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Molecular Cloning and Primary Structure of Rat Thyroxine-Binding Globulin[†,‡]

Shuji Imamura,[§] Yuichi Mori,[*,§] Yoshiharu Murata,[∥] Ikuo Yamamori,[§] Yoshitaka Miura,[§] Yutaka Oiso,[§] Hisao Seo,[∥] Nobuo Matsui,[∥] and Samuel Refetoff[⊥]

The First Department of Internal Medicine, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, 466 Japan, Department of Endocrinology and Metabolism, the Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-01 Japan, and Departments of Medicine and Pediatrics, University of Chicago, Chicago, Illinois 60637

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ABSTRACT: Rat thyroxine-binding globulin (TBG) cDNAs were isolated from a rat liver cDNA library by using a human TBG cDNA as a probe. From two overlapping cDNA inserts, an aligned cDNA sequence of 1714 nucleotides was obtained. There was 70% homology with human TBG cDNA over the span of 1526 nucleotides. In order to confirm that the cloned cDNA encodes rat TBG and to localize the NH_2-terminal amino acid of the mature molecule, the protein was purified by affinity chromatography and subjected to direct protein microsequencing. The NH_2-terminal amino acid sequence was identical with that deduced from the nucleotide sequence. The rat TBG cDNA sequenced consisted of a truncated leader sequence (35 nucleotides), the complete sequence encoding the mature protein (1194 nucleotides) and the 3'-untranslated region (485 nucleotides), containing two polyadenylation signals. It was deduced that rat TBG consists of 398 amino acids (M_r = 44 607), three NH_2-terminal residues more than human TBG, with which it shares 76% homology in primary structure. Of the six potential N-glycosylation sites, four are located in conserved positions compared to human TBG. Northern blot analysis of rat liver revealed an approximately 1.8-kilobase TBG mRNA. Its amount increased markedly following thyroidectomy and decreased with thyroxine treatment in a dose-dependent manner.

In many vertebrate species, thyroxine-binding globulin (TBG)[1] serves as a thyroid hormone transport protein in serum (Refetoff et al., 1970). In humans, it is a 54-kDa glycoprotein synthesized in the liver (Murata et al., 1985). Cloning and sequencing of human TBG cDNA revealed homology to another serum transport protein [cortisol-binding globulin (Hammond et al., 1987)] and to serine protease inhibitors (Flink et al., 1986).

The presence of TBG in rat serum was first demonstrated by polyacrylamide gel electrophoresis (Davis et al., 1970). Although similarities between rat and human TBG have been demonstrated in terms of electrophoretic mobility (Davis et al., 1970), structural microheterogeneity (Vranckx et al., 1986), and properties of binding to thyroxine and its analogues (Davis et al., 1970; Sutherland & Brandon, 1976), no direct or deduced information on rat TBG amino acid sequence has been available to explain its distinct immunological properties (Ain et al., 1987; Vranckx et al., 1990a).

Recent work has shown that the very low concentration of TBG in adult rat serum increases remarkably following thyroidectomy (Nanno et al., 1986; Young et al., 1988). This

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[*] Address correspondence to this author.
[§] The First Department of Internal Medicine, Nagoya University School of Medicine.
[∥] Department of Endocrinology and Metabolism, the Research Institute of Environmental Medicine, Nagoya University.
[⊥] Departments of Medicine and Pediatrics, University of Chicago.

[1] Abbreviations: TBG, thyroxine-binding globulin; M_r, molecular weight; 2DGE, two-dimensional gel electrophoresis; PVDF, poly(vinylidene difluoride).

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finding suggested that thyroid hormone may down-regulate the synthesis of TBG. However, the precise mechanism of such regulation remained unknown.

In this paper, we present the primary structure of the rat TBG molecule deduced by sequencing of two overlapping rat TBG cDNA clones. Rat TBG showed 76% amino acid sequence identity with human TBG. Moreover, using the cloned rat TBG cDNA as a probe, we show that TBG gene expression in rat liver is negatively regulated by thyroxine.

Experimental Procedures

Complementary DNA Cloning and Sequence Analysis. Human TBG cDNA (λcTBG8) (Flink et al., 1986), labeled with [^{32}P]dCTP (New England Nuclear) by nick translation (kit, Amersham), was used at high stringency to screen a λgt11 rat liver cDNA library (normal adult female Sprague-Dawley, Clontech Laboratories Inc.). Positive clones were isolated by a method described by Maniatis et al. (1982). Appropriate restriction fragments of two rat TBG cDNAs were subcloned into M13mp18 and M13mp19 (Messing et al., 1981) for the production of templates, which were sequenced by the dideoxy chain-termination method (Sanger et al., 1977). Search for a nucleotide sequence encoding the maximal length of amino acid sequence was carried out by using the genetic information processing software GENETYX (Software Development Co., Ltd.) for personal computer (PC-9801XA, NEC Corp.). The nucleotide and deduced amino acid sequences were compared to those of human TBG by using the same software.

