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Structures of Human Transthyretin Complexed with Thyroxine at 2.0 Å Resolution and 3′,5′-Dinitro-*N*-acetyl-L-thyronine at 2.2 Å Resolution

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Abstract

The molecular structures of two human transthyretin (hTTR, prealbumin) complexes, co-crystallized with thyroxine (3,5,3′,5′-tetraiodo-L-thyronine; T_4), and with 3′,5′-dinitro-*N*-acetyl-L-thyronine (DNNAT), were determined by X-ray diffraction methods. Crystals of both structures are orthorhombic, space group $P2_12_12$, and have two independent monomers in the asymmetric unit of the crystal lattice. These structures have been refined to 17.0% for 8–2.0 Å resolution data for the T_4 complex (I), and to $R = 18.4\%$ for 8–2.2 Å resolution data for the DNNAT structure (II). This report provides a detailed description of T_4 binding to wild-type hTTR at 2.0 Å resolution, as well as DNNAT. In both structures, the two independent hormone-binding sites of the TTR tetramer are occupied by ligand. A 50% statistical disorder model was applied to account for the crystallographic twofold symmetry along the binding channel and the lack of such symmetry for the ligands. Results for the co-crystallized T_4 complex show that T_4 binds deep in the hormone-binding channel and displaces the bound water previously reported for T_4 soaked into a native transthyretin crystal [Blake & Oatley (1977). *Nature (London)*, **268**, 115–120]. DNNAT also binds deeper in the channel toward the tetramer center than T_4 with the nitro groups occupying the symmetrical innermost halogen pockets. The *N*-acetyl moiety does not form polar contacts with the protein side chains as it is oriented toward the center of the channel. The weak binding affinity of DNNAT results from the loss of hydrophobic interactions with the halogen binding pockets as observed in T_4 binding. These data suggest that the halogen-binding sites toward the tetramer center are of primary importance as they are occupied by analogues with weak affinity to TTR, and are therefore selected over the other halogen sites which contribute more strongly to the overall binding affinity.

1. Introduction

Human transthyretin (hTTR, prealbumin) is one of three plasma proteins responsible for the serum transport of thyroid hormones such as thyroxine (T_4), or products of its enzymatic degradation. In man, TTR has been shown to carry about 20% of the circulating hormone whereas in the solar plexus, it is responsible for all of the available thyroxine (Braverman & Utiger, 1991; Robbins, 1991). It also binds retinol-binding protein that carries vitamin A. Although there are two sterically equivalent binding sites for thyroxine in the TTR tetramer which differ in their relative binding affinity, at physiological conditions there is only one hormone bound per tetramer (Robbins, 1991). A mechanism of negative cooperativity has been invoked to explain differences in affinity for binding the first hormone in the high-affinity site with K_a of $10^8\ M^{-1}$ and the second in the low-affinity site with K_a of $10^6\ M^{-1}$ (Cheng, Pages, Saroff, Edelhoch & Robbins, 1977). However, this mechanism is still poorly understood.

Structure–activity correlations have shown that ligands with the greatest binding affinity for TTR have a negatively charged phenyl substituent, a negatively charged or hydrogen-bonding function in the 4′-position, and a tetraiodo substitution pattern of the thyronine nucleus. Products of thyroxine metabolism have different affinities for TTR, with relative values ranging from 100% for T_4-thyroacetic acid, 39% for T_4, 0.07% for 3,3′-diiodothyronine, to less than 0.01% for 3-iodothyronine (Jorgensen, 1971).

Other structure–activity data show that rat liver microsomal type I iodothyronine deiodinase shares strong correlations with the binding requirements of hTTR since analogues with potent inhibition share structural features required for strong TTR binding affinities (Koehrle, Auf'mkolk, Rokos, Hesch & Cody, 1986). These data also indicated that replacement of the 3′,5′-I atoms by Br, OH or NO_2 reduces their activity while *N*-acetylation increases their potency. However, in TTR, *N*-acetylation greatly reduces binding affinity. *N*-acetyl-T_4 has only 8% of the binding affinity of T_4 to TTR (Jorgensen, 1971). The importance of 3,5-I substitution of activity in both proteins is revealed by

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the significant loss in activity of analogues without such tryrosyl substituents. The least active deiodinase inhibitor of a 34 compound series was 3′,5′-dinitrothyronine. Deiodinase activity was improved with *N*-acetylation (Koehrle *et al.*, 1986). Computer graphics modeling studies carried out using TTR as a target revealed that potent deiodinase inhibitors could be accommodated in the TTR hormone-binding site (Auf'mkolk, Koehrle, Hesch & Cody, 1986).

