Minireview

Transthyretin amyloidosis: a tale of weak interactions

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Received 24 April 2001; accepted 27 April 2001

First published online 16 May 2001

Edited by Gunnar von Heijne

Abstract Over 70 transthyretin (TTR) mutations have been associated with hereditary amyloidoses, which are all autosomal dominant disorders with adult age of onset. TTR is the main constituent of amyloid that deposits preferentially in peripheral nerve giving rise to familial amyloid polyneuropathy (FAP), or in the heart leading to familial amyloid cardiomyopathy. Since the beginning of this decade the central question of these types of amyloidoses has been why TTR is an amyloidogenic protein with clinically heterogeneous pathogenic consequences. As a result of amino acid substitutions, conformational changes occur in the molecule, leading to weaker subunit interactions of the tetrameric structure as revealed by X-ray studies of some amyloidogenic mutants. Modified soluble tetramers exposing cryptic epitopes seem to circulate in FAP patients as evidenced by antibody probes recognizing specifically TTR amyloid fibrils, but what triggers dissociation into monomeric and oligomeric intermediates of amyloid fibrils is largely unknown. Avoiding tetramer dissociation and disrupting amyloid fibrils are possible avenues of therapeutic intervention based on current molecular knowledge of TTR amyloidogenesis and fibril structure.

Key words: Transthyretin; Amyloid; Familial amyloidotic polyneuropathy; Thyroxine; Iododoxorubicin

1. Introduction

Transthyretin (TTR) amyloidosis is a systemic form of amyloidosis that is in the vast majority an hereditary disease. Over 70 different TTR mutations have been described associated with amyloid deposition; typical manifestations are peripheral neuropathy, cardiomyopathy, carpal tunnel syndrome and vitreous opacities. Peripheral neuropathy is the main symptom in familial amyloid polyneuropathy (FAP), while cardiomyopathy constitutes the main or the sole clinical symptom in familial amyloid cardiomyopathy (FAC) [1]. Non-mutated TTR can also deposit as amyloid in the heart of old people, a condition termed senile systemic amyloidosis. TTR is a plasma protein composed of four identical subunits with an extended β-sheet conformation, as revealed by X-ray crystal structure [2]. Several amyloidogenic variants have their crystal structure determined and while some of them do not show significant differences from the wild-type (WT) protein, others point to a transmission of structural events, associated with the mutation, which destabilize the quaternary protein structure. It is current opinion that the modified TTR represents an amyloidogenic intermediate, which integrates the fibril structure [3]; analyses of FAP fibrils have proved that TTR in the fibrils maintains a β-conformation and suggested that the TTR monomer is the building block in fibrils [4]. Further studies on native and synthetic TTR fibrils using high-resolution structural techniques will further elucidate fibril structure and the aggregation pathway. This review summarizes current trends in research in TTR aggregation towards prevention and treatment.

2. Amyloidogenic conformations

The 3D structure of TTR has an extensive β-sheet structure; each monomer contains two β-sheets, composed of strands DAGH and CBEF, which interact face-to-face through hydrogen bonds between strands HH′ and FF′ to form a dimer. In the tetramer (represented in Fig. 1), hydrogen bonds between main chain atoms belonging to loop AB of one monomer and strand H′ from the other monomer as well as hydrophobic contacts are important. The effects introduced by amyloidogenic mutations have been intensively studied in an attempt to identify conformational changes rendering the molecule vulnerable to aggregation. Among naturally occurring TTR mutations Val30Met is the most common and was the first to be crystallized and studied by X-ray diffraction. No major changes (as compared to the known 3D structure of the WT protein) were detected except for a movement of cysteine residue 10 opening a channel that runs through the molecule, where the thyroid hormone thyroxine (T4) binds [5]. One of the consequences of this change is an altered binding affinity of T4 for TTR. Another amyloidogenic mutation studied by X-ray crystallography was isoleucine 122, which lies in the region where two TTR dimers assemble to form a tetramer. Again in this case no drastic structural changes were observed, except for weaker bonding at dimer–dimer contacts [6]. This finding was in accordance with earlier speculations in the beginning of the decade that mutations in TTR may change subunit self-assembly properties, weakening interactions between subunits [7]. TTR Leu55Pro has received much attention because it is associated with a highly aggressive form of

