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Flavonoid interactions with human transthyretin: Combined structural and thermodynamic analysis

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ABSTRACT

Transthyretin (TTR) is a carrier protein involved in human amyloidosis. The development of small molecules that may act as TTR amyloid inhibitors is a promising strategy to treat these pathologies. Here we selected and characterized the interaction of flavonoids with the wild type and the V30M amyloidogenic mutant TTR. TTR acid aggregation was evaluated *in vitro* in the presence of the different flavonoids. The best TTR aggregation inhibitors were studied by Isothermal Titration Calorimetry (ITC) in order to reveal their thermodynamic signature of binding to TTRwt. Crystal structures of TTRwt in complex with the top binders were also obtained, enabling us to in depth inspect TTR interactions with these flavonoids. The results indicate that changing the number and position of hydroxyl groups attached to the flavonoid core strongly influence flavonoid recognition by TTR, either by changing ligand affinity or its mechanism of interaction with the two sites of TTR. We also compared the results obtained for TTRwt with the V30M mutant structure in the apo form, allowing us to pinpoint structural features that may facilitate or hamper ligand binding to the V30M mutant. Our data show that the TTRwt binding site is labile and, in particular, the central region of the cavity is sensible for the small differences in the ligands tested and can be influenced by the Met30 amyloidogenic mutation, therefore playing important roles in flavonoid binding affinity, mechanism and mutant protein ligand binding specificities.

1. Introduction

Aggregation and deposition of transtyretin (TTR) and its variants are involved in several severe amyloid diseases, such as senile systematic amyloidoses (SSA), familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC) and central nervous system amyloidoses (CNSA). SSA is characterized by wild type TTR (TTRwt) amyloid deposits (Connors et al., 2000; Westermark et al., 1990), whereas more than a hundred TTR point mutants are associated with FAP, FAC and CNSA (Connors et al., 2000). The V30M variant is the most prevalent cause of FAP and the mutation causes decreased tetramer stability, resulting in much more extensive TTR aggregation that accelerates the onset of FAP (Quintas et al., 1999).

TTR is a homo-tetrameric β-sheet-rich protein involved in the transport of the retinol-binding protein (RBP) and the thyroid hormone T_4 in human plasma and cerebral spinal fluid (Bartalena and Robbins, 1993). The tetramer is formed by subunits termed A, B, C and D. Each subunit is composed of 127 amino acids and has a molecular mass of 14 kDa.

Two TTR thyroxine binding sites (TBS) are located at the dimer–dimer interface, between the inner sheets of two TTR dimers. These TBS have a funnel-shaped morphology, with polar residues placed at the entrance and the bottom and a hydrophobic core located at the center. Three small depressions in TTR TBS are found and are responsible for accommodating the iodine atoms of T_4. These depressions are termed halogen binding pockets (HBPs) 1, 2 and 3. HBP1 is formed by TTR residues Lys15, Leu17, Thr106, Ala108 and Val121 at the TBS entrance. HBP2 is defined by Lys15, Val16, Leu17, Ala108, Ala109 and Leu110 at the center of the cavity; whereas HBP3 contains Leu17, Ala108, Ala109, Leu110, Ser117, Thr118 and Thr119, and is at the bottom of TTR TBS.

The TTR TBS is capable of accommodating several classes of chemicals including hormones and hormones analogues (Klabunde et al., 2000; Morais-de-Sa et al., 2004; Oza et al., 2002; Petrassi et al.,

Abbreviations: TTR, transthyretin; wt, wild type; V30M, Val30Met mutant; TTBS, thyroxin binding sites; HBP, halogen binding pocket; API, apigenin; LUT, luteolin; KAE, kaempferol; NAR, naringenin; CHR, chrisin; FIS, fisetin; QUE, quercetin; BAI, baicalein; T_4, 3,5,3′,5′-tetraiodothyronine.

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2005; Trivella et al., 2011; Wojtczak et al., 1992; Wojtczak et al., 1996; Zanotti et al., 1995), flavonoids (Green et al., 2005; Trivella et al., 2010) and non-steroidal anti-inflammatory drugs and their analogues, that interact in distinct manners with the polar and hydrophobic residues from TTR (Andricopulo et al., 2010; Klabunde et al., 2000; Oza et al., 2002; Petrassi et al., 2005), albeit with low specificity (Lima et al., 2010). Several bi-aryl and fused ring system derived compounds were reported as potent TTR inhibitors (Johnson et al., 2008a,b, 2009). In addition, directed linked bi-aryl TTR amyloid inhibitors were found as the most selective compounds to TTR in comparison to the off targets COX and thyroid hormone receptor (Johnson et al., 2008a), turning this class of compounds interesting targets for TTR amyloidosis drug development.

TTR amyloid inhibitors display high affinity for TTRwt and promote tetramer stabilization (Hammarstrom et al., 2003), which delays TTR aggregation and fibril formation, and are currently under investigation as potential inhibitors of TTR fibrosis and associated amyloid diseases (Connelly et al., 2010; Johnson et al., 2005b; Julius and Hawthorne, 2008; Klabunde et al., 2000; Oza et al., 2002; Petrassi et al., 2005).