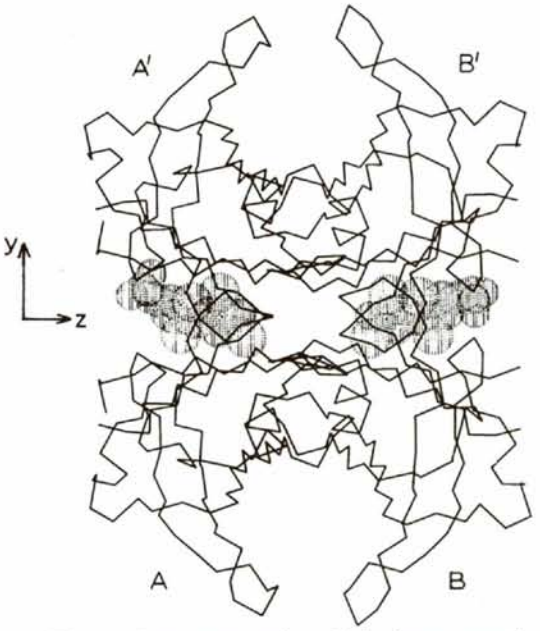


Fig. 1. The α-carbon representation of the human transthyretin quaternary structure showing the two independent monomeric subunits *A* and *B* forming the twofold-related tetramer with monomers labeled *A′* and *B′*. The tetramer is projected down the *a* axis. The van der Waals surface of thyroxine is shown in the TTR-binding sites.

Structural data for human transthyretin show that the 54 kDa protein is a tetramer with identical 127-residue monomers which are assembled around the central channel of the protein such that the tetramer possesses molecular 222 symmetry (Fig. 1). Each monomeric barrel is formed by eight strands, *A* to *H*, divided into two antiparallel β-sheets, one forming the channel surface (strands *A*, *D*, *G* and *H*, and the second one on the external surface of the tetramer (strands *B*, *C*, *E* and *F*) (Blake, Geisow, Oatley, Rerat & Rerat, 1978) (Fig. 2).

As part of a program to understand the role of the halogen pockets in determining the mechanism of molecular recognition for different thyroid hormone metabolites, as well as their binding competitors, we have determined the crystal structures of several human TTR-bound complexes (Cody, Wojtczak, Ciszak & Luft, 1991; Wojtczak, Luft & Cody, 1992, 1993; Ciszak, Luft & Cody, 1992). These data show that unusual binding modes can be accommodated by the protein and that multiple binding orientations are possible. For example, in the structure of the TTR-3,3′-diiodo-L-thyronine (3,3′-T_2) complex (Wojtczak *et al.*, 1992), the hormone metabolite binds deeper in the ligand channel than does T_4, and is twisted relative to that of thyroxine. In the case of the bromoflavones, two different binding modes were observed; a forward mode with the bromophenolic ring bound deeper in the channel than T_4, and reverse mode with the bromophenolic ring bound toward the channel entrance (Cody *et al.*, 1991).

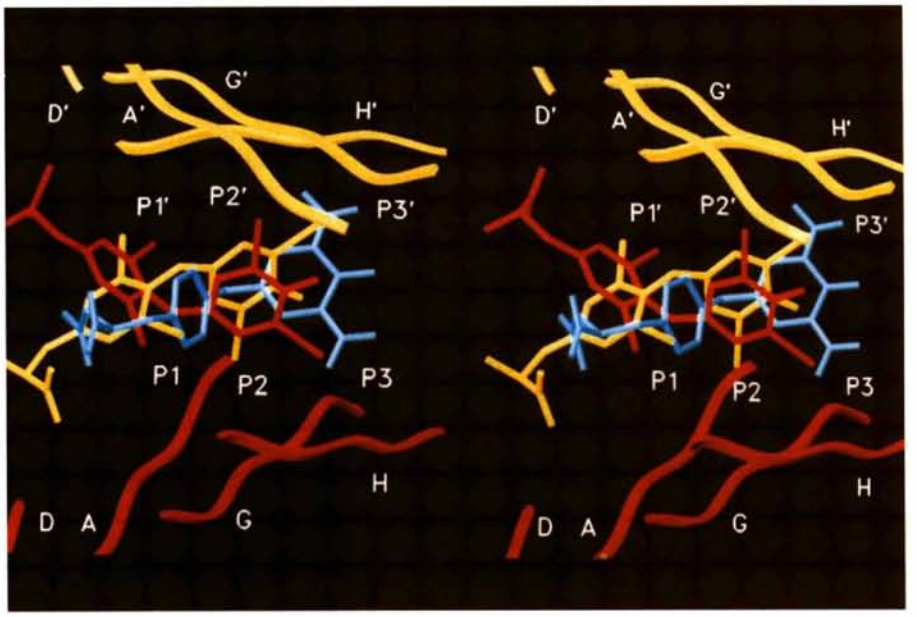
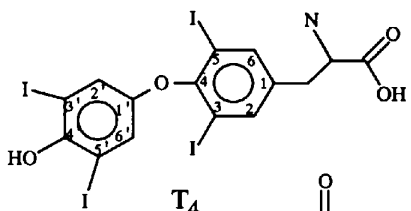


