THYROID HORMONE SERUM TRANSPORT PROTEINS

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ABSTRACT

Thyroid hormone (TH) effects are dependent on the quantity of the hormone that reaches the tissues, hormone activation, and the availability of unaltered TH receptors in the cell's nuclei and cytoplasm. Since TH enters the cell unbound, the concentration of free rather than total hormone reflects more accurately the activity level of TH-dependent processes. Under normal conditions, changes in free hormone level are adjusted by appropriate stimulation or suppression of hormone secretion and disposal. Total TH concentration in serum is normally kept at a level proportional to the concentration of carrier proteins, and appropriate to maintain a constant free hormone level.

INTRODUCTION

Most carrier protein dependent alterations in total hormone concentration in serum are due to quantitative changes in the hormone-binding proteins and less commonly to changes in affinities for the hormone. Since wide fluctuations in the concentration of TH carrier proteins does not alter the hormonal economy or metabolic status of the subject (1), their function is open to speculation. They are responsible for the maintenance of a large extrathyroidal pool of TH of which only the minute, <0.5 % fraction of free hormone is immediately available to tissues. It can be estimated that in the absence of binding proteins the small extrathyroidal T_4 pool would be significantly reduced, if not completely depleted in a matter of hours following a sudden cessation of hormone secretion. In contrast, in the presence of normal concentrations of T_4-binding serum proteins, and in particular thyroxine-binding globulin (TBG), a 24-h arrest in hormonal secretion would bring about a decrease in the concentration of T_4 and T_3 in the order of only 10 and 40 per cent, respectively. Thus, it seems logical to assume that one of the functions of T_4-binding proteins in serum is to safeguard the body from the effects of abrupt fluctuations in hormonal secretion. The second likely function of T_4-binding serum proteins is to serve as an additional protection against iodine wastage by imparting macromolecular properties to the small iodothyronine molecules, thus limiting their urinary loss (2). The lack of high affinity T_4-binding proteins in fish (3), for example, may be teleologically attributed to the greater iodine abundance in their natural habitat. Liver perfusion studies suggest a third function, that facilitating the uniform cellular distribution of T_4, allowing for changes in the circulating thyroid hormone level to be rapidly communicated to all cells within organ tissues (4). A fourth function, modeled after the corticosteroid-binding globulin (5), is targeting the amount of hormone delivery by site specific, enzymatic, alteration of TBG. Indeed neutrophil derived elastase transforms TBG into a heat resistant, relaxed, form with reduced T_4-binding affinity (6). TBG was found to have a putative role on the testicular size of the

boar. In fact, Meishan pigs with histidine rather than an asparagine in codon 226 have a TBG with lower affinity for T_4, smaller testes and earlier onset of puberty (7, 8).

In normal man, approximately 0.03 per cent of the total serum T_4, and 0.3 per cent of the total serum T_3 are present in free or unbound form (3, 9). The major serum thyroid hormone-binding proteins are thyroxine-binding globulin [TBG or thyropexin], transthyretin [TTR or thyroxine-binding prealbumin (TBPA)], and albumin (HSA, human serum albumin) (10). Several other serum proteins, in particular high density lipoproteins, bind T_4 and T_3 as well as rT_3 (9, 11) but their contribution to the overall hormone transport is negligible in both physiological and pathological situations. In term of their relative abundance in serum, HSA is present at approximately 100-fold the molar concentration of TTR and 2,000-fold that of TBG. However, from the view point of the association constants for T_4, TBG has highest affinity, which is 50-

fold higher than that of TTR and 7,000-fold that of HSA. As a result, TBG binds 75% of serum T_4, while TTR and HSA binds only 20% and 5%, respectively (Table 1). The distribution of the iodothyronine metabolites among the three serum binding proteins is distinct (12). According to their affinity, T_4 > tetraiodothyroacetic acid (TETRAC or T_4A) = 3,3',5'-triiodothyronine (reverse T_3 or rT_3) > T_3 > triiodothyroacetic acid (TRIAC or T_3A) = 3,3'-diiodothyronine (T_2) > 3-monoipdothyronine (T_1) = 3,5-T_2 > thyronine (T_0) for TBG (IC50-range: 0.36 nM to >100 IM) and T_4A > T_4 = T_3A > rT_3>T_3 > 3,3'-T_2 > 3-T_1 > 3,5-T_2 > T_0 for transthyretin (IC50-range: 0.94 nM to >100 IM). TBG, transthyretin, and albumin were not associated with T_0, 3-T_1, 3,3-T_2, rT_3, and T_4A. From evolutionary point of view, the three iodothyronine-binding serum proteins developed in reverse order of their affinity for T_4, HSA being the oldest (13).

