FEBS Letters 587 (2013) 2325–2331

journal homepage: www.FEBSLetters.org

# Structural evidence for native state stabilization of a conformationally labile amyloidogenic transthyretin variant by fibrillogenesis inhibitors

Giuseppe Zanotti [a,b,*], Laura Cendron [a,b], Claudia Folli [c], Paola Florio [d], Bruno Pietro Imbimbo [e], Rodolfo Berni [d,*]

[a] Department of Biomedical Sciences, University of Padua, Viale G. Colombo 3, 35131 Padova, Italy
[b] Venetian Institute of Molecular Medicine (VIMM), Via Orus 2, 35129 Padua, Italy
[c] Department of Food Science, University of Parma, Parco Area delle Scienze 23/A, 43124 Parma, Italy
[d] Department of Biosciences, University of Parma, Parco Area delle Scienze 23/A, 43124 Parma, Italy
[e] Research and Development Department, Chiesi Farmaceutici, Largo F. Belloli, 11/a, 43122 Parma, Italy

ARTICLE INFO

*Article history:*
Received 20 March 2013
Revised 20 May 2013
Accepted 6 June 2013
Available online 20 June 2013

Edited by Jesus Avila

*Keywords:*
Transthyretin
Amyloidosis
Protein misfolding
Fibrillogenesis inhibitor
Transthyretin stabilizer

ABSTRACT

**Several classes of chemicals are able to bind to the thyroxine binding sites of transthyretin (TTR), stabilizing its native state and inhibiting in vitro the amyloidogenic process. The amyloidogenic I84S TTR variant undergoes a large conformational change at moderately acidic pH. Structural evidence has been obtained by X-ray analysis for the native state stabilization of I84S TTR by two chemically distinct fibrillogenesis inhibitors. In fact, they fully prevent the acidic pH-induced protein conformational change as a result of a long-range stabilizing effect. This study provides further support to the therapeutic strategy based on the use of TTR stabilizers as anti-amyloidogenic drugs.**

*Structured summary of protein interactions:*
**TTR** and **TTR** bind by x-ray crystallography (View interaction)



## 1. Introduction

Protein misfolding and aggregation are involved in the pathogenesis of particularly important human diseases, the amyloidoses. Such diseases are characterized by the extracellular deposition of one of more than 30 amyloidogenic proteins as cross-β-sheet amyloid fibrils [1,2]. Specific mutations may induce or enhance the amyloidogenic potential of several amyloidogenic proteins, among which transthyretin (TTR) represents a notable example. Most mutations in TTR are involved in familial amyloidotic polyneuropathy (FAP) and cardiomyopathy (FAC), which are related to the predominant deposition of fibrillar aggregates of TTR in peripheral nerves and the heart, respectively [3].

TTR is a homotetramer of about 55 kDa involved in the transport of thyroxine (T4) in extracellular fluids, both plasma and cerebrospinal fluid. The assembly of the four identical subunits in TTR is highly symmetrical, being characterized by 222 symmetry. Each monomer is composed of eight antiparallel β-strands, arranged in a topology similar to that of the Greek key β-barrel, and a short α-helix. Two monomers are held together to form a stable dimer, and two dimers associate back to back, mainly through a limited number of hydrophobic contacts, to form the tetramer. A long channel, at the interface between the two dimers and coincident with one of the two-fold symmetry axes, transverses the tetrameric protein. Each symmetric half of the channel harbors a funnel-shaped binding site for T4.

A large body of evidence has been obtained to indicate that the rate-limiting dissociation of the native tetrameric state into monomers, followed by misfolding of TTR monomers and their downhill polymerization, is required to generate protein aggregates in vitro and presumably in vivo [4, and references therein]. Following these observations, the properties of a large number of compounds have been investigated in prospect of their use as drugs effective in the therapy of TTR amyloidoses [4]. A key feature they must possess is

*Abbreviations:* TTR, transthyretin; wt-TTR, wild type-TTR; T4, thyroxine; HBP, halogen binding pocket; PDB, Protein Data Bank

* Corresponding authors. Addresses: Department of Biomedical Sciences, University of Padua, Viale G. Colombo 3, 35131 Padova, Italy. Fax: +39 049 8073310 (G.Zanotti), Department of Biosciences, University of Parma, Parco Area delle Scienze 23/A, 43124 Parma, Italy. Fax: +39 0521 905151 (R. Berni).

*E-mail addresses:* giuseppe.zanotti@unipd.it (G. Zanotti), rodolfo.berni@unipr.it (R. Berni).