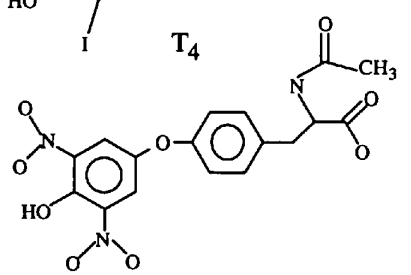
Fig. 2. TTR hormone-binding site, illustrated with the program *SETOR* (Evans, 1993), of the three halogen-binding pockets (P1, P2, P3) and their symmetry-related pairs (P1′, P2′, P3′) for domain *A* of the TTR tetramer. The binding orientation of T_4 (red) and its symmetry-related model (yellow) are shown, as well as the orientation of DNNAT (cyan) (symmetry-related molecule not shown). Also labeled are the β-strands comprising the binding site. Note that the tyrosyl 3,5-I atoms of T_4 occupy the symmetrical P1 and P1′ pockets, the distal 5′-I atom occupies the P3 pocket while the proximal 3′-I is in the P2′ pocket. In the case of the dinitro analogue, the 3′,5′-nitro groups occupy the P3 and P3′ pockets.

Because there are no published coordinates for hTTR-T_4 (Blake & Oatley, 1977), we have carried out the crystal structure determination of wild-type human TTR co-crystallized as the T_4 complex in order to compare its structural details with other ligand-bound complexes under investigation. Recently, coordinates for variant hTTR-T_4 complexes have been deposited in the Protein Data Bank (reference 1ETA and 1ETB); however, no details have been described. Furthermore, to optimize hormone binding, the TTR-T_4 complex was grown by incubating the protein with an excess of substrate before crystallization. Previous results from soaking experiments of a native crystal with hormone over a two month period resulted in only partial occupancy of the hormone-binding sites (Blake & Oatley, 1977).

In order to understand the mechanism of molecular recognition for thyroid hormone-binding sites by competitors, we have co-crystallized human TTR with thyroxine (T_4) and 3′,5′-dinitro-*N*-acetyl-thyronine, a weak thyromimetic analogue (Koehrle *et al.*, 1986), and report the results of their crystal structure determinations.



T_4



DNNAT

2. Experimental

2.1. *Crystallization and data collection*

Transthyretin was purified from pooled human sera obtained from the Red Cross (Tritsch, 1972). The TTR-T_4 complex (I) was obtained by the overnight incubation of purified transthyretin with T_4 at 277 K in 50 m*M* phosphate buffer pH 6.9. Hanging-drop crystallization was set up using *HANGMAN* (Luft, Cody & DeTitta, 1992; Luft & DeTitta, 1992). 5 µl protein solution was placed over the reservoir of 47% ammonium sulfate, 0.1 *M* phosphate buffer, pH 5.8. Data were collected on an Rigaku R-AXIS II imaging-plate system with a Rigaku 200 rotating-anode source from a 0.30 × 0.40 × 0.20 mm crystal at room temperature. The complex crystallizes in space group $P2_12_12$ with cell parameters $a = 43.410$, $b = 85.992$, $c = 65.552$ Å and with two independent monomers, *A* and *B*, in the asymmetric unit of the crystal structure. 51473 data were collected and 13485 reflections had $I > 2\sigma(I)$ (90.1% complete to 2.0 Å). R_{merge} for 13485 reflections with $F > 2\sigma(F)$ was 8.9%.

The thyronine analogue 3′,5′-dinitro-*N*-acetyl-L-thyronine (DNNAT) was obtained as a gift from Josef Koehrle. The TTR-DNNAT complex (II) was prepared by incubation overnight of 0.1 mg analogue at 277 K; in 10 m*M* phosphate buffer pH 6.9. Red crystals of the TTR-DNNAT complex were grown using the hanging-drop method in a reservoir with 55% ammonium sulfate, 0.1 *M* phosphate buffer, pH 5.9. Data were collected on an R-AXIS II imaging-plate system from a 0.30 × 0.30 × 0.50 mm crystal at room temperature. The complex crystallized in space group $P2_12_12$, with cell parameters of $a = 43.621$, $b = 86.041$, $c = 64.953$ Å, and two monomers in the asymmetric unit of the crystal lattice. 70011 data were collected with 12450 independent reflections at 2σ (96.2% complete to 2.2 Å). R_{merge} for 11520 reflections for $F > 1\sigma(F)$ was 6.9%,