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PII: S0014-5793(01)02480-2

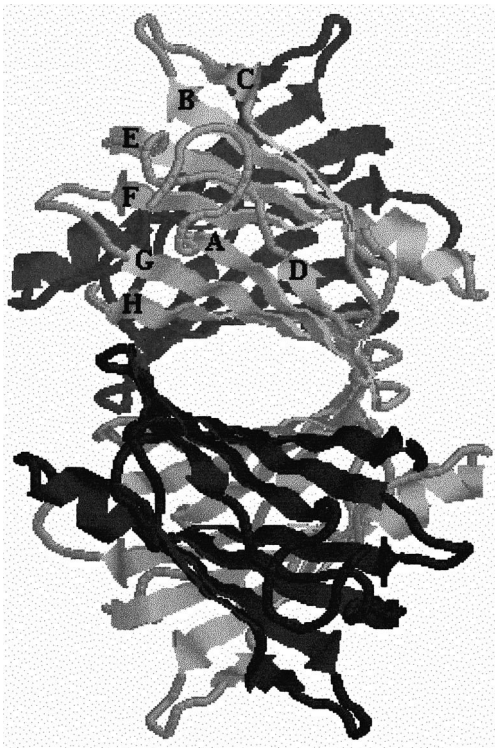

Fig. 1. 3D structure of TTR. The β-strands of one monomer are indicated by letters A to H.

FAP. X-ray studies on this mutant revealed drastic changes brought about by the mutation leading to aggregation of TTR having monomers as building blocks [8]. This is consistent with data from synchrotron analyses of 'ex vivo' fibrils [4] and indicated important changes in secondary structure by the disruption of strand D, which becomes part of a long loop that connects strands C and E. Disruption of the D strand affects the hydrogen bonding with the A strand, exposing new surfaces involved in aggregation; in particular, the contacts of the α-helix and the AB loop are different, suggesting these regions are important in amyloidogenesis. In fact, deletion or multiple substitutions in the D strand lead to highly amyloidogenic mutants. When monoclonal antibodies were raised against these aggressive mutants, one particular antibody did not recognize native TTR but only TTR fibrils [9]. Furthermore, this antibody was able to discriminate between sera from FAP patients and sera from control individuals [10], suggesting that TTR from carriers of amyloidogenic mutants circulates in a modified soluble form. In an attempt to identify early soluble intermediates of the fibrillogenesis cascade, a mutant TTR, TTR Thy78Phe, was generated by site-directed mutagenesis having the contacts between the α-helix and the AB loop destabilized (as evidenced in the X-ray structure of the Leu55Pro mutant). This mutant is recognized in its soluble form by the above-mentioned monoclonal antibody, specific for the amyloid fold, and represents a putative early modification in the cascade leading to TTR aggregation [11]. In the Tyr78Phe mutant, the hydrophobic interactions are changed at dimer–dimer interfaces and less stable tetramers with higher propensity for amyloid formation are generated. Thus, mutations in TTR that loose the AB loops of the tetramer and other dimer–dimer interactions increase the susceptibility of amyloid formation.

3. Self-assembly

Self-assembly properties of different TTR mutants have been widely investigated by different approaches.

Studies on the influence of TTR mutations on tetrameric stability showed dissociation into non-native monomeric species at physiological pH, followed by self-association of this intermediate into amyloid fibrils [3,12]. This was possibly due to subtle conformational modifications that occur in the TTR molecule before dissociation into monomers with altered tertiary structure. These authors had previously established a relation between the amyloidogenic potential of TTR variants and the decreased stability of the tetramer, with subsequent dissociation to monomeric species, with the Leu55Pro variant being the less stable mutant [13]. Colon and Kelly [14] were the first to present evidence for the existence of amyloidogenic intermediates after partial acid denaturation. This amyloidogenic intermediate was postulated to contain most of the native structure except for the rearrangement involving strands C and D. In this model, FAP mutations would not affect the structure of the folded state (tetramers) but would favor the denaturation pathway and/or degradation pathway(s) for TTR turnover.