Table 1. Some Properties and Metabolic Parameters of the Principal TH-Binding Proteins in Serum			
	TBG	**TTR**	**HSA**
Molecular weight (K daltons)	54*	55	66.5
Structure	Monomer	Tetramer	Monomer
Carbohydrate content (%)	20		
Number of binding sites for T_4 and T_3	1	2	4
Association constant, Ka (M^{-1})			
For T_4	1×10^{10}	2×10^{8}**	1.5×10^{6}**
For T_3	1×10^{9}	1×10^{6}	2×10^{5}
Concentration in serum			
(mean normal, mg/liter)	16	250	40,000
Relative distribution of T_4 and T_3 in serum (%)			
For T_4	75	20	5
For T_3	75	<5	20
In-Vivo Survival			
Half-life (days)	5***	2	15
Degradation rate (mg/day)	15	650	17,000

*Apparent molecular weight on acrylamide gel electrophoresis 60 K daltons.

**Value given is for the high affinity binding site only.

***Longer under the influence of estrogen.

The existence of inherited TH-binding protein abnormalities was recognized 1959, with the report of a family with TBG-excess (14) but it took 30 years before the first mutation in the TBG (serine protease inhibitor, SERPIN A7) gene was identified (15). Genetic variants of TH-binding proteins having different capacity or affinity for their ligands than the common type protein result in euthyroid hyper- or hypo-iodothyroninemia. The techniques of molecular biology have traced these abnormalities to polymorphisms or mutations in genes encoding TBG and TTR and HSA (see Chapter on Defects of Thyroid Hormone Transport in Serum).

THYROXINE-BINDING GLOBULIN (TBG)

The Molecule, Structure and Physical Properties

TBG is a 54 kD acidic glycoprotein migrating in the inter-α-globulin zone on conventional electrophoresis, at pH 8.6. The term, thyroxine-binding globulin, is a misnomer since the molecule also binds T_3 and reverse T_3. It was first recognized to serve as the major thyroid hormone transport protein in serum in 1952 (16). Since TBG binds 75% of serum T_4 and T_3,

quantitative and qualitative abnormalities of this protein have most profound effects on the total iodothyronine levels in serum. Its primary structure was deduced in 1989 from the nucleotide sequence of a partial TBG cDNA and an overlapping genomic DNA clones (17). However, it took 17 years to characterize its three dimensional structure by crystallographic analysis (18) (Fig. 1).