2.2. *Refinement*

Both structures are isomorphous to wild-type human transthyretin (Blake *et al.*, 1978), and its complexes with 3,3′-T_2 (Wojtczak *et al.*, 1992), milrinone (Wojtczak *et al.*, 1993), and bromoflavone (Ciszak *et al.*, 1992). The protein coordinates from the TTR-3,3′-T_2 complex were used as a starting model for initial rigid-body refinement with *CORELS* (Sussman, Holbrook, Church & Kim, 1977) against 12–3.0 Å resolution data. The *PROLSQ* least-squares procedure (Hendrickson & Konnert, 1980; Finzel, 1987; Smith, 1988) was used to refine both structures. Individual *B*-factor refinement was used with the resolution range gradually extended for data between 8 and 2.0 Å for the T_4 complex, and 8 and 2.2 Å for the DNNAT structure. After each series of refinements, the results were monitored by examination of difference electron-density maps utilizing the graphics programs *FRODO* (Bush & Jones, 1988) and *CHAIN* (Sack, 1988). The program package *PHASES* (Furey & Swaminathan, 1996) was used to calculate a 'complex-native' electron-density map phased by the native TTR model for each data set. This map and subsequent difference electron-density maps calculated during the refinement were used to verify the ligand position.

Data collection and refinement statistics are summarized in Table 1. As indicated from the Ramachandran plots prepared with the program *PROCHECK* (Laskowski, MacArthur, Moss & Thornton, 1993) (not shown), the main-chain conformation torsion angles are within 88% of the favorable core conformational region in both structures. The exceptions are primarily in surface regions of the structure with ill

Table 1. *Refinement statistics for the structures of TTR complexes*

	TTR-T_4 (I)	TTR-DNNAT (II)
Resolution range (Å)	8.0–2.0	8.0–2.2
Reflections used	11151	9664
R factor	0.170	0.184
Protein and ligand atoms	1964	1916
Water molecules	102	82
B factor (average) (Å^2)	24.09	20.63
B factor (water average) (Å^2)	39.87	31.38

	Target σ	R.m.s σ (observed) T_4 (I)	DNNAT (II)
Distances (Å)			
Bond distances	0.02	0.02	0.02
Angle distances	0.04	0.06	0.06
Planar 1–4 distances	0.05	0.06	0.07
Planar groups (Å)	0.02	0.01	0.02
Chiral volume (Å^3)	0.15	0.20	0.20
Non-bonded distances (Å)			
Single torsion	0.50	0.23	0.23
Multiple torsion	0.50	0.27	0.30
Possible hydrogen bonds	0.50	0.32	0.33
Torsion angles (°)			
Planar	5.0	3.1	3.2
Staggered	15.0	20.2	21.2
Orthonormal	15.0	20.8	18.6
Thermal restraints (Å^2)			
Main-chain bonds	1.75	1.78	1.72
Main-chain angles	2.50	2.85	2.66
Side-chain bonds	1.75	2.29	2.03
Side-chain angles	2.50	2.56	3.10

defined electron density. Residues 1–9 in both domains, and residues 126 and 127 for domain *B* were not observed in these electron-density maps for structure (I) while in structure (II), residues 1–6 for both domains and residues 126–127 were not observed. Difference electron-density maps ($2F_o - F_c$ and $F_o - F_c$) at the final stage of the refinement confirmed that there is no significant disagreement between the model and the electron density. The coordinates for these structures have been deposited with the Protein Data Bank.*

3. Results and discussion

In the orthorhombic space group $P2_12_12$, observed for all reported structures of human TTR (Blake *et al.*, 1978; Blake & Oatley, 1977; de la Paz, Burridge, Oatley & Blake, 1992; Cody *et al.*, 1991; Wojtczak *et al.*, 1992, 1993; Ciszak *et al.*, 1992; Hamilton *et al.*, 1993; Steinrauf, Hamilton, Braden, Murrell & Benson, 1993), there is twofold symmetry of the hormone-binding site formed by the monomers *A*–*A*′ along the channel axis. A non-crystallographic twofold axis relates the independent monomers *A* and *B* to form the second hormone-binding site, *B*–*B*′ (Fig. 1). The presence of a crystallographic twofold axis through the center of the two binding sites in the tetramer requires that the ligands either possess molecular twofold symmetry or bind in two orientations related by the crystallographic symmetry of the protein. Ligands without such symmetry occupy this site with a 50% statistical disorder. Because thyroid hormones have a skewed conformation (Cody, 1980, 1981), there is no single orientation of the hormone that can take advantage of the site symmetry.

