers the number of peptide-fibril HBs $\langle N_{hb} \rangle$ (their numbers are 7.0 and 10.5 in naproxen solution and water, respectively³¹). Therefore, naproxen interference eliminates approximately one-third of peptide-fibril interactions.

To explore the impact of naproxen on the energetics of fibril growth we computed the free energy landscapes of peptide-fibril interactions. In our previous studies, we have shown that equilibrium fibril elongation proceeds via two thermodynamically distinct transitions - docking and locking.³¹ Docking occurs upon binding of A β monomers to the fibril without their integration into the fibril structure. During locking incoming peptides adopt fibril-like conformation by forming parallel HBs with the fibril. Because side chain contacts measure any peptide-fibril interactions without regard of peptide structure, their number C is appropriate for probing the docking transition. Fig. 3a shows the free energies of incoming peptide $F(C)$ computed in water and naproxen solution. Both free energy profiles are

qualitatively similar featuring wide minimum associated with the bound state. To provide quantitative measure of naproxen anti-aggregation effect, we computed the binding free energies for incoming $A\beta$ peptide ΔF_{B-U} (Fig. 3a). In naproxen solution $\Delta F_{B-U}(npn) \approx -4.7RT$ compared to $\Delta F_{B-U}(w) \approx -9.9RT$ in water.³³ Therefore, due to naproxen interactions the free energy of peptide docked (bound) state is increased by $\Delta\Delta F_{B-U} = \Delta F_{B-U}(npn) - \Delta F_{B-U}(w) \approx 5.2RT$ (Fig. 3a). Furthermore, the free energy minimum in Fig. 3a associated with bound peptide states shifts to smaller C .

In our previous studies we showed that docking transition appears to be continuous occurring without significant free energy barriers.³¹⁻³⁷ Then, the temperature dependence of the system free energy $F(T)$ can be approximated by the quadratic fitting function $-\alpha(T - T_D)^2$, where α is a constant and T_D is an estimate of the docking temperature (data not shown). We found that in naproxen solution $T_D \approx 306K$, whereas in water it is considerably higher ($T_D = 380K$).³¹ Therefore, consistent with the analysis of free energy $F(C)$ naproxen exerts strong impact on peptide binding by decreasing the docking temperature by about 70K.

Locking transition, which involves the formation β -sheet structure in the bound $A\beta$ peptides, can be described by the free energy $F(N_{ahb}, N_{phb})$ computed as a function of the numbers of antiparallel N_{ahb} and parallel N_{phb} HBs (Fig. 3b and Methods). As in water environment³¹ four free energy basins can be distinguished in naproxen solution. The locked states L feature exclusively parallel β -sheets ($N_{phb} > 3, N_{ahb} = 0$), whereas the docked states D contain neither parallel or antiparallel HBs ($N_{phb} = N_{ahb} = 0$). Other free energy basins include states with antiparallel β -sheets AP ($N_{phb} = 0, N_{ahb} > 2$) and those with mixed (parallel and antiparallel) β -structure M ($N_{phb} > 0, N_{ahb} > 0$). Comparison of the two panels in Fig. 3b reveals that naproxen destabilizes all states (L , AP , and M) relative to the docked D . It follows from Fig. 3b that the probability of finding $A\beta$ peptide in L , P_L , is 0.38 in naproxen solution compared to 0.51 in water.³¹ Simultaneously, the probability of occupancy of D is increased from 0.06 in water to 0.25 in naproxen. Fig. 3b also allows us to compute the free energy gap between the L and D states, $\Delta F_{L-D} = F_L - F_D$, where $F_D = 0$ is the free energy of D and F_L is the free energy of L integrated over the entire locked basin. We found that ΔF_{L-D} are $-0.6RT$ and $-2.0RT$ in naproxen solution and water, respectively, thus implying considerable loss in the stability of the L state. For water environment the locking temperature T_L was defined from the condition $P_L(T_L) = 0.5$, from which $T_L \approx 360K$.³¹ Because the lowest temperature, at which conformational sampling in naproxen solution is available, is 330K and $P_L(T = 330K) < 0.5$, we estimate $T_L < 330K$.

