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Interaction-based evaluation of the propensity for amyloid formation with cross-β structure ☆

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Abstract

In order to reveal the requirements for amino acid sequences prone to form amyloid fibrils, a novel prediction method based on the original structural model of amyloids was developed. As a working hypothesis, two fundamental conditions were introduced into the design of the present system for the evaluation of the propensity for amyloidogenicity. The first of these two conditions was to ensure that the hydrophobic and hydrogen-bonding interactions between residues on neighboring antiparallel β-strands were formed along a fibril axis. The other condition was that the hydrophobic interacting residues appeared on both faces of the protofibril, which gave line-matching interactions. Most peptides with sequences exhibiting high scores, as evaluated by this method, were found to easily form amyloids with the aid of a turn-inducing structure designed as a connection of two β-strands. On the other hand, peptides with low-scoring native sequences and those modified by an internal residue–residue exchange (the latter yielding a null score) did not lead to amyloid formation. These data demonstrated the validity of this method for the prediction of amyloid structures. Moreover, the present study provided support for the proposed model of the essential structure associated with the above working hypothesis. The predicted high-scoring regions were in good agreement with the putative amyloid core regions reported thus far.

Keywords: Amyloid; Core cross-β; Propensity score; Line-matching; Hydrophobic interaction; Amyloidogenicity; Antiparallel; Amyloid β; β_2-Microglobulin; α-Synuclein; Prion

Knowledge about the structure of amyloid fibrils is crucial for the therapeutic treatment and clinical analysis of amyloid-related diseases. Recent X-ray analysis has revealed the atomic-resolution structure of the heptapeptide crystal with an assembly analogous to that in fibrils [1]. However, it should be noted that actual fibril structures remain unidentified by direct methods of analysis. Recently, H/D exchange NMR analysis has been successfully used to identify amyloid core regions in amino acid sequences, but not 3-dimensional structure [2,3]. Scanning mutagenesis has also proven to be a powerful technique in the assignment of amyloid core regions [4]. Based on the development of experimental methods that could provide valuable information regarding the sequences of core regions, theoretical methods will need to play complementary roles in the construction of real 3-dimensional structures of amyloids, as direct analysis of these structures remains extremely difficult.

As regards rule of the sequence that forms amyloids, a binary patterning model has been proposed that involves the alternation of polar and nonpolar residues [5]. More recently, methods for the prediction of the aggregation propensity of amino acid sequences have been developed. One such method is the TANGO algorithm, which was designed to calculate the partition functions of the phase-space including β-aggregate, α-helix, β-turn, random coil, and native conformation [6]. Another method was devised to calculate the Z-score for aggregation as based on the propensity score function, which is expressed by the optimized linear combination of the terms for the following: hydrophobicity,

☆ *Abbreviations:* ThT, thioflavin T; CD, circular dichroism.

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α-helical propensity, β-sheet propensity, hydrophobic patterning, and net charge effect [7]. Though these methods that make use of the statistical parameters have successfully predicted the regions of β-aggregation to some extent, a rational structural model for amyloids is necessary in order to clarify the structures of amyloid cores.

Recently, we developed a structural model that takes into account inter-strand stabilization with hydrophobic and/or hydrogen-bonding interactions between the side chains of neighboring hydrogen-bonded strands [8]. According to our model, the interacting side chains are aligned along a fibril axis to form lines such as $[(n+3),(n-3),(n+3)',(n-3)',\ldots]$, as indicated by the red line in Fig. 1A. The association of protofibrils to each fibril is most likely to be realized by an interaction between lines of hydrophobic residues, referred to as a "line-matching interaction" (Fig. 1B). This type of interaction plays an important role in coordinating the relative positions of protofibrils in the x–y plane, as illustrated in Fig. 1C. The orientation of the neighboring β-strands occurs in an antiparallel manner, and the neighboring protofibrils are orientated in C_2 symmetry around the axis parallel to the z-axis (Figs. 1B and C).

In the present study, we propose a novel method for the prediction of amyloid core regions that is based on our rational structural model. Using various synthetic peptides, the effectiveness and reliability of this method were verified in detail. This interaction-based prediction is expected to lead to a better understanding of amyloids, as well as to the identification of amyloid core regions.