TBG : INTACT S-FORM   **TBG: CLEAVED R-FORM**   **ABOVE VIEW OF R-FORM**
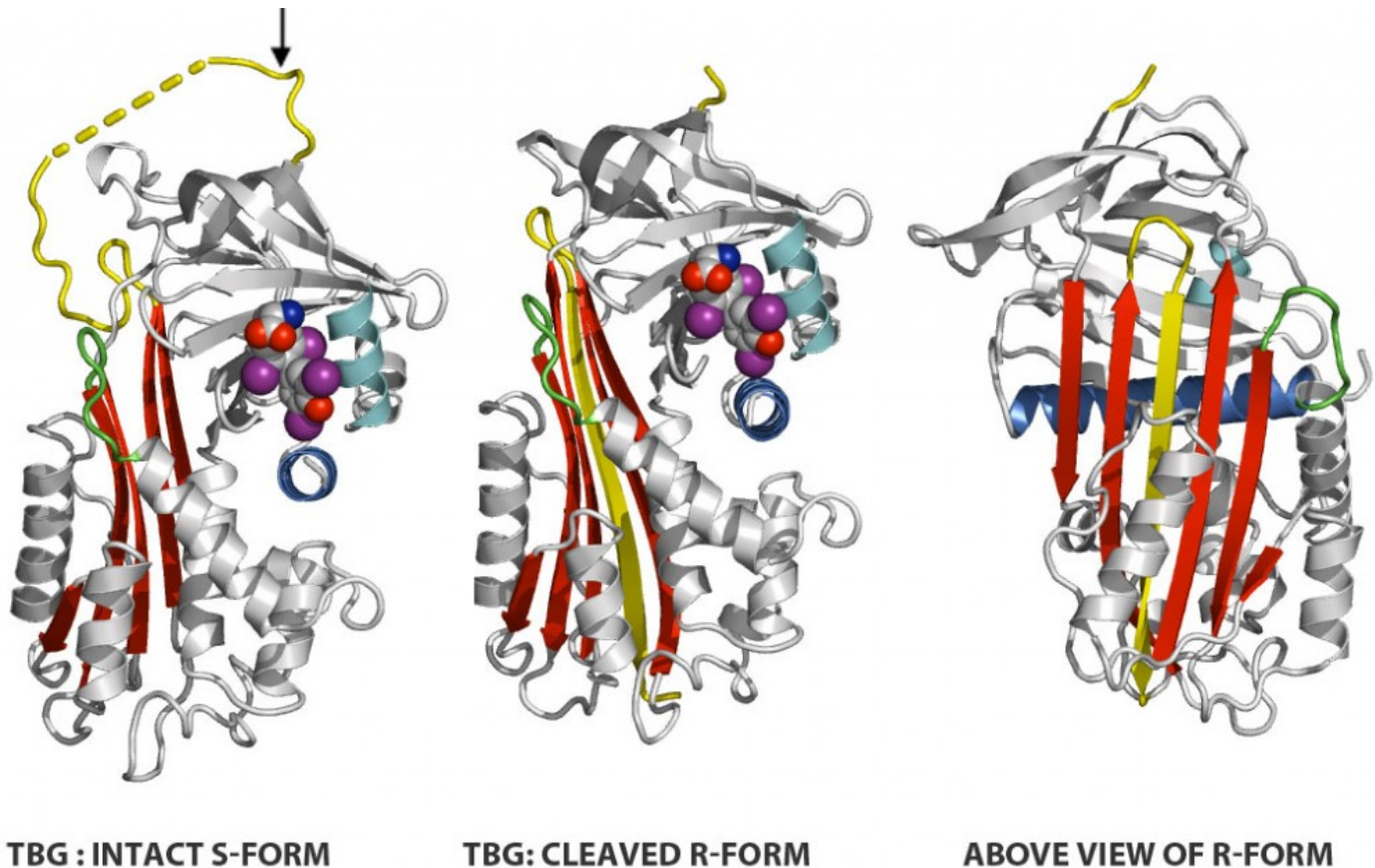
Figure 1. Structure of the TBG molecule: Reactive loop (in yellow). Insertion occurs following its cleavage by proteases to give an extra strand in the main sheet of the molecule but the T4-binding site can still retain its active conformation. This is in keeping with other findings showing that the binding and release of T4 is not due to a switch from an on to an off conformation but rather results from an equilibrated change in plasticity of the binding site. So, the S-to-R change in TBG results in a 6 -fold decrease but not a total loss of affinity. The important corollary is that that the release of thyroxine is a modulated process as notably seen in response to changes in temperature (19). (Courtesy of Dr, R.W. Carrell),

TBG is synthesized in the liver as single polypeptide chain of 415 amino acids. The mature molecule, minus the signal peptide, is composed of 395 amino acids (44 kD) and four heterosaccharide units with 5 to 9 terminal sialic acids. The carbohydrate chains are not required for hormone binding but are important for the correct post-translational folding and secretion of the molecule (20, 21) and are responsible for the multiple TBG isoforms (microheterogeneity) present on isoelectric focusing (22, 23). The isoelectric point

of normal TBG ranges from pH 4.2 to 4.6, however, this increases to 6 when all sialic acid residues are removed.

The protein is very stable when stored in serum, but rapidly loses its hormone binding properties by denaturation at temperatures above 55°C and pH below 4. The half-life of denaturation at 60°C is approximately 7 min but association with T_4 increases the stability of TBG (24-26). TBG can be measured by immunometric techniques or saturation analysis using one of its iodothyronine ligands (26-28).

