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Structural basis for the protective role of sulfite against transthyretin amyloid formation ☆

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Abstract

Transthyretin (TTR) is a plasma protein, which under conditions not yet completely understood, aggregates forming amyloid deposits that occur extracellularly. It is a protein composed of four identical subunits. Each monomer has a single cysteine residue (Cys10), which in the plasma is reduced (Cys-SH), oxidized (Cys-SO_3^-), sulfonated (Cys-S-SO_3^-) or bound to various sulfhydryls. There is evidence that these chemical modifications of the SH group alter the stability and the amyloidogenic potential of the protein. The sulfonated form was found to enhance the stability of the native conformation of TTR, avoiding misassembly of the protein leading to amyloid. Consequently, the potential treatment of TTR-type amyloidosis by sulfite has been suggested.

The structure of TTR pre-incubated with sulfite at physiological pH, was determined by X-ray crystallography to provide structural insight for the stabilizing effect of sulfite. Each subunit has a β-sandwich conformation, with two four stranded β-pleated sheets (DAGH and CBEF) and a small α-helix between strands. The sulfonated cysteines have two sulfite oxygens involved in intramonomer hydrogen bonds that bridge Cys10, the amino acid immediately before β-strand A, to the amino acids immediately after the edge β-strand D. Implications of the newly observed interactions in the inhibition of fibril formation are discussed in light of the recent structural models of TTR amyloid fibrils.

Keywords: Amyloid; Sulfite; Transthyretin; Familial amyloidotic polyneuropathy; X-ray crystallography

1. Introduction

The conversion of proteins from their folded functional form into self-assembled amyloid-like aggregates is intimately associated with a variety of neurodegenerative conditions, like Alzheimer's disease and prion-related transmissible spongiform encephalopathies [1]. More than 20 non-related proteins are involved in the formation of amyloid fibrils. These fibrils appear to share a common core structure, with cross-β conformation, consisting of a continuous array of β-sheets disposed parallel to the fibril axis, with the constituent β-strands running perpendicular to the fibril axis.

Transthyretin is the predominant protein found in extracellular protein deposits in the case of senile systemic amyloidosis (SSA) and familial amyloidotic polyneuropathy (FAP). X-ray fibre diffraction revealed that TTR fibrils share the cross-β structure seen in other amyloid materials [2]. In the native state, the four chemically identical monomers of TTR have a β-sandwich structure composed of two four-stranded β-sheets (DAGH and CBEF) [3]. The single helical region, comprising seven amino acids, is located between strands E and F. Each monomer has one cysteine at position 10 located near strand A.

It is widely accepted that TTR amyloid formation is a multi-step process involving the dissociation of the tetramer into non-native monomers [4]. Assembly of these intermediate species was described as comprising the formation of a new monomer interface when a major conformational change displaces the β-strand C, located in the edge of the CBEF β-sheet [1] and the

☆ Atomic coordinates have been deposited in the Protein Data Bank with an accession code: 2H4E.

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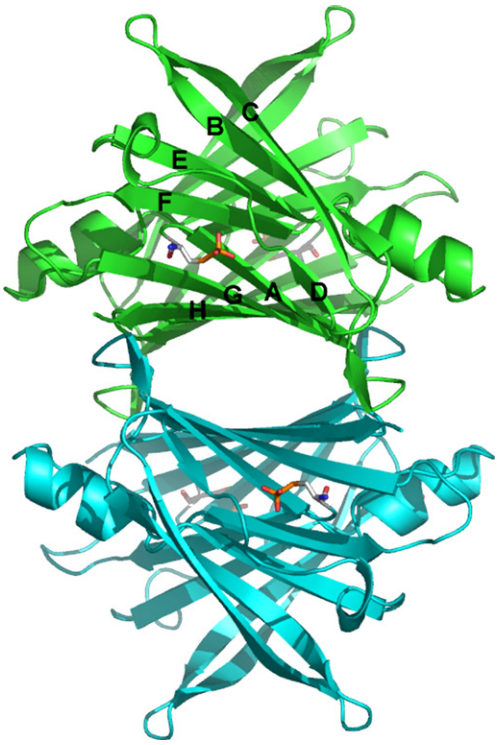