Materials and methods

Calculation of scores reflective of the propensity for fibril formation. The method described in the present paper is basically applicable to both parallel and antiparallel β-sheet formation with small modification in the expressions. However, we focused on the antiparallel system because of its relatively high frequency in globular proteins such as Greek-key motif [9]. In the case that two sequence regions, $(n \to n+k-1)$ and $(m \to m+k-1)$, form an antiparallel β-sheet, the degree of hydrophobic interactions (A) between the paired β-strands is estimated by the

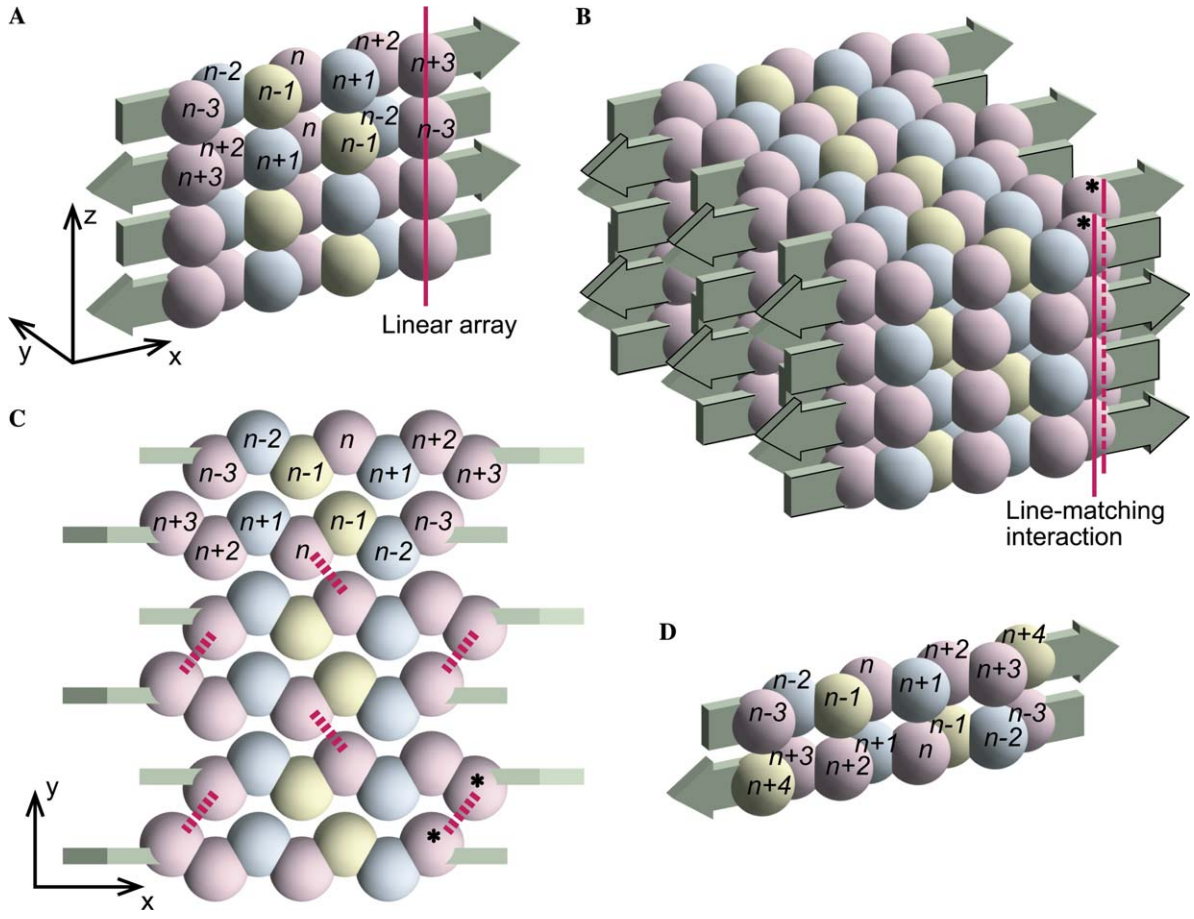

Fig. 1. Structural model of amyloid fibrils used for the evaluation of the propensity scores for amyloid formation. (A) Anti-parallel alignment of β-strands forming odd-length pairing. Each ball represents an amino acid residue, of which the general number is superimposed. The red line indicates the linear array of hydrophobic residues as an example. Cartesian coordinate system (x, y, z) is defined as in parallel to the directions of a thickness, a width, and an axis for the fibril, respectively. (B) Oblique projection of a part of the amyloid fibril. A couple of asterisks indicate the line-matching interaction between two linear arrays of hydrophobic residues. (C) Cross-sectional view of the amyloid fibril. The dotted line segments represent the line-matching interaction between the neighboring protofibrils. An example of the pattern with this interaction is shown. (D) Anti-parallel alignment of β-strands with even-length pairing.