The tertiary structure of TBG was solved by co-crystallizing the in-vitro synthesized non-glycosylated molecule with T_4 and speculations regarding the properties of TBG and its variants have been confirmed (18, 19). The molecule caries T_4 in a surface pocket held by a series of hydrophobic interactions with underlying residues and hydrogen bonding of the aminoproprionate of T_4 with adjacent residues (Figure 1). TBG differs from other members of the SERPIN family in having the upper half of the main ß-sheet completely opened. This allows the reactive center peptide loop to move in and out of the sheet, resulting in binding and release of the ligand without cleavage of TBG. Thus the molecule can assume a high-affinity and a low-affinity conformation, a model proposed earlier by Grasberger et al (29) and confirmed crystallographically (18). This reversibility is due to the unique presence of P8 proline in TBG, rather than a threonine in all other SERPINs, limiting loop insertion. The coordinated movements of the reactive loop, hD, and the hormone-binding site allow the allosteric regulation of hormone release.

Gene Structure and Transcriptional Regulation

The molecule is encoded by a single gene copy located in the long arm of the human X-chromosome (Xq22.2) (30, 31). The gene consists of 5 exons spanning 5.5kbp (Fig. 2). The first exon is a small and non-coding. It is preceded by a TATAA box and a sequence of 177 nucleotides containing an hepatocyte transcription factor-1 (HNF-1) binding motif that imparts to the gene a strong liver specific transcriptional activity (32). The numbers and size of exons, their boundaries and types of intron splice junctions as well as the amino acid sequences they encode are similar to those of other members of the SERPIN family, to which TBG belongs (32). These include cortisol-binding globulin and the serine protease inhibitors, α1-antitrypsin (α1AT) and α1-antichymotrypsin (α1ACT).
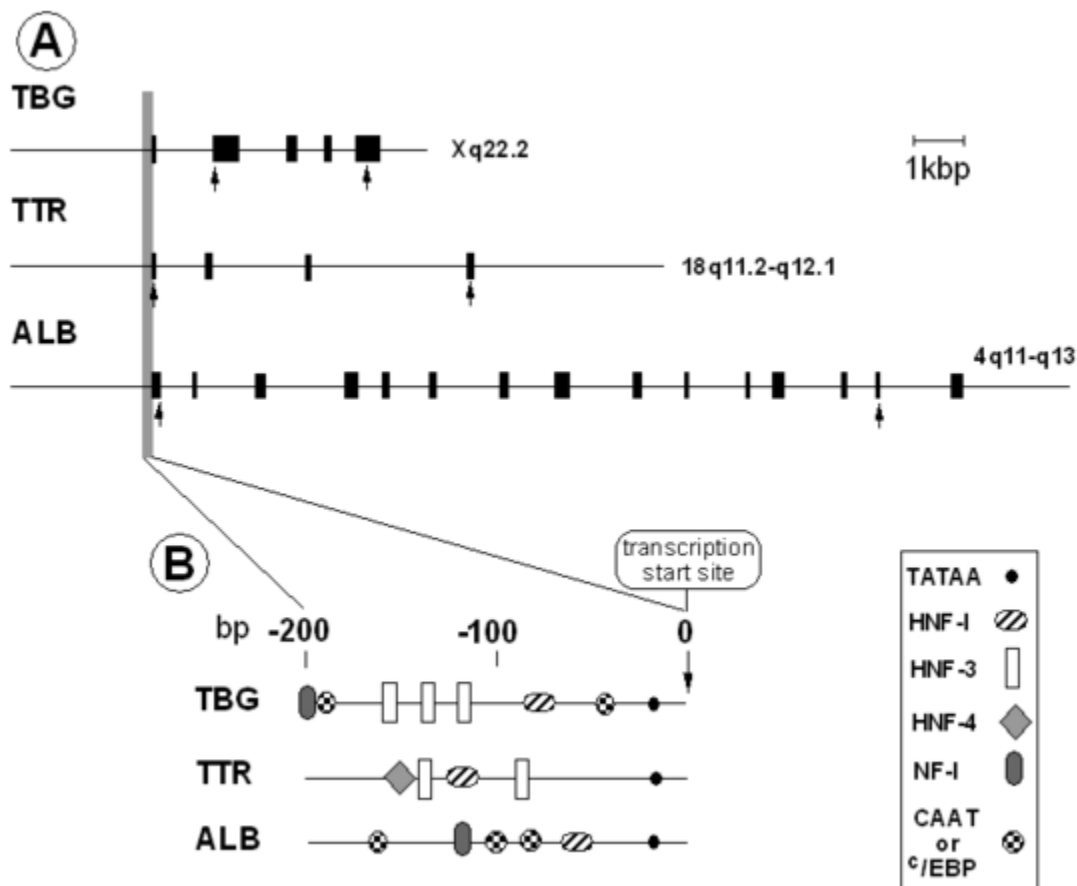
Figure 2. A. Genomic organization and chromosomal localization of thyroid hormone serum binding proteins. Filled boxes represent exons. Location of initiation codons and termination codons are indicated by arrows. B. Structure of promoter regions with the location of cis-acting transcriptional regulatory elements. Reproduced with permission from Hayashi and Refetoff, Molecular Endocrinology: Basic concepts and clinical correlations, Raven Press Ltd. 1995.

