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On the molecular mechanism of amyloid fibrillogenesis

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Amyloid fibrils are known to form out of partially unfolded human lysozyme through self-assembly of its disordered sequences into a cross-β structure. A new molecular mechanism for this process is proposed based on analogies with the mesophase and microsegregation behaviour of copolymers composed of rigid and flexible blocks. According to the model, α-helical sequences play an essential role on the stabilization of fibrils. The model ought to apply to globular–fibrillar transformations occurring for other proteins and conformational incompatibility might play a role on the stability of native tertiary structures.

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1. Introduction

The folded native structure of human lysozyme reveals the occurrence of several helical and less ordered segments within the sequence, see figure 1 (*a*). Perturbation of the native structure (by mutations, pH lowering or a slight temperature increase) favours the occurrence of so-called amyloidal fibrils[1–4]. These may be found in extracellular aggregates occurring in organs such as the brain, liver and heart, and are associated with amyloid diseases such as Alzheimer's and type II diabetes [5]. Studies of the fibrillar state by X-ray fibre diffraction suggest the occurrence of β-sheets oriented perpendicular to the fibre axis [6]. TEM studies on a variant of human lysozyme suggest that the fibres are unbranched, and attain lengths in the μm range with diameter *c.* 10 nm [4].

The current view on the mechanism of amyloid fibrillation has been recently summarized by Dobson [7]. Amyloidogenic mutations—e.g. Ile 56 Thr and Asp 67 His localized in the disordered regions of the sequence, figure 1 (*a*)—are regarded as a primary factor in the destabilization of the native structure. Out of the partially unfolded protein, the cross-β structure evolves by self-assembly of disordered sequences and an unknown organization of adjacent helical segments, figure 1 (*b*). Fragments of disordered segments readily aggregate into the cross-β structure [8]. On the other hand, fibrils are not readily produced when the protein is completely denatured. It transpires that the detailed driving force for the formation of the cross-β fibrils, and the role of the helical segments, are not completely understood.

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2. Proposed mechanism

A new approach to the assembly mechanism of amyloid fibrils is outlined here. The approach is based on an extension of mechanisms controlling the self-assembly of supramolecular polymers which were recently reviewed [9, 10]. The transformation of a globular structure such as that in figure 1 (*a*) into cross-β fibrils will be discussed on the basis of the incompatibility between residues along the sequence, with proper consideration for the occurrence of segments, or blocks, having different conformational order.

The role of hydrophobic and hydrophilic interactions and the analogy between globular and micellar structures in the presence of water has been frequently discussed. Charged and apolar groups are just an extreme example of chemical incompatibility. The customarily used compatibility parameter χ (from the Flory–Huggins theory of real solutions) is extremely sensitive to minute differences in the polarity of substituents, and can be calculated for simple sequences and block copolymers even in the absence of a selective solvent [11]. Values of χ larger than *c.* 0.5 (depending upon segment length) suggest poor compatibility, demixing of unconnected chains and microsegregation of copolymers [9].

The thermodynamic basis for microsegregation of two incompatible A and B blocks can be described using the basic concepts of a mean-field theory (SCFT) which successfully describes the behavior of coil–coil

