Mechanisms of Metabolite Amyloid Formation: Computational Studies for Drug Design against Metabolic Disorders

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S Supporting Information

ABSTRACT: Ordered self-organization of polypeptides into fibrillar assemblies has been associated with a number of pathological conditions linked to degenerative diseases. Recent experimental observations have demonstrated that even small-molecule metabolites can aggregate into supramolecular arrangements with structural and functional properties reminiscent of peptide-based amyloids. The molecular determinants of such mechanisms, however, are not clear yet. Herein, we examine the process of formation of ordered aggregates by adenine in aqueous solution by molecular dynamics simulations. We also investigate the effects of an inhibiting polyphenol, namely, epigallocatechin gallate (EGCG), on this mechanism. We show that, while adenine alone is able to form extended amyloid-like oligomers, EGCG interferes with the supramolecular organization process. Interestingly, acetylsalicylic acid is shown not to interfere with ordered aggregation, consistent with experiments. The results of these mechanistic studies indicate the main pharmacophoric determinants that a drug-like inhibitor should possess to effectively interfere with metabolite amyloid formation.

KEYWORDS: Self-organization, metabolites, metabolic disorders, molecular dynamics, drug design

The link between amyloidogenic self-assembly of peptides and proteins and degenerative phenotypes has been demonstrated for a number of pathologies, ranging from Creutzfeldt–Jakob disease to Parkinson’s disease, from Alzheimer’s to amyotrophic lateral sclerosis and type 2 diabetes.^{1,2} It comes as no surprise that a large number of basic and translational research initiatives have been dedicated to disentangle the molecular determinants of polypeptide aggregation and their relation to toxicity. The data have shown that such amyloid assemblies share common biochemical and biophysical properties, which consist of the presence of beta-sheet-rich secondary structures, the distinctive ability to bind thioflavin-T (ThT), and an extended twisted morphology of the fibrils, giving rise to characteristic X-ray reflections. Recent evidence supports the possibility for different sequences to establish cross-interactions, in the so-called “cross-amyloid interaction model”, potentially linking different amyloid diseases to each other.^{3,4} Finally, current findings have shown that amyloid fibrils display a unique surface reactivity endowing the aberrant sequestration of distinct molecules and secondary nucleation events.^{5–9} In this framework, it is reasonable to hypothesize that amyloidogenic aggregation may be a property of several types of peptides and that minimal aggregation determinants may exist. In the search for such fundamental determinants, short model peptides sequences have been shown to recapitulate the overall supramolecular behavior of more complex sequences.^{10,11} We characterized the

diphenylalanine (FF) peptide as a small module able to assemble into supramolecular assemblies, with biochemical and biophysical properties similar to amyloids.¹² Further investigation showed that even the single phenylalanine amino acid could give rise to ordered amyloid assemblies.¹³ This interesting finding was subsequently extended to other single amino acids (such as tyrosine) and other small metabolites (such as the nucleobase adenine): they were shown to accumulate in amyloid-like supramolecular structures and to induce cytotoxicity via apoptotic pathways, similar to their polypeptide counterparts.^{14–18}

Importantly, amyloid-like metabolite aggregates were observed in phenylketonuria patient brain tissues, whereby a mutation in the gene encoding phenylalanine hydroxylase results in its malfunctioning, which in turn causes the accumulation of phenylalanine and cells toxicity.¹³

It is clear that the availability of chemicals able to perturb the assembly of the amyloid-like metabolite aggregates could offer fresh perspective to the development of potential drugs for the treatment of metabolic disorders. To reach this goal, it is of primary importance to characterize, at the atomistic level of resolution, the mechanisms of metabolite aggregation and the

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Figure 1. Different types of aggregates formed in the ADE and EGCG simulations: (a,b) initial ordered adenine oligomers; (c) elongated adenine protofibril; (d) intercalation of EGCG molecules (in yellow); (e) EGCG (yellow) juxtaposes on preformed fibril and prevents more adenine molecules to participate to the elongation process.

effects that potential inhibitors may have on such mechanisms. While significant efforts have been spent and have reported success in explaining the mechanisms of peptide aggregation and inhibition via simulation^{3,19,20} as well as biochemical and biophysical characterizations of metabolite aggregates, a little is still known specifically on low molecular weight metabolite amyloid formation and disruption. Given the (even practical) complexities of such models, computational and simulative approaches can provide a viable means to elucidate the mechanistic details of metabolite amyloid formation, as well as those of potential inhibitors.^{9,21,22}