Biological Properties

The TBG molecule has a single iodothyronine binding site with affinity slightly higher for T_4 than for T_3 (33) (Table 1). Optimal binding activity requires the presence of the L-alanine side chain, an unsubstituted 4'-hydroxyl group, a diphenyl ether bridge, and halogen (I or Br) constituents at the 3,5,3' and 5' positions (34). Compared to L-T_4, 3,3',5'-triiodothyronine (rT_3) binds to TBG with ~40% higher affinity, D-T_4 with half that of the L-isomer and

tetraiodothyroacetic acid with ~25%. A number of organic compounds compete with thyroid hormone-binding to TBG. Most notable are: 5,5-diphenylhydantoin (35), 1,8-anilinonaphthalenesulfonic acid, and salicylates (36). While reversible flip-flop conformational changes of TBG allow for binding and release of the hormone ligand, cleavage of the molecule by leukocyte elastase produces a permanent change in the properties of the molecule. This modified form has reduced T_4-binding and increased heat stability (6).

Denatured TBG does not bind iodothyronines but can be detected with antibodies that recognize the primary structure of the molecule (26). In euthyroid adults with normal TBG concentration, about one-third of the molecules carry thyroid hormone, mainly T_4. When fully saturated, it carries about 20 µg of T_4/dl of serum. The biologic half-life is about 5 days, and the volume of distribution is similar to that of albumin (37, 38) (Table 1). TBG is cleared by the liver. Loss of sialic acid accelerates its removal through interaction with the asialo-glycoprotein receptors reducing the half live by 500-fold (24). However, it is unknown whether desialylation is a required in the normal pathway of TBG metabolism.

Physiology

TBG concentration in the serum of normal adults ranges from 1.1 to 2.1 mg/dl (180 - 350 nM), 14 - 26 µg T_4/dl in terms of maximal T_4-binding capacity. The protein is present in serum of the 12th week old fetus and in the newborn until 2-3 years of age it is about 1.5 times the normal adult concentration (39-41). TBG levels decline slightly reaching a nadir during mid-adulthood and tend to rise with further advance in age (42). Variable amounts of TBG, though much smaller than those in serum, have been detected in amniotic fluid (43), cerebrospinal fluid (44) and urine (45).

Estrogen excess, either from an endogenous source (hydatidiform mole, estrogen-producing tumors, etc.) or exogenous (therapeutic or birth control use) is the most common cause of increased serum TBG concentration. The level of several other serum proteins such as corticosteroid-binding globulin, testosterone-binding globulin, ceruloplasmin, and transferrin, are also increased (46). This effect of estrogen is mediated through an increase in the complexity of the oligosaccharide residues in TBG together with an increase in the number of sialic acids

resulting in prolonged biological half-life (47, 48). Androgens and anabolic steroids produce an opposite effect (49, 50). Although sex hormones affect the serum level of TBG, gender differences are small except during pregnancy during which concentrations are on the average 2.5-fold the normal value (28, 51). Extreme changes in TBG concentration (low or high) alters the accuracy of immunometric measurements of free iodothyronines and particularly that of T_3 (52).