Data for the thyroxine-bound complex (Blake & Oatley, 1977) showed that each of the two twofold-related monomers which form the hormone-binding site has three sets of halogen pockets. As illustrated (Fig. 2), the innermost pocket to the tetramer center, P3, is located between the side chains of Ser117 and Thr119 of strand *H*, and Ala108 and Leu110 of strand *G*. Its surface is formed by aliphatic methyl and methylene groups, as well as the Ser117 Oγ hydroxyl, the carbonyl groups of Ser117, Thr118 and Ala108, and the main-chain NH groups of Thr119, Ala109 and Leu110 (Blake & Oatley, 1977; de la Paz *et al.*, 1992). The central P2 pocket is formed between side chains of Leu110, Ala109, Lys15 and Leu17. Similar to P3, it is primarily hydrophobic with nucleophilic contributions from the carbonyl groups of Lys15, Ala108 and Ala109. The outermost P1 pocket is formed between the side chains of Ala108, Thr106, Met13 and Lys15 and is positioned between strands *A* and *G*, as is P2. The pocket is lined with the methyl and methylene groups of Lys15, Ala108 and Thr106 which are exposed to the channel surface. A significant contribution to ligand binding comes from the charged surface region near the P1 pocket formed by Glu54 and Lys15.

Since co-crystallization of wild-type hTTR was carried out in the presence of excess hormone, full saturation of both binding domains was assumed with a stoichiometry of two hormones bound per tetramer. Each domain binds one hormone in two twofold-related orientations at 50% occupancy. The electron density

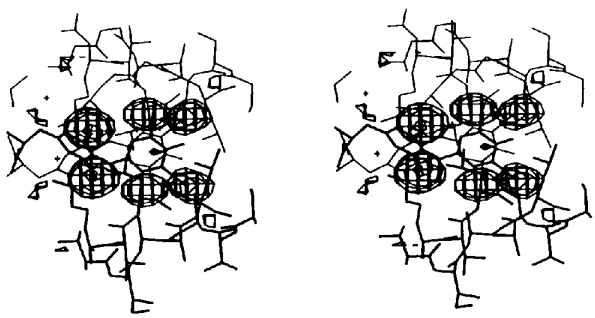


Fig. 3. Omit ($F_o - F_c$) electron-density map, contoured at 5σ, for hTTR-T_4 indicating the iodine positions of thyroxine in binding domain *A*. This model shows that the hormone binds with its phenolic ring near the tetramer center.

*Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1ROY, R1ROYSF). Free copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Reference: PT0002).

observed in the channel for TTR–T_4 complex (I), which appears elongated, indicated the presence of thyroxine bound in both binding domains (Fig. 3). In the DNNAT complex (II), the difference Fourier maps clearly show density for the ligand ring system with the dinitro groups in domain *A*, and for the complete ligand structure in domain *B* (Fig. 4). These results showed that DNNAT binds in a forward binding mode similar to that observed for 3,3′-T_2 (Fig. 5) (Wojtczak *et al.*, 1992).

Similar to other T_4 complexes (Blake & Oatley, 1977, PDB references 1ETA, 1ETB), this co-crystallized TTR complex shows that thyroxine is bound deep in the cleft of the channel surface between the side chains of residues Leu17, Ala18 and Leu110, with interactions of its phenolic ring with Ser117 and Thr119