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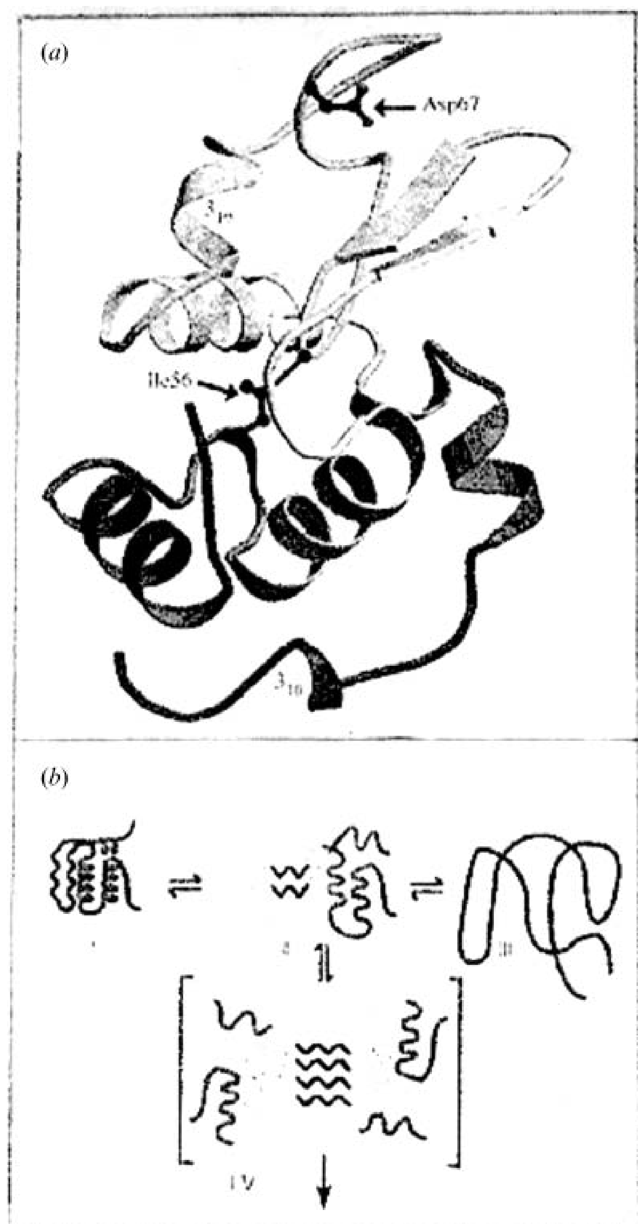

Figure 1. (*a*). Schematic represectation of the folded structure of human lysozyme including the amyloid mutations Ile56Thr and Asp67His. Four disulfide bonds are not shown. (*b*). Schematic represectation of current view: during amyloid fibrillogenesis the disordered segments of the partially unfolded protein (II) enter the cross-β structure of the fibrils. The latter is not formed by the native (I) or denatured (III) protein. Adapted from [1].

(chemically homogeneous) diblock copolymers in the bulk state [10, 12] (see [13] for extension to solutions). As shown schematically in figure 2 (*a*), chemically compatible blocks only produce structurless melts. However, if the two blocks are chemically incompatible, each block stretches out, promoting longitudinal recognition and growth of neighbouring domains

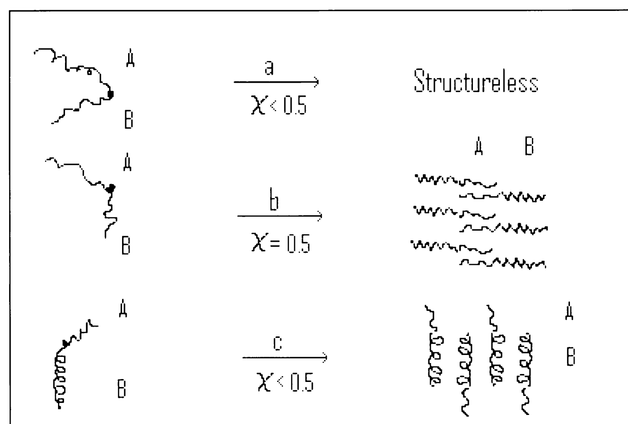

Figure 2. Schematic represectation of the self-assembly in the molten state of A and B *segments* into corresponding A and B *domains* for: (*a*) coil–coil copolymers formed from compatible segments, (*b*) coil–coil copolymers formed from incompatible segments; (*c*) helix–coil copolymers formed from compatible segments. Microsegregation is driven by chemical incompatibility in case (*b*) and conformational incompatibility in case (*c*).

separated by a surface containing the intersegmental bonds, figure 2 (*b*). Such an assembly prevents the occurrence of energetically unfavourable contacts between the incompatible A and B segments. The cost is a conformational entropy loss (controlled by chain flexibility and the total number of residues) due to chain elongation.

The application of this approach to the fibrillogenesis of lysozyme entails an evaluation of the chemical compatibility between its helical and disordered blocks. However, a complication arises from the fact that each block includes different amino acid residues and therefore is not chemically homogeneous. This problem could be circumvented by assigning average χ parameters to each block or by focusing on the parameters pertaining to the pair of residues joining the blocks. Significant incompatibility is anticipated upon closer inspection of the sequence. The conformational difference between the two blocks also supports their considerable compositional difference. However, since cross-β fibrils were not readily observed for the structurless (denatured) protein, one must conclude that the distribution of chemically incompatible residues may be a contributing but not a sufficient condition for the microsegregation of its disordered blocks into cross-β fibrils.