Acquired TBG Abnormalities

Altered synthesis, degradation, or both are responsible for the majority of acquired TBG abnormalities (38). Severe terminal illness is undoubtedly the most common cause for acquired decrease in TBG concentration. Interleukin-6, a stimulator of acute phase reactants, is a candidate for mediation of this effect (53). In vivo studies in man showed a reduction in the turnover of TBG in hypothyroidism and an increase in hyperthyroidism (37, 38). Thus, alterations in the degradation rate, rather than changes in the rate of synthesis, may be responsible for the changes of TBG concentration observed in these two conditions.

Partially desialylated TBG, has slow electrophoretic mobility (sTBG, not to be confused with the variant TBG-S), and was found in the serum of some patients with severe liver disease (54) and may be present in relatively higher proportion than TBG in serum of patients with a variety of non-thyroidal illnesses and particularly those with compromised hepatocellular function (55). This is not surprising considering that sTBG is removed by the asialoglycoprotein receptors present in abundance on liver cells (24, 56).

Patients with the carbohydrate-deficient glycoprotein (CDG) syndrome show a characteristic cathodal shift in the relative proportion of TBG isoforms compatible

with diminished sialic acid content (57). This inherited syndrome presenting psychomotor retardation, cerebellar hypoplasia, peripheral sensorimotor neuropathy, and variably, retinitis pigmentosa, skeletal abnormalities and lipodystrophy (58), manifests also abnormalities of charge and mass in a variety of serum glycoproteins (59).

TRANSTHYRETIN (TTR)

The Molecule, Structure and Physical Properties

TTR is a 55kD homotetramer which is highly acidic although it contains no carbohydrate. Formerly known as thyroxine-binding prealbumin (TBPA), for its electrophoretic mobility anodal to albumin, was first recognized to bind T_4 in 1958 (60). Subsequently it was demonstrated that TTR also forms a complex with retinol-binding protein and thus plays a role in the transport of vitamin A (retinol, or trans retinoic acid) (61, 62).