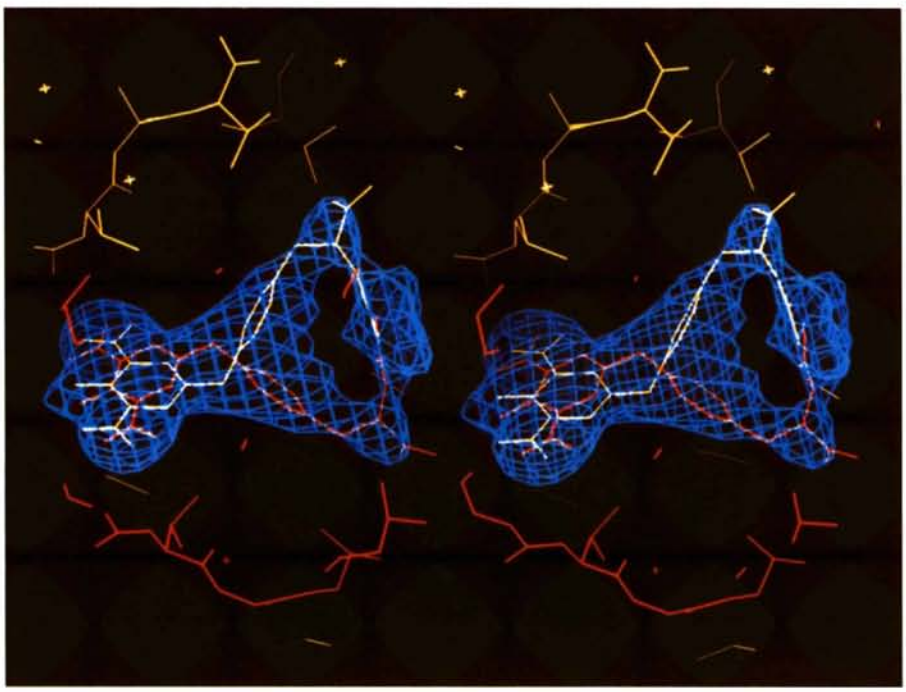


Fig. 4. Omit map ($F_o - F_c$) for structure (II), calculated without the ligand contributions, contoured at 2.5σ, illustrating the orientation of the dinitro analogue (red) and its symmetry-related orientation (yellow) in domain *B* for which the complete structure is mapped. These data reveal that the dinitro analogue binds deeper in the channel than T_4 and that the *N*-acetyl group does not make polar interactions with the protein side chains.

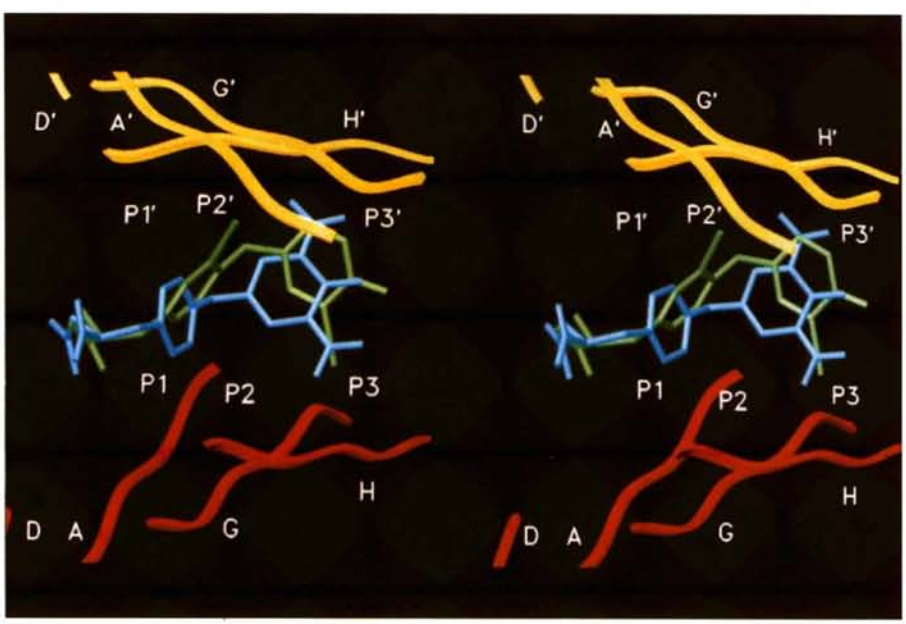


Fig. 5. Stereoview of the superposition of 3,3′-diiodothyronine (green) (Wojtczak *et al.*, 1992) on that of the dinitro analogue (cyan) which show occupation of the hormone site by a significantly different conformation of the hormone analogue.

at the tetramer center, and with its alanyl moiety interacting with Glu54 and Lys15 near the channel entrance. Because the twofold symmetry axis is coincident with the channel axis, the electron density in the channel is an average of two symmetry-related orientations of T_4 with 50% occupancy (Fig. 3). The distal 5′-I makes short contacts to the carbonyl of residue Ala109 (I···O 3.2 Å). Similar short contacts have been observed in many iodinated small molecules in which directional contacts were shorter than the sum of their van der Waals radii (I···O 3.55 Å) (Cody & Murray-Rust, 1984).

Although complete density was not observed for the thyronine nucleus of thyroxine (Fig. 3), the conformation of the hormone fit to the observed iodine peaks is not significantly different from that observed in its crystal structure (Cody, 1981). Examination of T_4 interactions with TTR side chains shows that it can make good hydrogen-bonding contacts with Lys15 (NH_2···K15NZ, 3.09 Å) and Glu54 (O9···E54O2, 2.92 Å), in agreement with earlier computational studies which showed the zwitterionic hormone amino acid interacts *via* hydrogen bonds with Lys15 and Glu54 (Blaney *et al.*, 1982).