To make progress along this fascinating route, herein we study the mechanisms of ordered self-assembly of the purine adenine, which accumulates due to defects in the enzyme adenine phosphorybosil transferase, and how such mechanisms can be perturbed by a polyphenolic compound, epigallocatechin gallate (EGCG) via molecular dynamics simulations that was proved to be a useful tool to study complex mechanisms with the aspect of drug design.²³ The polyphenols family is known to inhibit the formation of protein amyloid fibrils.^{24,25} In that aspect we have shown that EGCG can interfere with ordered aggregation mechanisms, hijacking accumulated adenine to nontoxic, nonordered species.²⁶ EGCG concentrations of 0.5 and 1 mM were found to be able to inhibit fibrils of adenine obtained at 60 mM adenine concentration.²⁶ As a negative control in our simulations, we probe the effects of acetylsalicylic acid (ASA), which was shown not to have an effect on adenine aggregation.²⁶ The overarching goal of this study is to investigate, at atomistic resolution, the main traits of metabolite aggregation starting from completely random structures in solution and to shed light on the possible ways by which potential inhibitory leads may interfere with such mechanisms. In this context, it is important to notice that complementary simulations studying the binding of inhibitors to preformed amyloid nuclei had previously been carried out showing that EGCG tends to bind

to preformed fibrils with a more favorable interaction energy than ASA.²⁶

The data obtained by these studies can aptly be used to understand the main pharmacophoric features responsible for adenine amyloid like formation and to derive characterization of potential inhibitors of the fibrillation process.

With these aims in mind, we started molecular dynamics (MD) simulations of a pure adenine solution (simulation labeled ADE), in conditions mimicking the experimental ones in terms of metabolite and salt concentration (see [Supporting Information](#) for simulation details). Each simulation system was prepared by filling a cubic box with a side length of 100 Å with 44 molecules of adenine for simulation ADE, 40 molecules of adenine plus 4 molecules of acetylsalicylic acid for simulation ASA, 41 molecules of adenine, and 3 molecules of epigallocatechin gallate for simulation EGCG. In the initial configuration, adenine molecules were randomly distributed in a cubic box filled with water. After equilibration and thermalization, three independent copies of 300 ns long simulations are produced for each condition, providing 900 ns of total all atom sampling for each system to analyze.

The pure adenine solution simulation immediately showed the tendency for the nucleobase to form ordered structures. During the progress of the simulation, we observed the formation of ordered aggregates of different sizes, ranging from initial trimers to larger aggregates as large as hexamers and decamers. This type of hierarchical aggregation is largely reminiscent of what was previously observed in the case of peptide aggregation,^{3,9,21,22} whereby the formation of smaller oligomers and their rearrangements appeared to precede the formation of larger amyloid structures.

In both the small and in the larger aggregates of the ADE simulation, the aromatic rings pack on top of each other, forming parallel layers ([Figure 1a–c](#)). Interestingly, once a stacked motif is formed, other adenine molecules can establish hydrogen bonds across two monomers with motifs and geometries reminiscent of those observed for facing base

Figure 2. Time evolution of the number of free monomers in solution, dimers, trimers, and tetramers formed during the simulations.

Figure 3. (a) Distributions of the angles between the normal vectors to the plane of the purine bases, when the latter are in contact. (b) Dimensions of largest possible aggregate formed at distinct time points, defined in terms of the numbers of molecules with their respective COM-distances below 0.6 nm, irrespective of their identity and their relative orientations.

pairs in double-stranded nucleic acids (Figure 1a,b). As the ordered aggregate grows in dimensions, a clear elongated morphology for the supramolecular arrangement emerges (Figure 1c). While the limited simulation time does not allow to observe the formation of “real” fibril-like structures, it is tempting to suggest that the combination/juxtaposition of elongated, ordered oligomers may lead to the final formation of the amyloid metabolite aggregate. Interestingly, the observed behavior is consistently observed in the three replicates.

To investigate the effects of the presence of the generic fibrillation modifying polyphenol epigallocatechin gallate (simulation labeled EGCG), we added to the initial randomly arranged adenine molecules the number of EGCG molecules necessary to mimic the experimental concentration. In this context, it is interesting to observe that EGCG can establish a number of interactions with several adenine molecules, both through the formation of hydrogen bonding interactions (thanks to the relatively high abundance of hydroxyl functionalities in the molecules) and through pi-stacking type packing and van der Waals type of interactions. Analysis of the trajectories indicates that EGCG may act via two complementary mechanisms: on the one hand, the polyphenol can hijack adenine from the formation of amyloid-like ordered aggregates, trapping them in disordered oligomers in which the adenine planes are not optimally oriented for subsequent growth of supramolecular aggregates. On the other hand,

EGCG may intercalate in partially organized and elongated fibril like arrangements of adenine, blocking their potential seeding effects (Figure 1d,e).