We have shown in previous studies that the affinities of the fibril edges (Fig. 1c) with respect to binding $A\beta$ peptides are different.³¹ To check if naproxen changes the edge affinities, we computed the free energy of a peptide along the fibril axis z , $F(z)$, at 360K. Fig. 3c reveals two free energy minima of unequal depth associated with the peptide binding to the convex (CX) and concave (CV) edges. The free energy gap between the edges is $\Delta F_{CV-CX} \approx -1.2RT$, which is significantly narrower than ΔF_{CV-CX} in water ($\approx 2.6RT$).³¹ To complement these findings we also computed the probabilities of occurrence of incoming $A\beta$ peptide on the two fibril edges as a function of temperature, $P_{CX}(T)$ and $P_{CV}(T)$ (Supporting Information). At 360K $P_{CX} \approx 0.22$, whereas $P_{CV} \approx 0.78$. For comparison, in water $P_{CX} \approx 0.08$ and $P_{CV} \approx 0.92$.³¹ Thus, although the affinities of the edges for $A\beta$ binding remain unequal, naproxen interactions reduce their difference.

Naproxen competes with incoming $A\beta$ peptides for binding to the fibril

Strong impact of naproxen on peptide-fibril interactions implicates binding of this ligand to $A\beta$ species. This inference can be illustrated by the free energy $F(r_b)$ of a ligand computed as a function of the distance between ligand and $A\beta$ surface, r_b . The free energy $F(r_b)$ in Fig.

4a displays a deep minimum at $r_b \approx 5 \text{ \AA}$, from which the binding free energy ΔF_b is found to be $-7.8RT$. We also computed the probability of binding naproxen molecule to $A\beta$, P_b (inset to Fig. 4a). At 360K $P_b \approx 0.78$ that implies that, on an average, the number of bound ligands is $\langle L \rangle \approx 46.9$ (out of the total of 60). About 30% of the bound ligands (14.1) interact simultaneously with the incoming peptides and the fibril, i.e., they mediate peptide-fibril interactions. In all, the number of contacts between ligands and $A\beta$ side chains is $\langle C_l \rangle \approx 189.9$, i.e., each naproxen molecule upon binding interacts with about four amino acids. Interestingly, the total number of HBs between the ligands and $A\beta$ backbone is only $\langle N_{lhb} \rangle \approx 9.3$ that is 20 times smaller than $\langle C_l \rangle$. These calculations show that ligand binding is mainly driven by the interactions with amino acid side chains.

Competition of peptides and ligands for binding to $A\beta$ fibril follows from the analysis of amino acid and ligand radial distribution functions, $g_p(r)$ and $g_l(r)$ (see Fig. 4b and Methods). Their comparison suggests three conclusions. First, $g_p(r)$ obtained in water and naproxen solution are qualitatively similar. Both feature two maxima, of which the first is associated with the amino acids from incoming peptides directly bound to the fibril (a first bound shell, FBS, $r < 7 \text{ \AA}$) and the second is formed by the amino acids interacting with the FBS (a second bound shell, SBS). Second and more important observation is that there is a noticeable decrease in the amplitude of the first peak (the probability of finding an amino acid in the FBS is decreased from 0.70 to 0.51), whereas the SBS is not affected. Slower decay of $g_p(r)$ in naproxen solution compared to water indicates that some amino acids from the FBS are pushed away from the fibril implicating destabilization of peptide bound state. In fact, the average separation between amino acid from incoming peptide and the fibril $\langle r \rangle$ increases from 6.5 Å in water to 8.5 Å in naproxen solution. Third, the ligand $g_l(r)$ reveals a single peak attributed to naproxen molecules bound to the fibril. Interestingly, the location of $g_l(r)$ peak approximately coincides with that of the amino acid FBS. Therefore, the redistribution of amino acids detected by $g_p(r)$ is likely to be the consequence of direct competition between the ligands and amino acids for binding to the fibril.

Naproxen impact on $A\beta$ aggregation interface

If naproxen destabilizes binding of $A\beta$ peptides to the fibril, then the ligand interference may also change the aggregation interface by redistributing peptide-fibril interactions. To check this possibility we computed the matrix of side chain contacts $\langle C(s1, s2) \rangle$ formed between the fibril sequence region $s1$ and the region $s2$ from incoming peptide (Table 1). Because the Nt-Nt peptide-fibril interactions retain about 80% of the interactions formed in water, they are the least affected by naproxen. In comparison, naproxen eliminates about half of side chain contacts between other sequence regions. Similar conclusion follows from the analysis of peptide-fibril HBs. Table 1 shows that the number of Nt-Nt HBs is somewhat increased in naproxen solution (by 15%), whereas there are fewer HBs formed between other sequence regions. For instance, the number of HBs formed between the peptide Ct and the fibril Nt regions is reduced almost two-third.