Fig. 1. Structure of Cys10-sulfonated TTR, showing a two-fold symmetry axis running along the hormone-binding channel. Monomers A and B (green) form the crystallographic asymmetric unit and monomers A′ and B′ (blue) are generated by a two-fold symmetry operation. Each monomer has a β-sandwich structure composed of two four-stranded β-sheets (DAGH and CBEF). The sulfonated cysteines (shown as solid sticks) are positioned in the vicinity of β-strand A and are involved in hydrogen bond interactions with residues in the vicinity of β-strand D. All figures were generated using The Pymol Molecular Graphics System [35].

edge β-strand D (DAGH β-sheet), with a consequent increase in the exposition of the cysteine to the solvent [5]. Altland and co-workers studied the stability of TTR after preliminary isolation from serum through isoelectric focusing studies [6–9]. It was reported that the cysteine is frequently chemically modified in the plasma, both in healthy humans and in amyloidosis patients. The prevalent modifications are cysteinylation, oxidation (Cys-SO_3^-), sulfonation (Cys-S-SO_3^-), glutathionylation, cysteinylglycylation, and homocysteinylation. While sulfonation of the reactive cysteine was found to have a stabilizing effect on TTR tetramers, the binding of sulfhydryls increased the misfolding propensity of the protein [6–11]. These modifications were described as important in the amyloidogenesis cascade of human TTR Val30Met "in vivo" [12] and it was also shown that they have a drastic effect in the protein stability as well as in the kinetics of acid-induced amyloid formation [11].

TTR is a plasma protein that transports thyroxine and binds the retinol binding protein for the transport of retinol. There are two thyroxine binding sites that are located in a channel running through the molecule, that results from the assembly of the four monomers into the tetrameric structure (Fig. 1). Present studies concerning promising therapeutical strategies rely on the use of small molecules that bind in the TTR channel and induce new stabilizing intermonomer interactions. Among the compounds tested are some NSAIDs (diflunisal, diclofenac, flufenamic acid, and derivatives) [13–16], plant-derived flavones [17,18] and xanthones [19], and the synthetic estrogen diethylstilbestrol [20]. Many of these compounds were shown to have a high affinity to TTR and to impair amyloid formation "in vitro" by acidification. However, only a few of those, like iododiflunisal, were successfully tested for their selectivity in the plasma for TTR [15] and the use of this class of compounds in animal models has not been proved yet.

In order to provide the structural basis for the protective action of sulfite against transthyretin amyloid formation, we determined the crystal structure of WT-TTR pre-incubated with sulfite at physiological pH.

2. Materials and methods

2.1. Protein complex preparation and crystallization

Recombinant TTR-WT was produced in an *Escherichia coli* expression system and isolated and purified as reported previously [21]. The protein solution was dialyzed against 10 mM HEPES buffer (pH 7.4), concentrated to

Table 1
Data collection and refinement statistics

Data collection	
Space group	$P2_12_12$
Unit cell dimensions (Å)	a=42.3 b=85.9 c=63.4
Resolution range (Å)	50.97–1.45
No. of observations (total/unique)	396885/39998
Multiplicity (overall/last shell)	9.5/6.7
Rmerge (overall/last shell)	5.4/30.3
Completeness (%)(overall/last shell)	100.0/100.0
$I/\sigma(I)$ (overall/last shell)	28.5/5.9
Mathews coefficient (Å^3 Da^{-1})	2.05
Solvent content (%)	39.4
Structure refinement	
R_{factor}/R_{free}	18.4/19.7
No. of unique reflections (working/test set)	41712/2105
Water molecules	221
Residues with alternate conformations (A and B refer to the two monomers in the asymmetric unit)	S23A, S85A, S117A, M13B, N27B, E72B, N98B, S115B
Total number of atoms	2014
Average overall B-factor (Å^2)	17.3
Average protein B-factor (Å^2)	15.7
Average main-chain B-factor (Å^2)	14.5
Average side-chain B-factor (Å^2)	16.9
Average water B-factor (Å^2)	30.9
Average sulfite B-factor (Å^2)	31.9
R.m.s. bonded B's (Å^2)	1.1
R.m.s. deviations from ideal values	
Bonds (Å)	0.008
Angles (°)	1.2
Ramachandran plot statistics	
Most favoured regions (%)	92.5
Additionally allowed regions (%)	7.5
TLS groups	group 1: monomer A group 2: monomer B