0014-5793/$36.00 
http://dx.doi.org/10.1016/j.febslet.2013.06.016

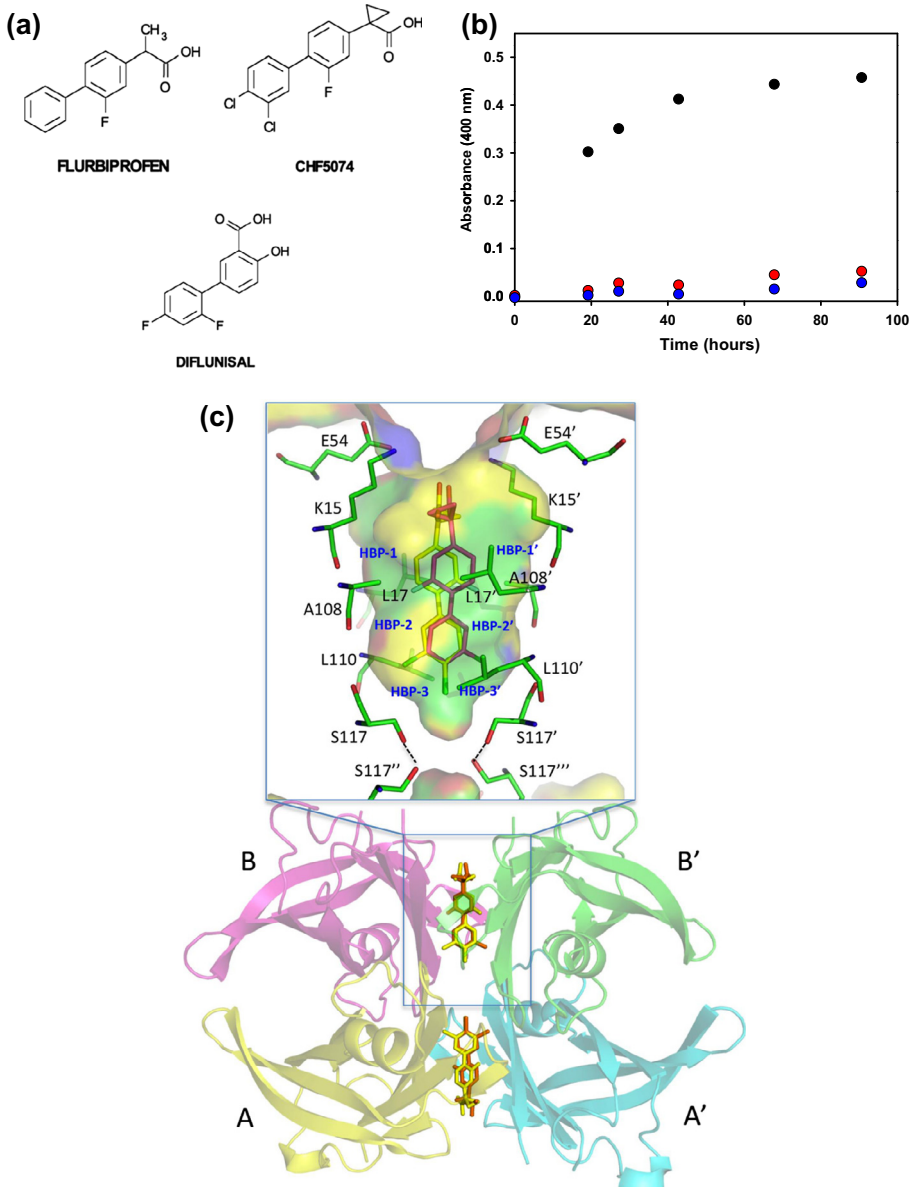


**Fig. 1.** (a) Structural formulae of TTR ligands. (b) Inhibition of fibrillogenesis for I84S TTR at acidic pH. Time-courses of fibril formation for uncomplexed I84S TTR (black) and in the presence of CHF5074 (red) or diflunisal (blue), as determined by monitoring spectrofotometrically the increase in turbidity at 400 nm. In the case of uncomplexed I84S TTR the fibrillogenesis assay solution contained about 0.5% (v/v) DMSO, as in the case of the assay solutions containing fibrillogenesis inhibitors. See Section 3 for experimental details. (c) Crystal structure of wt-TTR in complex with CHF5074 at pH 7.0. The ribbon diagram of tetrameric TTR with bound ligand within the two T4 binding pockets, at the weak dimer–dimer interface, is shown. The close-up view of one binding site depicts the TTR ligand as it is bound in the two symmetry-related binding modes (yellow and red, stick model). The carboxylate end group of CHF5074 is positioned at the entry port of the T4 binding pocket (outer binding subsite), mimicking the mode of binding of T4 (forward binding mode). The positions of the two symmetry-related, respectively primed and non-primed, HBPs forming the T4 binding cavity are shown. The side chains of residues that have at least an atom at a distance shorter than 4 Å from the ligand are shown (stick model). The H-bond interactions of the two Ser117 of monomers B and B′ with those of monomers A and A′ are explicitly indicated.

their ability to fit into the TTR T4 binding sites, establishing interactions with residues of the couple of subunits that line each hormone binding cavity present in the central channel. As a result, such interactions would bridge neighboring subunits at the dimer–dimer interface, thereby stabilizing the TTR tetramer. Indeed, one such compound, tafamidis, was found to possess quite favorable features in vitro and in vivo, such as a highly selective binding to TTR in human plasma, and was recently approved by EMA (European Medicines Agency) for the treatment of TTR FAP [5].

The crystal structures of amyloidogenic TTR variants are generally well conserved under native conditions [6]. On the other hand, structural alterations in the crystal structures of some amyloidogenic TTR variants crystallized at moderately acidic pH (pH 4.6) [7,8] and of wt-TTR crystallized at pH 4.0 [9] have been revealed. It should be noted that an acidic pH is also required to promote in vitro TTR fibrillogenesis, which is mediated by protein destabilization occurring at this pH [10]. Most evident is the alteration affecting I84S TTR, in which the lowering of the pH (pH 4.6) causes the unwinding of the TTR short EF-helix (residues 75–82) and the change in conformation of the adjacent EF-loop hosting the mutation (residues 83–90) in one (B subunit) of the two subunits (A and B subunits) present in the asymmetric unit [7]. Through the use of X-ray analyses we show here that the interactions of two chemically distinct fibrillogenesis inhibitors with I84S TTR prevent the