To further illustrate the impact of naproxen Fig. 5 compares the numbers of peptide-fibril HBs $\langle N_{hb}(i) \rangle$ formed by the residues i in incoming peptide in naproxen solution and water. Naproxen mainly affects the HBs in the C-terminal, leaving those in the Nt region largely intact. For example, it follows from Fig. 5 that the numbers of HBs formed by the Nt and Ct are $\langle N_{hb}(Nt) \rangle \approx 4.0$ and $\langle N_{hb}(Ct) \rangle \approx 2.0$. Because in water the respected numbers of HBs are 4.6 and 4.5, naproxen eliminates only 13% of them in the Nt region, but 56% in the Ct. We have shown earlier that the Nt region in the incoming peptide represents the primary interface involved in fibril growth.³⁷ The simulations reported here suggest that naproxen further enhances the polarization of aggregation interface by selectively targeting the peptide-fibril interactions formed by the incoming peptide C-terminal.

Discussion

Molecular basis of naproxen anti-aggregation effect

In this study we investigated the effect of naproxen ligands on the binding of A β peptides to the amyloid fibril. We showed that naproxen interference eliminates about one-third of peptide-fibril interactions. As a result the docked (bound) states of incoming A β peptide experience considerable destabilization as their free energy increases by $\Delta\Delta F_{B-U} \approx 5.2RT$ and the temperature of docking is decreased 70K with respect to water environment.³¹ Naproxen also compromises the stability of the locked state reducing the free energy gap between the locked and docked states by $1.4RT$. Accordingly, the probability of occupancy of the locked state is reduced and the temperature of locking the incoming peptide into the fibril-like state is decreased at least 30K. Therefore, naproxen shifts the equilibrium from structured (locked) A β states to disordered (docked) states.

We also showed that naproxen binds with high affinity to A β peptides and the fibril. In fact, the free energy gain upon ligand binding is $\Delta F_b \approx -7.8RT$ and about 78% of the ligands are bound at 360K. Interestingly, about 30% of the bound naproxen molecules directly interfere with the peptide binding to the fibril because they occur at the peptide-fibril interface. The distributions of amino acids and ligands near the fibril surface shown in Fig. 4b reveal that (i) compared to water the amplitude of the amino acid FBS is decreased in naproxen solution and (ii) the ligand distribution peaks at about the same distance as the amino acid one does. This results in a redistribution of amino acids away from the fibril. These observations point to direct competition between amino acids and ligands for binding to the fibril.

To substantiate this conclusion we consider the location of binding sites for the peptides and ligands on the fibril surface. Fig. 3c implicates the concave (CV) edge is the primary binding site for incoming A β peptides. Indeed, in water the ratio of the probabilities of peptide binding to the edges is $P_{CV}/P_{CX} \approx 9$ (Supporting Information)³¹. Using the free energy profile $F(z)$ along the fibril axis for A β peptide in water we showed that binding to the fibril sides is disfavored.³¹ On the other hand, we found earlier that naproxen demonstrates a strong preference to bind to the CV edge.³⁸ For example, in the absence of incoming peptides the ratio of the numbers of ligands bound to the CV and CX edges is $\langle L_{CV} \rangle / \langle L_{CX} \rangle = 2.1$. The high affinity of the CV is due to strong interligand interactions induced by the confinement of bound ligands to the CV groove (Fig. 1c). As a result when A β peptides and the ligands are coincubated, they compete for binding to the same location on the fibril surface. Thus, we propose that the competition for the same binding site makes the basis for naproxen anti-aggregation effect observed experimentally^{18,19}

We have also explored the impact of naproxen on the peptide-fibril aggregation interface (Table 1 and Fig. 5). Naproxen ligands mainly compromise the interactions formed by the C-terminal of incoming A β peptide, which are already the weakest in the aggregation interface.³⁷ In contrast, those formed by the N-terminal remain largely unchanged. Therefore, although naproxen destabilizes peptide-fibril interactions, it does not dramatically changes their distribution in the aggregation interface.

Does naproxen change the structure of A β monomer?

It is possible that one of the factors contributing to naproxen anti-aggregation propensity are the changes in the secondary structure of A β monomer caused by ligand binding. To test this possibility, we first computed the fractions of β -strand $\langle S \rangle$ and helix $\langle H \rangle$ structure in A β incoming peptides in naproxen solution. We obtained that at 360K $\langle S \rangle \approx 0.49$ and $\langle H \rangle \approx 0.12$. For comparison, in water $\langle S \rangle$ and $\langle H \rangle$ are 0.52 and 0.11,³¹ respectively, i.e., the changes in $\langle S \rangle$ and $\langle H \rangle$ are comparable to the computational error of REMD sampling. Therefore, naproxen appears to have little effect on the secondary structure of bound A β

peptides. However, to enhance sampling of aggregated states our REMD simulations of fibril elongation used elevated A β concentrations. Consequently, the thermodynamic equilibrium is shifted toward bound states causing the probability of A β peptide binding to the fibril to reach 0.99 at 360K. Large binding probability masks the impact of naproxen on A β monomers.