TTR circulates in blood as a stable tetramer of identical subunits, each containing 127 amino acids (63). Although the tetrameric structure of the molecule was demonstrated by genetic studies (64, 65), detailed structural analysis is available through X-ray crystallography (66, 67) (Fig. 3). Each TTR subunit has 8 ß-strands four of which form the inner sheet and four the outer sheet. The four subunits form a symmetrical ß-barrel structure with a double trumpeted hydrophobic channel that traverses the molecule forming the two iodothyronine binding sites. Despite the apparent identity of the two iodothyronine binding sites, TTR usually binds only one T_4 molecule because the binding affinity of the second site is greatly reduced through a negative cooperative effect (69). The TTR tetramer can bind four molecules of RBP that do not interfere with T_4-binding, and vice versa (70). TTR can be measured by densitometry after its separation from the other serum proteins by electrophoresis, by hormone saturation, and by immunoassays.
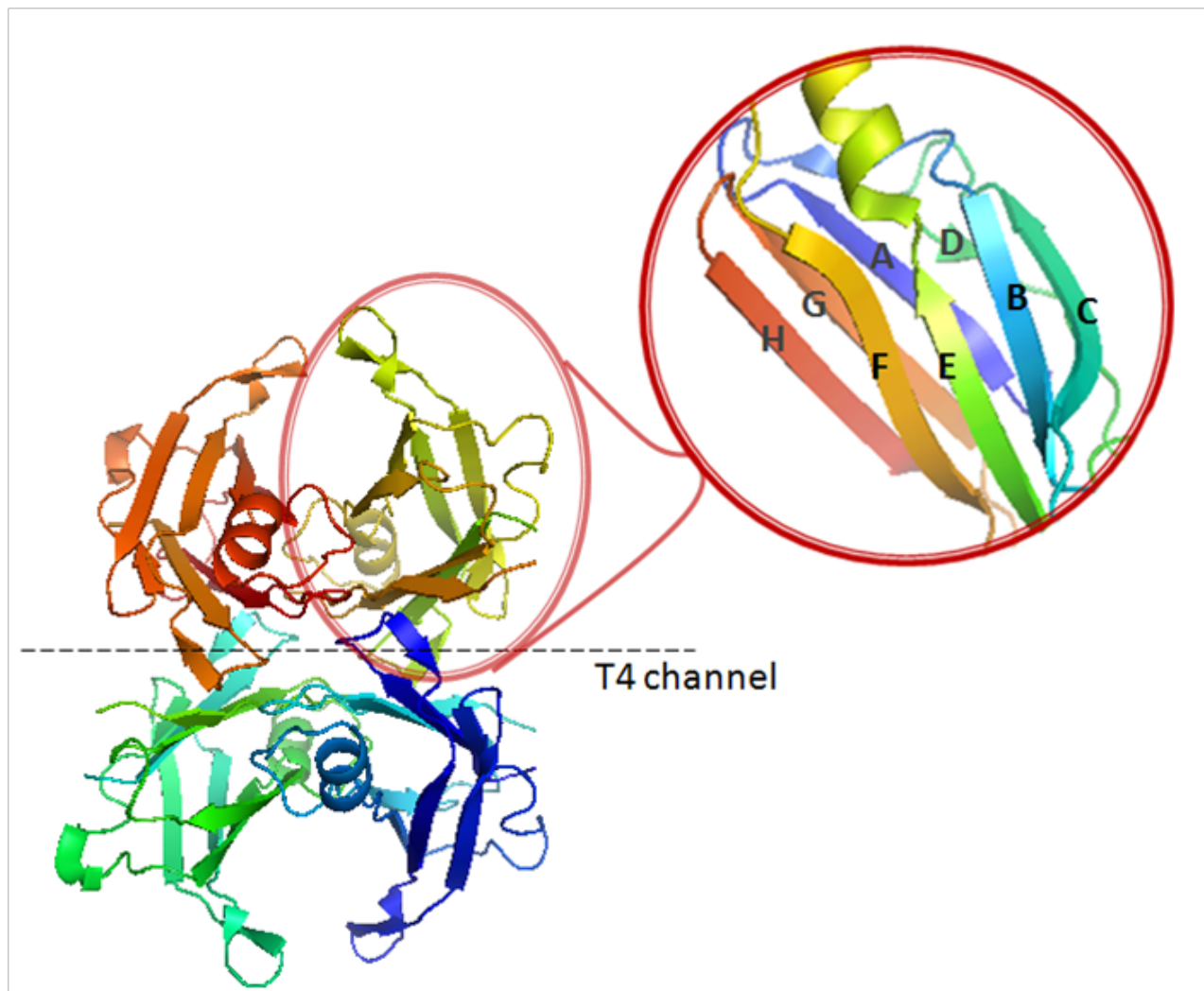
Figure 3. X-ray structure of TTR. The molecule is a homotetrameric protein composed of four monomers of 127 amino acids. Structurally, in its native state, TTR contains eight stands (A-H) and a small α-helix. The contacts between the dimers form two hydrophobic pockets where T4 binds (T4 channel). As shown in the magnified insert, each monomer contains one small α-helix and eight β-strands (CBEF and DAGH). Adapted from a model; PDB code 1DVQ (68).

Gene Structure and Transcriptional Regulation

TTR is encoded by a single gene copy located on human chromosome 18 (18q11.2-12.1) (63, 71) (Fig. 2). The gene consists of 4 exons spanning for 6.8kbp. Knowledge about the transcriptional regulation of the human *TTR* gene comes from studies of the mouse gene structural and sequence homology which

extends to the promoter region (72, 73). In both species a TATAA box and binding sites for HNF-1, 3 and 4 are located within 150 bp from the transcription start site.

Although TTR in serum originates from the liver (74), TTR mRNA is also found in kidney cells, the choroid plexus, meninges, retina, placenta, pancreatic islet

cells and fetal intestine (75-78). TTR constitutes up to 25% of the total protein present in ventricular cerebrospinal fluid where it binds 80% of T_4 (79).

Biological Properties

Despite the 20-fold higher concentration of TTR in serum relative to that of TBG, it plays a lesser role in iodothyronine transport. In the presence of normal levels of TBG, wide fluctuations in TTR concentration or its removal from serum by specific antibodies has little influence on the concentration of free T_4 (80). Some of the properties of TTR are summarized in Table 1.

