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A Molecular Dynamics Study of the Interaction of D-Peptide Amyloid Inhibitors with Their Target Sequence Reveals a Potential Inhibitory Pharmacophore Conformation

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The self-assembly of soluble proteins and peptides into β-sheet-rich oligomeric structures and insoluble fibrils is a hallmark of a large number of human diseases known as amyloid diseases. Drugs that are able to interfere with these processes may be able to prevent and/or cure these diseases. Experimental difficulties in the characterization of the intermediates involved in the amyloid formation process have seriously hampered the application of rational drug design approaches to the inhibition of amyloid formation and growth. Recently, short model peptide systems have proved useful in understanding the relationship between amino acid sequence and amyloid formation using both experimental and theoretical approaches. Moreover, short D-peptide sequences have been shown to specifically interfere with those short amyloid stretches in proteins, blocking oligomer formation or disassembling mature fibrils. With the aim of rationalizing which interactions drive the binding of inhibitors to nascent β-sheet oligomers, in this study, we have carried out extensive molecular dynamics simulations of the interaction of selected D-peptide sequences with oligomers of the target model sequence STVIIE. Structural analysis of the simulations helped to identify the molecular determinants of an inhibitory core whose conformational and physicochemical properties are actually shared by nonpeptidic small-molecule inhibitors of amyloidogenesis. Selection of one of these small molecules and experimental validation against our model system proved that it was indeed an effective inhibitor of fibril formation by the STVIIE sequence, supporting theoretical predictions. We propose that the inhibitory determinants derived from this work be used as structural templates in the development of pharmacophore models for the identification of novel nonpeptidic inhibitors of aggregation.

Keywords: aggregation; amyloid inhibition; molecular dynamics; pharmacophore structure; drug design

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Abbreviations used: MD, molecular dynamics; Aβ, amyloid β; IAPP, islet amyloid polypeptide; PR, phenol red; 3D, three-dimensional; EGCG, epigallocatechin gallate; EM, electron microscopy.

Introduction

Protein misfolding, amyloid oligomerization, and further fibril formation are central events in the pathogenesis of more than 20 human disorders known as amyloid diseases.[1] Drugs that are able to interfere with these processes may be able to prevent and/or cure these conditions. Indeed, "amyloid aggregation inhibition" is currently a widely explored therapeutical strategy against amyloidosis.[2] This approach relies on the identification of molecules that can interfere at different stages of the aggregation process.

Active research in recent years has identified a number of molecules that are able to prevent not only the protein/protein interactions leading to amyloid formation but also their associated cellular toxicity.[3–6] However, only in a few cases has a mechanism of inhibition been proposed.[7,8]

Recently, soluble β-sheet oligomeric species have been identified as the main cause of cellular toxicity,[9–14] making them clear targets for the design of antiamyloidogenic drugs. However, the transient nature of these early oligomers has hampered the characterization of their structural–dynamical properties at the atomic level of resolution. The lack of detailed structural models for the different species on—and off—the amyloid pathway that might represent potential drug targets has seriously limited the potential of rational drug design and has hindered efforts to rationally improve the efficacy of lead molecules.

A successful strategy to untangle important aspects of amyloid fibril formation has been the identification or design of small model systems that recapitulate the biophysical and toxic properties of naturally occurring amyloid proteins.[15–18] Studies using a *de novo*-designed peptide, STVIIE, revealed the importance of net charge in driving the fibrillization process[15] and allowed us to propose a detailed structural model[15] and also to extract an amyloid sequence pattern[19] that can detect amyloidogenic fragments in proteins. These short amyloidogenic stretches have been proven to trigger amyloid formation by nonamyloidogenic proteins[20] and also to form aggregates as toxic in cell culture as the ones from naturally occurring amyloids.[21] Their small sizes make them also ideally suited for theoretical investigations. Indeed, molecular dynamics (MD) simulations of different point mutants of the *de novo*-designed STVIIE helped explain sequence effects on amyloidogenic behavior in terms of molecular interactions.[22] Moreover, the toxic amyloid oligomeric state that should be ideally targeted is particularly suited for computer simulations. For example, the aggregation of small oligomers, ranging from dimers to octamers, has been examined using a number of computational methods and protein energy models.[23–29]

The combination of experimental and theoretical studies on small amyloidogenic systems that allow pinpointing of the effect of the perturbation of single interactions on the final aggregated state may actually prove to be an ideal approach also for the development of inhibitors of the amyloid process. Indeed, MD simulations of the interaction of amyloid inhibitors with short peptides have recently started to tackle this problem.[30,31]

In this context, we have recently demonstrated that short amyloidogenic protein stretches can be used as targets for the screening or design of antiamyloid compounds that can revert amyloid formation and induced cytotoxicity by the full-length amyloid protein.[32] Based on the concept that amyloid formation is stereospecific,[33,34] we hypothesized that an appropriate D-amino acid sequence that is able to interact with these short amyloidogenic stretches in proteins will break the stereospecific contacts of the L-amyloid species and impair their growth.[32] In fact, D-peptides have been previously reported as potent inhibitors of the amyloid formation of the amyloid β (Aβ) 42 peptide, an Alzheimer's disease peptide.[35–38] D-Peptides containing the key amyloidogenic motif of the Aβ42 peptide KLVFFA have been shown to be more effective inhibitors of Aβ amyloid formation and toxicity than the respective L-diastereoisomers.[36] The purpose of our prior work was to explore the D-amino acid sequence space to identify molecules that can disrupt the stereochemical requirements for the self-assembly of our peptide model system, STVIIE. To this end, we used a combinatorial approach to identify D-peptide sequences that can interfere with the process of STVIIE amyloid formation. Analysis of the sequences of the D-peptides as a function of their inhibitory effect suggested that the location of aromatic residues modulates the efficacy and the inhibitory activity exerted by the D-peptide. With the aim of rationalizing these results, in this study, we have carried out extensive MD simulations of the interaction of selected D-peptide sequences that exhibit different inhibitory activities with small β-sheet oligomers of STVIIE, representing the initial oligomerization states of the target sequence.[22] Structural analysis of the simulations suggests a possible inhibitory core whose conformational, stereochemical, and physicochemical properties are actually shared by known small-molecule inhibitors of the amyloid formation and cytotoxicity of islet amyloid polypeptide (IAPP) and Aβ.[39–41] These observations have been experimentally challenged by performing inhibition assays of one of these molecules, phenol red (PR), against the amyloid peptide STVIIE used in this study. The results support the hypothesis that PR is indeed an effective inhibitor of fibril formation for this sequence as well. We have also generated pharmacophore models based on the common chemical features of a diverse training set of 32 nonpeptidic small amyloid inhibitors. Interestingly, the inhibitory core identified in the active D-peptides matches them.

Hence, we propose that the stereospecific organization of structures and functionalities for D-peptides identified in this work may be shared by known inhibitors of the amyloid formation of other peptides such as Aβ and IAPP. The stereospecific arrangement of chemical groups displayed by the inhibitory core can, in turn, be used as template for the identification and/or design of new small molecules with amyloid-inhibiting capabilities.

Results

D-Peptides selected for simulation

In a previous work, we studied the effect of 32 defined D-hexapeptide sequences on different oligomerization states of STVIIE.[32] These D-peptides were synthesized based on the results of the screen-