To correct this problem, we performed separate REMD simulations of A β monomer in the solution of naproxen ligands (see Methods). The binding temperature T_b was obtained using the probability of ligand binding to A β monomer, P_b . From the condition $P_b(T_b) = 0.5$ T_b is 348K implying that at $T < T_b$ naproxen bound state is preferred. Because the conformational ensemble of A β monomer in water has been investigated in our previous study,⁴¹ we can evaluate the changes in A β structure induced by naproxen binding. At 330K ($< T_b$) the number of intrapeptide HBs $< N_{ihb} >$ is reduced from 17.1 in water to 12.4 in naproxen solution. More important are the changes in the secondary structure. The fraction of β -strand residues $< S >$ is increased from 0.18 in water to 0.33 in naproxen solution (an almost two-fold change), whereas the helix fraction $< H >$ declines from 0.36 to 0.23. Fig. 6 shows that the changes in secondary structure along the sequence of A β monomer are largely confined to the N-terminal Nt. For example, the strand content in the Nt region increases three-fold (from 0.12 to 0.38), but only by 25% in the Ct (from 0.22 to 0.27). Similarly, an almost two-fold decrease in the helix structure in the Nt (from 0.57 to 0.32) is accompanied by minor change in the Ct (from 0.18 to 0.15). Therefore, naproxen binding strongly enhances the β -strand propensity in A β monomer.

These findings allow us to rationalize the non-monotonic temperature dependence of naproxen anti-aggregation effect. The inset to Fig. 2 shows the ratio of the numbers of peptide-fibril side chain contacts in naproxen solution $< C(T) >$ and water $< C(T; w) >$. Strikingly, this ratio increases at $T \lesssim 400\text{K}$ indicating that anti-aggregation effect of naproxen becomes weaker with the decrease in temperature. It is likely that the decline in naproxen anti-aggregation propensity is due to superposition of two opposing factors. First, as temperature is lowered naproxen starts to compete with incoming A β peptides for binding to A β fibril. Second, naproxen ligands also start to bind to A β monomers in the bulk causing a shift to β -structure in their conformational ensemble. Importantly, the most profound enhancement of β -structure in A β monomer occurs in the Nt region, which is also the primary aggregation interface in fibril growth.³⁷ As a result A β monomer is expected to bind to the fibril with elevated β -structure content that, in turn, should narrow the free energy gap between the docked and locked A β states (the enhancement of β -structure in the docked state is shown in Supporting Information). The second factor promotes A β fibril growth, while the first tends to inhibit it.

Comparing ibuprofen and naproxen anti-aggregation effects

It is interesting to compare the anti-aggregation effects of naproxen and ibuprofen. In our previous study we showed that ibuprofen impedes A β fibril elongation by competing with incoming A β peptides for the same binding site, the CV fibril edge.³³ Ibuprofen interactions also shift the thermodynamic equilibrium from fibril-like locked states to disordered docked states. However, ibuprofen destabilizes A β bound and locked states to a lesser extent than naproxen (for example, ibuprofen $\Delta\Delta F_{B-U} \approx 2.4RT$ compared to $5.2RT$ for naproxen). From this perspective, the anti-aggregation actions of the ligands are qualitatively similar.

The difference emerges when we consider the impact of the ligands on the structure of A β monomer. According to our earlier REMD simulations³² ibuprofen binding causes minor changes in A β secondary structure (less than 10% at 330K). Small impact of this ligand on A β monomer is apparently related to its weaker binding affinity (Supporting Information). Compared to ibuprofen naproxen binds with stronger affinity to the monomer and forms

large bound clusters facilitated by favorable interactions between naproxen ligands.³⁸ To illustrate this Fig. S3 in Supporting Information compares the distributions of bound naproxen and ibuprofen ligands $\langle L(S_c) \rangle$ with respect to the clusters of the size S_c , i.e., the number of ligands in the cluster. (Note that a cluster is defined as an isolated group of bound ligands.) The naproxen distribution $\langle L(S_c) \rangle$ is bimodal implying that, on an average, about 55% of bound ligands form large clusters ($S_c > 6$). In contrast, the distribution $\langle L(S_c) \rangle$ for ibuprofen is unimodal and large clusters are extremely rare (involving just 1% of all bound ligands). In our previous study we have showed that ibuprofen forms few large clusters compared to naproxen due to the difference in their chemical structure. Naproxen molecule contains a conjugated naphthalene ring, which is responsible for strong (mostly, vander-Waals) attractive interligand interactions. On the contrary, ibuprofen has a single phenyl ring, which forms substantially weaker interligand interactions. Consequently, $A\beta$ monomer structure is largely unaffected by ibuprofen.

