Crystal structure of human transthyretin in complex with iodo-diflunisal, a potent amyloid inhibitor

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Transthyretin (TTR) is a plasma protein, with an extensive β-sheet conformation, implicated in the transport of thyroxine and vitamin A. The misfolding of this protein, due to a point mutation or change in the environment, and subsequent aggregation into amyloid fibrils is related to Familial Amyloidotic Polineuropathy diseases. TTR is a homotetramer, with β sandwich monomers composed of two four-stranded β sheets. The four monomers assemble, around the central channel of the protein, where two thyroxine molecules can accommodate simultaneously. Several small molecules, that bind in the hormone binding cavity, are being tested as inhibitors TTR amyloid fibril formation, since they stabilize the protein native tetrameric fold. One of the most potent inhibitors was proved to be diflunisal [1]. This encouraged us to look for diflunisal structure-related compounds with even higher TTR affinity and determine their protein-drug three-dimensional structure. Here we report the 1.7 Å crystal structure of TTR in complex with one of the most promising ligands: iodo-diflunisal. Iodo-diflunisal binds very deep in the hormone binding channel. The fluorine-substituted phenyl ring is positioned deeply in the interior of the binding cavity. The two fluorine atoms are involved in extensive hydrophobic interactions with Ala 108, Leu 110, Ser 117, Thr 118 and Thr 119 of two adjacent TTR monomers that may help to stabilize the native tetrameric structure of the protein. The iodine-substituted phenyl ring is positioned at the entry of the hormone binding channel. The iodine atom establishes hydrophobic interactions with the side chains of Thr 106, Ala 108, Thr 119 and Val 121 and the carboxylate substituent forms a weak hydrogen interaction with Nε of Lys 15 of one of the monomers (distance of 3.5 Å). Furthermore, the ligand binding induces the rotation of the side chains of Ser 117 and Thr 119 leading to formation of new intersubunit hydrogen bonds. The results clearly show why this compound stabilizes the native structure of TTR.

[1] Klabunde, T., Petrassi, H.M., Oza, V.B., Raman, P., Kelly, J.W. & Sacchettini J.C. Nature Struct. Biol. **7**, 312-320 (2000).