TTR ligands can interact with the two TTR TBS following cooperative or non-cooperative binding mechanisms (Andrea et al., 1980; Cheng et al., 1977; Johnson et al., 2005a). In the last years, we have studied, using structural and thermodynamic approaches, the molecular basis of TTR interactions with small molecules (Trivella et al., 2011, 2010). Our findings pinpointed structural changes in TTR tetramers and ligand features that may account for TTR allosterism. Comparing the binding of T_4 and T_4 analogues, which act by distinct mechanisms of interaction with TTR, it was possible to predict ligand features and interactions responsible for TTR allosterism in this class of TTR amyloid inhibitors (Trivella et al., 2011). We also reported that subtle changes in TTRwt tetramer are responsible for the cooperative mechanism of genistein binding to TTRwt and that alterations induced by the V30M mutation may interfere in the V30M mutant biding to this isoflavone (Trivella et al., 2010). However, it is not entirely clear the origin of the changes caused by genistein and if the observed conformational changes of the bottom of the binding cavity apply and also influence the binding of other flavonoids to TTR.

Aiming to better understand the mechanisms that guide TTR interaction with flavonoids, here we report the study of TTR interactions with several flavonoids (Fig. 1), to probe the influence of the number and position of hydroxyl groups in flavonoid scaffold compounds, as well as the B ring position, in their binding to TTR and amyloid inhibition. Both TTRwt and the most frequent TTR amyloidogenic variant, the V30M TTR mutant were studied in this work. We gain insights into the molecular and thermodynamic basis of these interactions using isothermal titration calorimetry and X-ray crystallography.

We also compared the results obtained to the wild type protein with the V30M mutant structure in the apo form and in complex with other ligands, which allowed us to propose structural features in the ligands that may facilitate or hamper their binding to the V30M mutant. These findings complement our previous results with genistein and T_4 analogues, allowing better understanding of TTR interactions with inhibitors.

2. Methods

2.1. Chemicals

Flavonoids were purchased from Sigma–Aldrich; columns and resins were from GE Health Care; and crystallization kits were from Hampton Research. All chemicals used in the experiments were of reagent grade quality.

2.2. Protein expression and purification

Recombinant TTRwt and the V30M variant were expressed and purified as described previously (Lai et al., 1996). Protein concentrations were determined by using the absorption extinction coefficient of $7.76 \times 10^4\ M^{-1}\ cm^{-1}$ at 280 nm. Protein quality was accessed by native gel electrophoresis (Phast system® – GE Healthcare) and dynamic light scattering (DynaPro® – Protein Solutions) prior to experiments.

2.3. TTR acid-mediated aggregation assay

In vitro acid-mediated aggregation assays were carried out following well-established protocols (Green et al., 2005; Petrassi et al., 2005; Trivella et al., 2010). Aggregation curves were

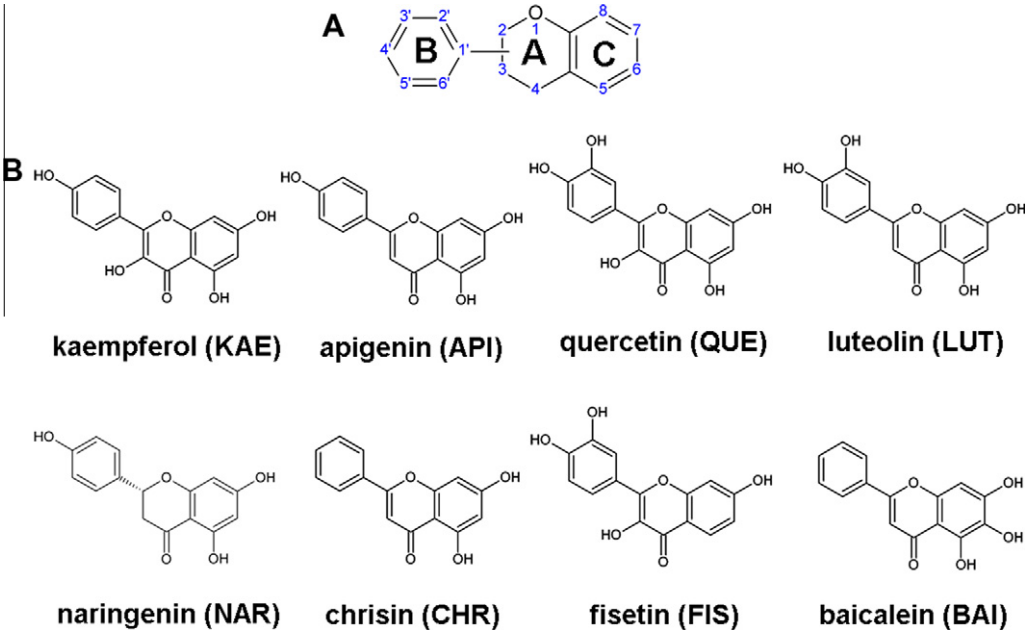

Fig. 1. Flavonoids: (A) general formula and (B) flavonoids selected in the present study.