Although ibuprofen has weaker anti-aggregation action, it does not appear to show a non-monotonic temperature dependence indicative for naproxen. Indeed, the ratio $\langle C(T) \rangle / \langle C(T; w) \rangle$ for ibuprofen in the inset to Fig. 2 remains almost flat at low temperatures, presumably because there is no contribution to ibuprofen anti-aggregation propensity from the changes in $A\beta$ monomer structure. Therefore, extrapolating the trends seen in $\langle C(T) \rangle / \langle C(T; w) \rangle$ we predict that ibuprofen becomes stronger anti-aggregation agent than naproxen at $T < 330K$.

Comparison with experiments

In vitro experimental studies have investigated the anti-aggregation action of naproxen.^{18,19} It was found that naproxen can reduce the accumulation of $A\beta$ fibrils and even inhibit their elongation.^{18,19} Naproxen has also an ability to destabilize, but apparently not to depolymerize, existing $A\beta$ fibrils.¹⁹ Similar conclusions follow from our simulations, in which naproxen destabilizes the bound state of incoming $A\beta$ peptides, but does not dissociate them from the fibril. (According to our previous study the constraints applied to fibril peptides have no qualitative impact on the mechanism of fibril growth.³¹) Several studies have also demonstrated that amyloid fibrils grow via monomer addition to the edges.⁵⁰⁻⁵² Therefore, if a ligand impedes or inhibits fibril extension, it is likely to bind to their edges as it occurs in our simulations. Hence, our simulations of the growth of $A\beta$ fibril coincubated with naproxen appear to be qualitatively consistent with experiments.

Using our *in silico* results we can compare the anti-aggregation actions of naproxen and ibuprofen with the experimental data. According to the experiments naproxen has stronger binding affinity than ibuprofen.¹⁸ This observation was reproduced in our previous study, in which we investigated binding of these ligands to $A\beta$ fibril without considering incoming peptides.³⁸ However, it is intriguing that compared to ibuprofen naproxen has weaker anti-aggregation effect.¹⁹ We believe that our findings allow us to provide an explanation to this puzzle. At elevated temperatures (e.g., at 360K) naproxen has higher affinity of binding to $A\beta$ fibril and, accordingly, demonstrates stronger anti-aggregation action. However, this result cannot be extrapolated to lower temperatures closer to those used experimentally (300K) without taking into account the impact of ligands on the secondary structure of $A\beta$ monomer. We have showed that ibuprofen binding causes minor changes in $A\beta$ monomer conformational ensemble in a wide range of temperatures starting from 310K and above.³² This study suggests that naproxen, in contrast, strongly impacts the conformational ensemble of $A\beta$ monomers by promoting the formation of β -structure. As described above this factor should reduce the anti-aggregation potency of this ligand making it less efficient than ibuprofen. This analysis indicates that the relation between binding affinity of a ligand and its anti-aggregation action is not straightforward as various contributions such as the

impact on the structure of A β monomer must be factored in predicting the anti-aggregation efficiency of a ligand.