Self-assembly properties were particularly investigated with studies on a non-amyloidogenic TTR, TTR Thr119Met. This mutant is frequent in Portugal, and compound heterozygotic individuals for Met30/Met119 have been found to exhibit a more benign form of the disease. Comparative studies of amyloidogenic TTR Val30Met and the non-amyloidogenic TTR Thr119Met by semi-denaturing isoelectric focusing revealed that TTR Val30Met has a higher tendency for dissociation of the tetramer into monomers than the WT TTR. In contrast, Thr119Met showed higher resistance to dissociation into monomers than the WT protein, in addition, TTR from compound heterozygotes Met30/Met119 behaved like WT TTR [15]. Thus, one possible way by which Thr119Met can exert anti-amyloidogenic effects is by counteracting the weaker subunit interactions of Met30 tetramers. Recent X-ray studies on the Thr119Met mutant revealed that new hydrogen bonds within each monomer and monomer–monomer intersubunit contacts increase protein stability possibly leading to the protective effect of the TTR Met30/Met119 variant when compared to the single variant TTR Val30Met [16].

4. Drug design: tetrameric stabilizers and fibril disrupters

The protective stabilizing effect of the Thr119Met mutation on the Val30Met weaker subunit interaction is, in fact, the basis for the rational design of drugs in TTR amyloidosis. The

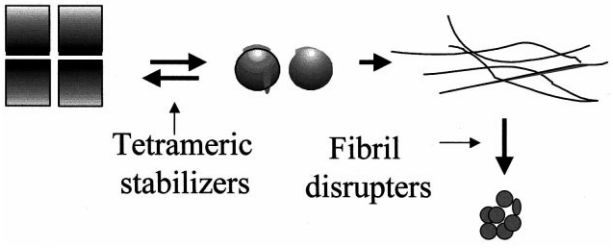

Fig. 2. TTR tetrameric stabilizers and fibril disrupters as potential drugs in the treatment of TTR amyloidosis.

aim is at binding TTR in the central hydrophobic channel that runs through the molecule, where the hydrophobic hormone T4 binds, to prevent dissociation into monomers (see Fig. 2). X-ray crystallographic analyses of different TTR–stabilizer complexes have elucidated the binding modes in the hormone binding cavity and contacts between adjacent TTR subunits [17]. So far, these drugs have been shown to act 'in vitro' but 'in vivo' studies to make them relevant for clinical use are much awaited. Sulfite has been shown both 'in vitro' and 'in vivo' to prevent TTR tetramers from dissociating but the structural basis for this effect has not been elucidated [18].

Another class of drugs with potential application in TTR amyloidosis are fibril disrupters (see Fig. 2). Recently, 4′-deoxy-4′iododoxorubicin (IDOX) has been proved useful as a tool for disrupting TTR amyloid fibrils [19]. The amyloid disrupters might also prove useful for amyloidoses associated with other protein precursors, such as the β protein in Alzheimer and the prion scrapie protein. Due to its cardiotoxicity, IDOX analogues able to disrupt amyloid fibrils are needed. The molecular interactions between IDOX and Leu55Pro TTR 'amyloid-like' oligomer have been theoretically modeled [20], allowing the design of analogues with fibril disrupting properties.

5. Final remarks

Growing knowledge on the molecular events leading to TTR aggregation makes possible the development of therapeutic agents; however, better therapeutics will be possible when more is known on other aspects of this fascinating group of diseases, namely on genetic and environmental modulators of phenotypic expression and on the mechanisms of cell death, aspects not reviewed here.

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