In the TTR–DNNAT complex (II), the absence of 3,5-substituents on the tyrosyl ring increases the conformational flexibility of this analogue compared to thyroxine (Cody, 1980, 1981). As indicated from the electron-density profiles for DNNAT (Fig. 4), the conformation about the ether bridge is perpendicular and differs significantly from that of thyroxine in structure (I) which has a skewed conformation (Fig. 2). The *N*-acetyl alanyl moiety is positioned near the charged surface region formed by Lys15 and Glu54 and forms a salt bridge between the carboxylic O9 atom of DNNAT and NZ of Lys15 (O···N 2.35 and 2.90 Å, for domains *A* and *B*, respectively). The acetyl group participates only in van der Waals interactions with the surrounding residues and is positioned near the channel center. The phenolic ring of DNNAT binds deep in the channel close to the symmetry axis of the lattice, similar to that observed for the structures of 3,3′-diiodothyronine (Fig. 5) (Wojtczak *et al.*, 1992), milrinone (Wojtczak *et al.*, 1993), and bromoflavone (Ciszak *et al.*, 1992). The nitro group in the P3 pocket forms hydrogen-bond interactions with Ser117 Oγ (O···O 2.9 and 3.2 Å, for domains *A* and *B*, respectively), while the nitro group in the P3′ pocket forms interactions with Ser117 hydroxyl and the main-chain N atom of Thr119 (O···N 3.0 and 3.3 Å, for domains *A* and *B*, respectively). The phenolic hydroxyl forms hydrogen bonds to Ser117 Oγ.

In the co-crystallized hTTR–T_4 complex reported here, the electron density in both binding domains (Fig. 3) shows the hormone positioned deeper in the channel than previously reported (Blake & Oatley, 1977). In this alternate position, contacts of the tyrosyl I atoms are nearly the same but the phenolic ring I atoms occupy slightly different positions placing the 4′-hydroxyl, distal 5′-I, and the tyrosyl 5-I closer to the tetramer center by 1.43, 1.45 and 1.15 Å, respectively (Fig. 6). As a result, the distal 5′-I in the P3 pocket is close to the water position reported for the hormone complexes with wild-type hTTR (Blake & Oatley, 1977) and from coordinates deposited for the variants V30M and A109T (PDB numbers 1ETA and 1ETB). However, there is no evidence for a water molecule bound in the P3 pocket in this structure.

In order to verify the absence of water in the P3 pocket and to explain the shape of the electron density for the halogen positions (Fig. 3), a cross-phased electron-density map was calculated with the A109T variant TTR-T_4 coordinates which had the hormone set at one quarter occupancy (PDB number 1ETB), and the

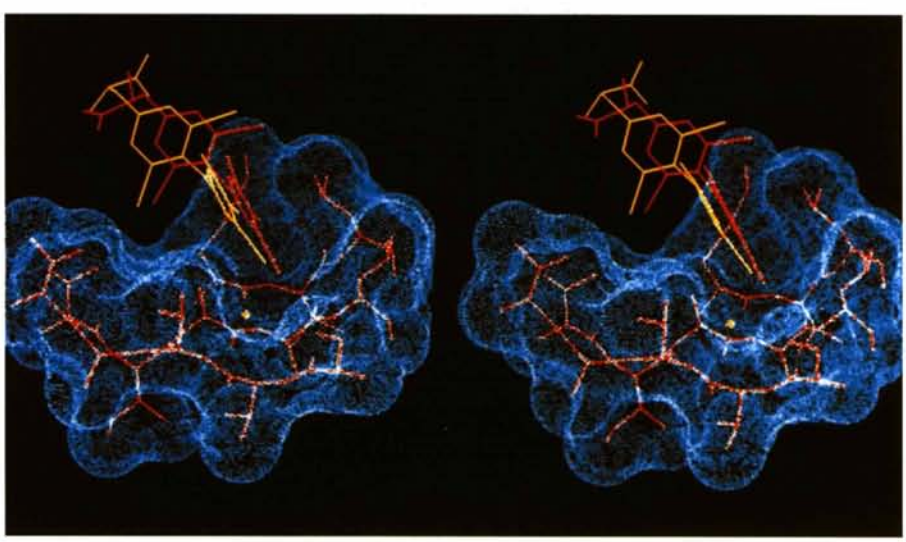


Fig. 6. Stereoview of the superposition of thyroxine (yellow) soaked into a native crystal (Blake & Oatley, 1977) on that observed in this co-crystallized complex (red). A Connolly surface (Connolly, 1983) of the hormone binding site is shown (cyan). The bound water observed in the hormone soaked crystal is shown as '+'.

diffraction data from this experiment. The orientation of T_4 in this variant is the same as observed for this co-crystallized wild-type TTR complex. As illustrated (Fig. 7), there is significant density remaining above 3σ near all the I-atom positions with that near the proximal I-atom positions being less populated. The positive density represents contributions not present in the model reported for the variant TTR hormone position, but present in this data. This density can be interpreted as a second low occupancy (<20%) position deeper in the channel than that of the major population already included in the refinement. Also, this extra iodine density is located near the water in the previously reported structures. It is concluded that there is no evidence for bound water in the P3 site in this co-crystallized complex. The presence of more than one population in the same binding domain may explain the elongation of the halogen density and the lack of complete electron density for the entire molecule in the $2F_o - F_c$ difference Fourier maps.