Finally, it is important to comment on the ratio of ligand and peptide concentrations. Barrio and coworkers have reported that naproxen and its structural derivative, the molecular imaging probe ¹⁸FFDDNP, share the binding sites on the surface of A β fibril.⁵³ It has been estimated that the number of ¹⁸FFDDNP binding sites is from 3.5 to 7.1 per 10,000 fibril peptides.⁵³ Given the similarity between the ligands this estimate is likely to hold for naproxen. But such small number of binding sites implies that these ligands are bound to very few specific locations on the fibril surface, which are likely to be associated with the structural features such as its edges. In those locations a large local excess of ligand concentration over that of peptides should occur. These arguments are in accord with our results showing that naproxen molecules tend to concentrate in specific locations on the fibril surface such as the CV edge as shown in Fig. 1c.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- (1). Selkoe DJ. Nature. 2003; 426:900–904. [PubMed: 14685251]
- (2). Dobson CM. Nature. 2003; 426:884–890. [PubMed: 14685248]
- (3). Shoji M, Golde TE, Ghiso J, Cheung TT, Estus S, Shaffer LM, Cai XD, McKay DM, Tintner R, Frangione B. Science. 1992; 258:126–129. [PubMed: 1439760]
- (4). Hardy J, Selkoe DJ. Science. 2002; 297:353–356. [PubMed: 12130773]
- (5). Petkova AT, Leapman RD, Guo Z, Yau W-M, Mattson MP, Tycko R. Science. 2005; 307:262–265. [PubMed: 15653506]
- (6). Petkova AT, Yau W-M, Tycko R. Biochemistry. 2006; 45:498–512. [PubMed: 16401079]
- (7). Yoshiike Y, Akagi T, Takashima A. Biochemistry. 2007; 46:9805–9812. [PubMed: 17676931]
- (8). Haass C, Selkoe DJ. Nature Rev. Mol. Cell. Biol. 2007; 8:101–112. [PubMed: 17245412]
- (9). Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. Nature Medicine. 2008; 14:837–842.
- (10). Murphy RM, Pallitto MM. J. Struct. Biol. 2000; 130:109–122. [PubMed: 10940219]
- (11). Carulla N, Caddy GL, Hall DR, Zurdo J, Gair M, Feliz M, Giralto E, Robinson CV, Dobson CM. Nature. 2005; 436:554–558. [PubMed: 16049488]
- (12). Martins IC, Kuperstein I, Wilkinson H, Maes E, Vanbrabant M, Jonckheere W, van Gelder P, Hartmann D, Hooge RD, de Strooper B, Schymkowitz J, Rousseau F. EMBO J. 2008; 27:224–233. [PubMed: 18059472]
- (13). Cole GM, Frautschy SA. CNS & Neurolog. Disorders - Drug Targets. 2010; 9:140–148.
- (14). Vlad SC, Miller DR, Kowall NR, Felson DT. Neurology. 2008; 70:1672–1677. [PubMed: 18458226]
- (15). Imbimbo BP. Expert Opin. Investig. Drugs. 2009; 18:1147–1168.
- (16). Gasparini L, Ongini E, Wenk G. J. Neurochem. 2004; 91:521–536. [PubMed: 15485484]
- (17). Varvel NH, Bhaskar K, Kounnas MZ, Wagner SL, Yang Y, Lamb BT, Herrup K. J. Clin. Invest. 2009; 119:3692–3702. [PubMed: 19907078]