We should therefore consider a direct role of conformational differences in the compatibility of lysozyme blocks. The first consideration is that the recognition and microsegregation of disordered blocks is enhanced when the adjacent blocks are in the helical form. In terms of the mean-field theory, the entropy penalty should be smaller when one block is in the α-helical rather than coiled conformation.

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There is, however, an additional subtler contribution of conformational differences to segmental compatibility. The conventional analyses of compatibility parameters, and the free energy of mixing in isotropic solutions, do not require an assessment of solute conformation. However, the large volume fraction and the axial ratio of the α-helical blocks within the partially unfolded protein should favour their liquid crystalline order [14, 15]. For instance, the axial ratio (X) of helical segments containing up to 20 amino acids residues is ≈ 6. In terms of the virial theory ($v^* \approx 3.3/X$) [15], this corresponds to a critical volume fraction $v^* \approx 0.5$, certainly smaller than reported compositions of α-forming residues (cf. Protein Data Bank). In the LC state, a large conformational incompatibility between rigid and flexible segments is known to occur [14]. If the connections between the blocks were severed, demixing would be expected, with the phase rich in helical blocks displaying liquid crystallinity above a critical concentration. A corresponding increase of the critical concentration was reported for copolymers of rigid and flexible blocks [16], revealing that the flexible blocks are excluded from the mesophase of the rigid ones. Microsegregation does occur, figure 2 (*c*), even for chemically compatible blocks, and promotes aggregation of less ordered segments of several protein molecules spaced by LC domains of α-segments [15, 17].

It is to be noted that the predictions of the SCFT theory include the occurrence of instability modes that direct the formation of mesophases such as the familiar hexagonal columnar and lamellar phases. In these phases, the distribution of A and B blocks is well defined and can be verified by TEM [18]. Lysozyme should however be regarded as a multiblock system exhibiting the additional complication of variable length and composition of each block. In such a situation it is not easy to predict or verify the overall morphology. Only the organization of β-blocks of lysozyme is easily verified due to the propensity of their fragments to form the cross-β structure [8].

Attempts to elucidate the molecular packing of the sequence for SH3 domains of an alternative protein (largely unfolded bovine PI3 kinase) have been made using high resolution cryo-electron microscopy [19]. Fibrils appear, composed of four twisted protofilaments defining the surface of a hollow tube in which the β-sheet domains are interconnected by the remaining domains in an unspecified conformation. The role of both helical and less ordered blocks, in terms of the mechanism outlined here, could be assessed by simulation or by characterization of the compatibility and morphological features of model diblock and multiblock copolymers based on segments able to assume different conformations under controlled conditions [20].

3. Conclusions

The present approach suggests an answer to some unsolved issues regarding amyloid formation, particularly the mechanism of fibrillation and the role of helical sequences. The latter appear much more important than anticipated. Fibrillation is prevented in the native state when the net balance of supramolecular interactions (including four disulphide bonds) prevails over conformational incompatibility and locks-in the folded structure. Fibrillation is also prevented in the fully denatured state since closer contacts when *all* segments are randomly coiled hinder recognition (including H-bond formation [21]) of similar sequences. Incompatibility between helical and disordered segments is however enhanced within the partially folded protein. This leads to transient segregation into adjacent domains and ensuing fibrillation.

As pointed out by Dobson [7], fibrillation is a process relevant to a wide variety of amyloidal diseases [5] and also to intercellular deposition as occurring in Parkinson's and Huntington's diseases [22]. Fibrillation *in vitro* was also observed for proteins unrelated to known diseases [7]. In fact, the assembly mechanism based on block-copolymer recognition outlined here might be relevant to a variety of transformations from globular to fibrous proteins. More detailed study should investigate the possibility that conformational incompatibility affects the distribution and clustering of helical segments, even within the native structure, and that the latter is not in a thermodynamically stable state. The author has already suggested that conformational incompatibility drives the age-induced segregation and loss of properties for natural systems based on blends of rigid and flexible polymers (e.g., the vitreous body of the eye) [23].

A final point concerns the general issue of prevention or treatment of amyloid disorder. The therapeutic strategies of increasing the stability of the protein [24], hindering variants [25], gene therapy, and blocking fibril growth [26] have been discussed [7]. The approach presented here suggests that a reduction of the stability of α-helices ought to be a way to improve block compatibility, thereby hindering (or reversing) microsegregation and the ensuing fibrillation.

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