In order to understand the mechanism of recognition for the binding of thyroid hormones and their metabolites, we have compared the results for the T_4 and DNNAT complexes with that of 3,3′-diiodothyronine which binds weakly to TTR (Wojtczak *et al.*, 1992). The 3′,5′-dinitro-*N*-acetyl-thyronine analogue binds deeper in the channel than T_4 with the nitro groups occupying both the P3 and P3′ sites simultaneously forming close contacts to Ser117 (Fig. 2). Comparison of DNNAT with 3,3′-diiodothyronine shows that although the two hormone analogues have similar perpendicular thyronine conformations, they occupy the binding site with significantly different orientations (Fig. 5). Removal of one tyrosyl iodine has released the conformational constraints and permits the hormone metabolite to bind deeper in the channel with a different orientation than thyroxine. In this case, the tyrosyl 3-I occupies the P2′ pocket, as does the proximal 3′-I of thyroxine, while the phenolic 3′-I of 3,3′-T_2 occupies the P3 pocket near the water binding site reported previously (Blake & Oatley, 1977). In both DNNAT and 3,3′-T_2, electron density for the entire ligand was observed suggesting that these analogues are more strongly anchored in the binding site than T_4. As illustrated (Fig. 5), the alanyl moiety of both ligands have similar interactions with the protein side chains. *N*-acetylation results in a loss of binding affinity by weakening the polar interactions with Glu54 observed with T_4 binding.

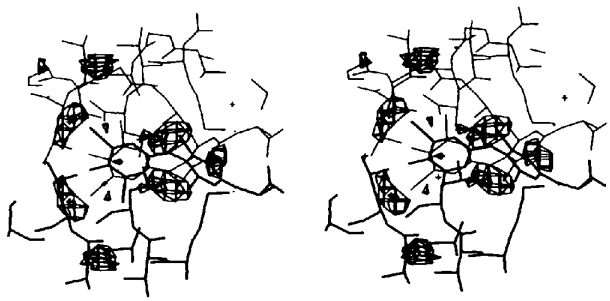


Fig. 7. Difference electron density, contoured at 3σ, phased with the A109T variant TTR–T_4 complex coordinates with reflections from wild-type hTTR–T_4 co-crystallized complex. Monomer *A* (dark lines) and its twofold-related monomer *A*′ (light lines) are shown. These data show significant density near the iodine positions which can be fit by translation of the model toward the channel center. Also shown is the difference density for the A109T substitution. The water molecule reported in the variant structure is shown as '+' in the innermost density.

A unique feature of the thyroid hormones is their iodine substituents. Previous studies have shown the covalently bound iodine can form intermolecular contacts with nucleophiles which are shorter than the sum of their van der Waals radii (I···O 3.55 Å, I···N 3.65 Å) and that the shortest contacts have a directional specificity (Cody & Murray-Rust, 1984). These data showed that the average I···O contact distance was 3.2 Å with an angle of 163°. Analysis of these TTR hormone complexes reveals similar contacts for the phenolic ring iodines binding in the P2 pocket which has a more nucleophilic character than the P1 pocket. In this wild-type TTR–T_4 complex, the distal 5′-I in the P3 pocket forms close contacts to Leu110 backbone N atom (I···N 3.5–3.7 Å), while the shortest contacts for the proximal 3′-I are formed with the carbonyl of Ala109 (I···O 2.8–3.3 Å). These contacts for the low-occupancy model of the hormone are shifted toward the tetramer center and toward the carbonyl and hydroxyl of Ser117, as well as Ala109. In the 3,3′-T_2 complex (Wojtczak *et al.*, 1992), the distal 3′-I in the P3 pocket of both domains makes short contacts to Ser117 Oγ (I···O 2.9–3.2 Å) while the 3-I in the P2′ pocket makes contacts to the carbonyl of Ala109. These observations suggest that these interactions are selective for binding, but that the contributions of the P1 pocket is of greater importance for strong binding affinity.

These data suggest that the weak binding affinity of 3,3′-T_2 and DNNAT is related primarily to the loss of hydrophobic interactions in the P1 halogen pocket, and to a lesser degree, the P2 and P3 pockets, although the innermost pockets are more selective for binding. Further loss of affinity comes from the loss of hydrogen-bonding interactions with Glu54 by the *N*-acetyl substitution as *N*-acetylation of T_4 greatly weakens its binding affinity. Furthermore, close contact to Ser117 at the tetramer center is only observed for the weakly binding analogues. Movement along the channel, as observed by the different binding positions for thyroxine in this co-crystallized data and that observed previously, may be related in some way to the negative cooperativity observed for the binding of the second hormone to the tetramer. These results suggest that further study is needed to fully understand the mechanism of

ligand recognition and selectivity for binding to human transthyretin.

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