- (18). Agdeppa ED, Kepe V, Petric A, Satyamurthy N, Liu J, Huang S-C, Small GW, Cole GM, Barrio JR. *Neurosci.* 2003; 117:723–730.
- (19). Hirohata M, Ono K, Naiki H, Yamada M. *Neuropharmacology.* 2005; 49:1088–1099. [PubMed: 16125740]
- (20). Ma B, Nussinov R. *Curr. Opin. Chem. Biol.* 2006; 10:445–452. [PubMed: 16935548]
- (21). Sgourakis NG, Yan Y, McCallum SA, Wang C, Garcia AE. *J. Mol. Biol.* 2007; 368:1448–1457. [PubMed: 17397862]
- (22). Yang M, Teplow DB. *J. Mol. Biol.* 2008; 384:450–464. [PubMed: 18835397]
- (23). Lu Y, Derreumaux P, Guo Z, Mousseau N, Wei G. *Proteins Struct. Funct. Bioinform.* 2009; 75:954–963.
- (24). Bellesia G, Shea J-E. *Biophys. J.* 2009; 96:875–886. [PubMed: 19186127]
- (25). Takeda T, Klimov DK. *Proteins Struct. Funct. Bioinf.* 2009; 77:1–13.
- (26). Nandel FS, Anand P, Hansmann UHE. *J. Chem. Phys.* 2008; 129:195102. [PubMed: 19026087]
- (27). Urbanc B, Betnel M, Cruz L, Bitan G, Teplow DB. *J. Amer. Chem. Soc.* 2010; 132:4266–4280. [PubMed: 20218566]
- (28). Cecchini M, Rao F, Seeber M, Caflisch A. *J. Chem. Phys.* 2004; 121:10748–10756. [PubMed: 15549960]
- (29). Nguyen PH, Li MS, Stock G, Straub JE, Thirumalai D. *Proc. Natl. Acad. Sci. USA.* 2007; 104:111–116. [PubMed: 17190811]
- (30). Krone MG, Hua L, Soto P, Zhou R, Berne BJ, Shea J-E. *J. Amer. Chem. Soc.* 2008 doi: 10.1021/ja8017303.
- (31). Takeda T, Klimov DK. *Biophys. J.* 2009; 96:442–452. [PubMed: 19167295]
- (32). Raman EP, Takeda T, Klimov DK. *Biophys. J.* 2009; 97:2070–2079. [PubMed: 19804739]
- (33). Chang WE, Takeda T, Raman EP, Klimov DK. *Biophys. J.* 2010; 98:2662–2670. [PubMed: 20513411]
- (34). Sugita Y, Okamoto Y. *Chem. Phys. Lett.* 1999; 114:141–151.
- (35). Brooks BR, Brucoler RE, Olafson BD, States DJ, Swaminathan S, Karplus M. *J. Comp. Chem.* 1982; 4:187–217.
- (36). Ferrara P, Apostolakis J, Caflisch A. *Proteins Struct. Funct. Bioinform.* 2002; 46:24–33.
- (37). Takeda T, Klimov DK. *Biophys. J.* 2009; 96:4428–4437. [PubMed: 19486667]
- (38). Takeda T, Chang WE, Raman EP, Klimov DK. *Proteins Struct Funct Bioinform.* 2010 doi: 10.1002/prot.22804.
- (39). Hou L, Shao H, Zhang Y, Li H, Menon NK, Neuhaus EB, Brewer JM, Byeon I-JL, Ray DG, Vitek MP, Iwashita T, Makula RA, Przybyla AB, Zagorski MG. *J. Amer. Chem. Soc.* 2004; 126:1992–2005. [PubMed: 14971932]
- (40). Bednarek E, Bocian W, Dobrowolski JC, Kozerski L, Sadlej-Sosnowska N, Sitkowski J. *J. Mol. Struct.* 2001; 559:369–377.
- (41). Takeda T, Klimov DK. *J. Phys. Chem. B.* 2009; 113:6692–6702. [PubMed: 19419218]
- (42). Paravastu AK, Petkova AT, Tycko R. *Biophys. J.* 2006; 90:4618–4629. [PubMed: 16565054]
- (43). Whittemore NA, Mishra R, Kheterpal I, Williams AD, Wetzel R, Serspersu EH. *Biochemistry.* 2005; 44:4434–4441. [PubMed: 15766273]
- (44). Bitan G, Vollers SS, Teplow DB. *J. Biol. Chem.* 2003; 278:34882–34889. [PubMed: 12840029]
- (45). Zhou Y, Vitkup D, Karplus M. *J. Mol. Biol.* 1999; 285:1371–1375. [PubMed: 9917381]
- (46). Sasahara K, Naiki H, Goto Y. *J. Mol. Biol.* 2005; 352:700–711. [PubMed: 16098535]
- (47). Thomas T, Nadackal TG, Thomas K. *NeuroReport.* 2001; 12:3263–3267. [PubMed: 11711868]
- (48). Kabsch W, Sander C. *Biopolymers.* 1983; 22:2577–2637. [PubMed: 6667333]
- (49). Ferrenberg AM, Swendsen RH. *Phys. Rev. Lett.* 1989; 63:1195–1198. [PubMed: 10040500]
- (50). Esler WP, Stimson ER, Jennings JM, Vinters HV, Ghilardi JR, Lee JP, Mantyh PW, Maggio JE. *Biochemistry.* 2000; 39:6288–6295. [PubMed: 10828941]
- (51). Cannon MJ, Williams AD, Wetzel R, Myszkowski DG. *Anal. Biochem.* 2004; 328:67–75. [PubMed: 15081909]

- (52). Ban T, Hoshino M, Takahashi S, Hamada D, Hasegawa K, Naiki H, Goto Y. *J. Mol. Biol.* 2004; 344:757–767. [PubMed: 15533443]
- (53). Agdeppa E, Kepe V, Liu J, Flores-Torres S, Satyamurthy N, Petric A, Cole GM, Small GW, Huang S-C, Barrio JR. *J. Neurosci.* 2001; 21:1–5.
- (54). Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. *J. Comp. Chem.* 2004; 25:1605–1612. [PubMed: 15264254]

Fig. 1.

Sequence of $A\beta_{10-40}$ peptide. The N-terminal Nt spans the sequence positions 10 to 23. The C-terminal Ct includes the residues 29 through 39. (b) Naproxen molecule has two polar moieties, methoxy and carboxylate groups (G2 and G3), linked to the central hydrophobic naphthalene ring (G1). Carbon and oxygen atoms are shown in grey and red. (c) Snapshot of incoming $A\beta_{10-40}$ peptides interacting with amyloid fibril in naproxen solution at 360K. Four $A\beta$ peptides in orange form a fibril fragment. Two incoming peptides in red are bound to the fibril edge. $A\beta$ fibril protofilament is built of four in-register parallel β -sheets formed by the Nt and Ct sequence regions and has two distinct edges - concave (CV) and convex (CX) as shown on the drawing. Ct terminals are indented on the CV edge resulting in the appearance of the groove. The CV edge is the main binding site of $A\beta$ peptides and naproxen.³¹⁻³⁸ (d) $A\beta_{10-40}$ monomer in naproxen solution at 330K. Bound naproxen ligands induce β -strand formation in the peptide. In (c-d) naproxen ligands are in light grey/red.

Fig. 2.