Finally, we estimated the effects of the presence of acetylsalicylic acid in the adenine solution (simulation labeled ASA). It is worth noting here that acetylsalicylic acid was shown experimentally to be ineffective as inhibitor of adenine amyloid fibrillation.²⁶ Indeed, it was used as negative control, e.g., in the analysis of the cytotoxic effects of aggregates. Consistent with these experimental observations, our simulations show limited interactions of acetylsalicylic acid both with small adenine aggregates and with larger fibril-like supramolecular arrangements. Furthermore, no intercalation similar to that observed for EGCG is observed in this case. Replicate simulations for the EGCG case and for the ASA case confirm this observation.

To put these observations on a more quantitative ground, we set out to define a set of parameters able to report on the dimension of the forming aggregates as well as on the relative orientations of the adenine monomers. First of all we calculated all the distances between the centers of mass (COM) of adenine molecules and defined two adenine molecules to form a stable complex that may lead to metabolite fibril formation if the distance between their two COMs is below 0.6 nm (Figure 2) and if the angle between the normal vectors to the plane of the purine is close to zero. The latter

criterion is aimed to define the orientation leading to the formation of stacked arrangements. We then clustered the distributions of distances with a cutoff of 0.6 nm and evaluated the number of elements present in each cluster at different simulation times along the trajectories: such number provides an indication of how many dimers, trimers, etc., are present in the simulation box at any given time. Analogous graphs for the independent replicas are presented in the [Supporting Information](#) (Supplementary Figure SI1), supporting the validity of the observation.

Interestingly, the ADE simulation shows the minimum number of single adenine molecules free in solution. This is paralleled by the presence of a significant number of dimers (around 10 on average), trimers (around 5), and tetramers (around 3). In the presence of EGCG, these parameters are significantly modified: the number of free adenine molecules raises, with a sharp decrease in particular in the numbers of trimers and tetramers. The ASA simulation shows a situation similar to that of the ADE simulation, once more indicating the low tendency for acetylsalicylic acid to interfere with the initial steps of adenine amyloid aggregation. Overall, in the ADE simulation, tetramers are present in more than 95% of the frames, in the ASA simulation they are present in about 80% of the frames, while this number decreases to about 70% in the presence of EGCG.

We next looked at the distribution of the angles between the normal vectors to the plane of the purine bases, when the latter are in contact ([Figure 3a](#)): interestingly, the values of the angles defined by such vectors are always smaller in the case of the pure adenine simulation, compared to the larger values consistently observed in the presence of EGCG. This result indicates that the presence of EGCG influences the relative orientations of the adenine monomers, disfavoring the parallel arrangement of aromatic planes necessary for stacking and growth of the aggregates.

One final interesting piece of observation comes from the evaluation of the dimensions of largest possible aggregate formed at distinct time points, defined in terms of the numbers of molecules with their respective COM-distances below 0.6 nm, irrespective of their identity and their relative orientations. This analysis shows that large yet disordered aggregates may form in the EGCG simulation, suggesting that the polyphenol establishes a number of interactions with a number of monomers and with a geometric arrangement that is not compatible with the stacked geometry hypothesized as relevant to give rise to the amyloidogenic aggregation ([Figure 3b](#)). In such disordered oligomers, EGCG sequesters adenine monomers by establishing extensive h-bonding interactions that outcompete ordered oligomer formation via parallel-plane stacking. In this case, too, the results of the three independent replicas for each system are presented in the [Supporting Information](#) (Supplementary Figure SI2).

Overall, our results shed light on the possible mechanism of metabolite amyloid formation and provide molecular details on the potential inhibition mechanisms that may contrast/block such phenomena. From a drug design and discovery point of view, we suggest that candidate inhibitors should interfere with both stacking, through aromatic/hydrophobic moieties, and hydrogen bonding, through functionalities that can sequester adenine molecules from ordered aggregation. One potentially viable strategy of intervention could be represented by the use of drug-multipresentation strategies: considering the complex molecularity and the diversity of possible "targets" in the

aggregation process, displaying multiple copies of the inhibitors through multivalent supports could aptly increase the effectiveness of metabolite fibrillization inhibition.^{27–29} On this basis, we are currently pursuing drug-like molecules that will inhibit the self-assembly process of adenine.

In summary, we have shown that it is possible to study the complex processes of metabolite aggregation and inhibition at atomistic levels of detail via molecular simulations. These data can be used to extract the fundamental chemical determinants necessary to design potential new drugs able to interfere with adenine aggregation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the [ACS Publications website](#) at DOI: [10.1021/acsmmedchemlett.9b00024](https://doi.org/10.1021/acsmmedchemlett.9b00024).

Detailed description of the methodology and the computational setup ([PDF](#))

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

MD, molecular dynamics simulation; EGCG, epigallocatechin gallate; ASA, acetylsalicylic acid.

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