**Introduction**

Transthyretin (TTR) is one of the more than 20 known amyloidogenic proteins. It is thought that the aggregation pathway of TTR into amyloid fibrils occurs by tetramer dissociation, a process which may be blocked by stabilization of the tetramer via ligand binding. In this regard, several small molecules have been reported as tetramer stabilizers.

There are more than 25 crystallographic TTR-ligand complexes deposited in the Protein Data Bank. However, until now, no attempt to critically review the relevant information for ligand binding that is embedded on them has been performed. In this context, the aim of the present work was the computational analysis of these structures in order to deduce useful information for drug discovery and design.

**Previous work**

The binding site of TTR is characterized by the presence of three-related pairs of pockets named in the literature as HBP1/1’, HBP2/2’ and HBP3/3’ (figure 1). It was demonstrated that the affinity of HBP1/HBP1’ and HBP3/HBP3’ pockets for halogens is greater than that of HBP2/HBP2’.

![Figure 1. Schematic representation of the three-related pairs of pockets at the A-A interface.](image)

**Methods**

Preparation of the crystallographic complexes. Crystal coordinates of all studied TTR-ligand complexes were directly extracted from the Protein Data Bank. Hydrogen atoms were added to the X-ray structures by using the EDIT module from the AMBER v6 package.

Hydrogen atoms refinement. Added hydrogen atoms were energy-minimized by using the *parm94* force field. Ligand partial charges were calculated by computing the electrostatic-potential around the optimized structures at the RH6-31G* level using Gaussian 94 and then fitting the charges using the RESP method. It was used a distance dependent dielectric constant of 10 Å and a cutoff distance of 12 Å for van der Waals interactions. Minimization was accomplished using 5000 cycles of steepest descents followed by conjugate gradient until the maximum gradient of the AMBER energy was smaller than 0.001 kcal/mol Å2.

Energy decomposition. Molecular mechanics interaction energy between TTR and the ligand was decomposed on a residue basis by using the module ANAL module of AMBER.

**Concluding results**

1. The binding site of TTR is a large and flexible cavity formed by three regions with different chemical features.

![Figure 2. Superposition of the crystallographic ligands into the binding site of TTR (see text).](image)

2. TTR is able to accommodate a wide diversity of ligands into the binding site.

3. Comparison of the crystallographic complexes show that:
   a. ligands adopt multiple positions and conformations into the binding site of TTR (figure 2a).
   b. ligands can bind in forward or reverse mode depending on the conformation of the serine and threonine residues located at the end of the binding site (figure 2b).
   c. a same functional group can be found in different placements, being not possible to identify a unique zone into the binding site of TTR where its placement is favorable (figure 2b).

6. The most active compounds are characterized by the presence of at least one halogen atom in the HBP1/HBP1’ or HBP3/HBP3’ pockets (figure 2).

**References**


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