Numbers of peptide-fibril side chain contacts $\langle C(T) \rangle$ (thick lines) and parallel HBs $\langle N_{phb}(T) \rangle$ (thin lines) probe the binding of incoming $A\beta$ peptides to amyloid fibril in naproxen solution (in black) and in water (in grey). The values of $\langle C(T) \rangle$ are scaled by a factor of three. The plot shows that naproxen destabilizes $A\beta$ binding to the fibril. The inset compares the anti-aggregation effects of naproxen (thick line) and ibuprofen (thin line) by plotting the contact ratios $\langle C(T) \rangle / \langle C(T; w) \rangle$, where $\langle C(T; w) \rangle$ and $\langle C(T) \rangle$ are the numbers of peptide-fibril contacts in water and in naproxen or ibuprofen solutions.

Fig. 3.

(a) The free energy of incoming peptide $F(C)$ as a function of the number of peptide-fibril side chain contacts C : naproxen solution (*npxn*, data in black), water (*w*, data in grey). The free energy of $A\beta$ binding to the fibril is $\Delta F_{B-U} = F_B - F_U$, where F_B and $F_U = 0$ are the free energies of bound (B) and unbound (U , $C = 0$) states. To compute F_B we integrate the B states (shaded in light grey) with $F(C) \leq F_{min} + 1.0RT$, where F_{min} is the minimum in $F(C)$. (b) Locking of bound $A\beta$ peptide is described by the free energy contour plot $F(N_{ahb}, N_{phb})$, where N_{ahb} and N_{phb} are the numbers of peptide-fibril antiparallel and parallel HBs. The

locked (*L*), antiparallel (*AP*), docked (*D*), and mixed (*M*) basins are indicated. The panels (a) and (b) show that naproxen destabilizes *Aβ* docked and locked states. (c) The free energy of *Aβ* peptide along the fibril axis *z*, $F(z)$. Two minima are associated with *Aβ* binding to the convex (CX) and concave (CV) fibril edges. The free energy gap between the CV and CX bound states, $\Delta F_{CV-CX} = F_{CV} - F_{CX}$, is computed by integrating over the states in the CV and CX minima (using the same procedure as in (a)). All panels are computed at 360K.

Fig. 4.

(a) The free energy $F(r_b)$ of naproxen molecule as a function of the distance between ligand and $A\beta$ surface (fibril or incoming peptides), r_b . The free energy of binding ΔF_b is computed by integrating over the ligand bound states (shaded in grey) as it is done in Fig. 3a. The values of F at $r_b > 29$ Å are set to zero. The profile $F(r_b)$ implicates naproxen binding to $A\beta$. The inset shows the probability $P_b(T)$ of binding naproxen molecule to $A\beta$ vs temperature T . (b) The radial distribution functions, $g_p(r)$ and $g_l(r)$, measure respectively the probabilities of finding an amino acid from incoming peptide and naproxen ligand at the distance r from $A\beta$ fibril surface. Filled black and grey circles represent $g_p(r)$ in naproxen solution and water, empty circles display $g_l(r)$. The plot suggests a competition between the ligands and amino acids for binding to $A\beta$ fibril. The peaks in $g_p(r)$ attribute the first and second bound shells (FBS and SBS). Both panels are computed at 360K.

Fig. 5.

The numbers of peptide-fibril HBs $\langle N_{hb}(i) \rangle$ formed by the residues i in incoming peptide in naproxen solution (in black) and in water (in grey). The N- and C-terminals Nt and Ct are boxed. The plots computed at 360K show that naproxen deletes mainly the HBs in the C-terminal.

Fig. 6.

The distributions of the fractions of helix $\langle H(i) \rangle$ and β -strand $\langle S(i) \rangle$ structure formed by the residues i in $A\beta_{10-40}$ monomer at 330K: naproxen solution (in black), water (in grey). Naproxen binding promotes β -strand structure in the N-terminal. The N- and C-terminals, Nt and Ct, are boxed.

Table 1 $A\beta$ aggregation interface in naproxen solution [‡] †(a) Number of peptide-fibril side chain contacts $\langle C(s1, s2) \rangle$

	Nt	Ct
Nt	7.6 (80%)	6.6 (58%)
Ct	4.5 (51%)	1.8 (55%)

(b) Number of peptide-fibril HBs $\langle N_{hb}(s1, s2) \rangle$

	Nt	Ct
Nt	2.3 (115%)	1.5 (65%)
Ct	1.5 (39%)	0.3 (50%)

[‡] s1 and s2 denote $A\beta$ sequence regions Nt and Ct.[†] numbers in parenthesis indicate the percentage of interactions retained in naproxen solution compared to water environment.