The first T_4 molecule binds to TTR with a Ka of about 100-fold higher than that for HSA and about 100-fold lesser than that for TBG. Properties necessary for optimal binding activity include iodines at the 3' and 5' positions and a desamino acid side chain which explain the lower T_3 and higher T_4A affinities relative to that of T_4 (34, 81). Non-iodothyronine ligands are also differentially bound, the most notable example being the flavonoid compounds which have a markedly higher binding affinity for TTR than for TBG (82). Among drugs that compete with T_4-binding to TTR are ethacrynic acid, salicylates, 2,4-dinitrophenol, penicillin (83, 84) and perfluoroalkyl substances (85). The latter have with near equal affinity to TTR and TBG. Barbital also inhibits iodothyronine binding to TTR.

Only 0.5% of the circulating TTR is occupied by T_4. TTR has a relatively rapid turnover (t1/2 = 2 days) and a distribution space similar to that of HSA and TBG (86, 87) except that it also exists in CSF. Hence, acute diminution in the rate of synthesis is accompanied by a rapid decrease of its concentration in serum.

Physiology

Normal average concentration in serum is 25 mg/dl, and corresponds to a maximal binding capacity of approximately 300 μg T_4/dl. Changes in TTR concentration have relatively little effect on the serum concentration of serum iodothyronines (80, 88). There is a distinct reciprocal relationship between acquired changes in TBG and TTR concentration related to gender, age, glucocorticoids, estrogen and androgens (42, 51, 89-91).

Acquired TTR Abnormalities

The reduction or serum TTR concentration surpasses that of TBG in major illness, nephrotic syndrome, liver disease, cystic fibrosis, hyperthyroidism, and protein-calorie malnutrition (10, 92-94). Increased serum TTR concentration can occur in some patients with islet cell carcinoma (95). Studies on the metabolism of TTR in man, utilizing radioiodinated purified human TTR, indicate that diminished TTR concentration associated with severe illness or stress is due to a decrease in the rate of synthesis or an increase in the rate of degradation, or both (86, 87).

HUMAN SERUM ALBUMIN (HSA)

The Molecule, Structure and Physical Properties

HSA is a 66.5 kD protein synthesized by the liver. It is composed of 585 amino acids with high content of cystines and charged amino acids but no carbohydrate (96). The three domains of the molecule can be conceived as three tennis balls packaged in a cylindrical case.

Gene Structure and Transcriptional Regulation

HSA is encoded by a single gene copy located on human chromosome 4 (4q11-q13) (97). The gene contains 15 exons, 14 of which are coding (98) (Fig. 2). The promoter region of the HSA gene has been most intensive studied. The transcriptional regulation has been best characterized in rodents that share 90% sequence homology with the corresponding human gene, including a distal enhancer element 10 kbp upstream from the promoter region (99). Binding sites for hepatocyte enriched nuclear proteins, such as HNF-1, C/EBP, and DBP have been identified (100-102).

Biological Properties

HSA associates with a wide variety of substances including hormones and drugs possessing a hydrophobic region, and thus the association of TH to HSA can be viewed as nonspecific. Of the several iodothyronine-binding sites on the HSA molecule, only one has a relatively high affinity for T_4 and T_3. Yet these are 10,000-fold inferior to those of TBG (27). Fatty acids and chloride ions decrease their binding to HSA (27). The biologic t1/2 of HSA is relatively long (103). Some of its properties are summarized in Table 1.

More than half of the total protein content in serum is HSA. As a result, it is the principal contributor to the maintenance of the colloid osmotic pressure (96). It has been suggested that HSA synthesis may be, in part, regulated by a feedback mechanism involving alteration in the colloid osmotic pressure. Indeed, down-regulation of HSA gene expression has been recently observed during the infusion of macromolecules in the rat (104).

Physiology

Because of the low affinity and despite the high capacity of HSA for iodothyronines, its contribution to thyroid hormone transport is relatively minor. Thus, even the most marked fluctuations of serum HSA concentration, including analbuminemia, have no significant effects on thyroid hormone levels (105).

LIPOPROTEINS

Lipoproteins bind T_4, and to some extent T_3 (9, 106). The affinity for T4-binding is similar to that of TTR. These proteins are estimated to transport roughly 3% of the total T_4 and perhaps as much as 6% of the total T_3 in serum. The binding site of apolipoprotein A1 is a region of the molecule that is distinct from that portion which binds to the cellular lipoprotein receptors, and the physiological role of such binding is still unclear.

ACKNOWLEDGMENTS

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