Purification of Rat TBG and Determination of Its NH_2-Terminal Sequence. Thyroxine (Sigma Chemical Co.) was fixed onto Affi-Gel 15 (Bio-Rad Laboratories) as described in the manufacturer's manual. Thyroxine (130 μmol) was dissolved in 15 mL of ethanol and combined with 5 mL of the gel. The gel was washed with 2 mM NaOH and 0.1 M phosphate-buffered saline (PBS), pH 7.6, successively. The amount of thyroxine attached to the gel was estimated to be approximately 5 μmol/mL of gel.

Thyroxine affinity chromatography was carried out by the method of Pensky and Marshall (1969) with some modifications. After removal of the endogenous thyroxine by anion-exchange resin, pooled serum from thyroidectomized rats was applied to the affinity column at a flow speed of about 3 mL/h. After a washing with 0.1 M PBS, the protein retained on the column was eluted with 2 mM NaOH. These procedures were carried out at 4 °C.

Two-dimensional gel electrophoresis (2DGE) was carried out by the method of Manabe et al. (1979) with minor modifications. Isoelectric focusing was first performed on a 4% polyacrylamide disk gel containing 0.25% ampholines (pH 3.5–5 and pH 3.5–10 in a 3:2 ratio; Pharmacia LKB Biotechnology). The second dimension utilized a 3–17% gradient nondenaturing polyacrylamide slab gel. Approximately 0.4 mg of the partially purified TBG was resolved by 2DGE and electroblotted for 2 h at 20 V onto a poly(vinylidene difluoride) (PVDF) membrane (Immobilon, Millipore Co.) (LeGendre & Matsudaira, 1988). Proteins transferred onto the membrane were visualized by Coomassie blue staining. For the localization of TBG, another sample processed in parallel was incubated with ^{125}I-thyroxine (Dainabot Co.) and exposed to Kodak X-AR film (Eastman Kodak) for 24 h at room temperature.

The area on the PVDF membrane corresponding to the radioautographed spot was excised and applied to a gas-phase protein sequencer (Model 470A, Applied Biosystems) equipped with an on-line PTH analyzer (Model 120A, Applied Biosystems) (LeGendre & Matsudaira, 1988).

Animal Treatment. Four-week-old male Wistar rats (Nakajima Experimental Animal Institute) were rendered hypothyroid by surgical thyroidectomy. Rats were then given standard chow diet and water containing 0.9% $CaCl_2$ ad libitum. They were housed for 28 days under controlled conditions (22 °C; constant humidity; 12-h light, 12-h dark cycle). Hypothyroidism was confirmed by the cessation of weight gain and by serum thyroxine levels less than 1 nM.

For TBG purification, rats were killed by exsanguination under ether anesthesia 28 days after thyroidectomy. Serum was pooled and stored at −20 °C until used. Groups of four thyroidectomized rats each were treated with different amounts of thyroxine in order to induce various thyroid states. The low thyroxine group (LT_4 group) was given daily intraperitoneal injections of 1.15 nmol of thyroxine per 100 g of body weight; the median thyroxine group (MT_4 group), 2.25 nmol; and the high thyroxine group (HT_4 group), 4.5 nmol. The thyroidectomized group (Tx group) received vehicle only (50 mM PBS containing 0.03% BSA), and the control (C group) consisted of sham-operated intact rats. Treatment with thyroxine was begun on the 28th day after thyroidectomy. On the 5th day of this treatment and 24 h after the last thyroxine injection, rats were killed by exsanguination under ether anesthesia. Excised livers were frozen immediately in liquid nitrogen and kept at −80 °C until RNA extraction.

Analysis of Rat TBG mRNA. RNA was extracted from frozen liver by the method of Chomczynski and Sacchi (1987). For Northern blot analysis, 10 μg of total RNA were resolved by electrophoresis through a 0.8% agarose gel and transferred onto Gene Screen Plus (GSP, New England Nuclear). Hybridization was carried out under the conditions previously described (Kambe et al., 1988). The probe was λrcTBG12 labeled with [^{32}P]dCTP by using a random-primed labeling kit (Boehringer Mannheim). After being washed, GSP was exposed to Kodak X-AR film for 2 weeks at −80 °C.

For dot-blot analysis, total RNA was denatured and serially diluted as previously described (Murata et al., 1990). The highest concentration of RNA was 10 μg/well. Denatured RNA was blotted onto GSP by using a Manifold (Schleicher and Schuell). Hybridization and washing were under the conditions described previously (Kambe et al., 1988). GSP was exposed to an imaging plate (Fuji Photo Film Co.) (Amemiya & Miyahara, 1988) for 2 days at room temperature and analyzed by using a bioimage analyzer (BAS2000, Fuji Photo Film Co.).

Results

Fifteen positive clones were obtained by screening of 5×10^5 clones of a rat liver cDNA library with a human TBG cDNA probe (λcTBG8) (Flink et al. 1986) at high stringency conditions. The cDNA inserts were excised from the λ vector, and the restriction maps of each insert were compared. The two largest cDNA inserts (approximately 1.3 kilobase each) were found to overlap by about 0.8 kb and to contain all the sequences of the 13 smaller clones. These two inserts, designated as λrcTBG12 and λrcTBG13, were sequenced according to the strategy shown in Figure 1. An 826-nucleotide sequence was found to be common to λrcTBG12 and 13. Besides this overlapping region, λrcTBG12 contained 448 nucleotides extending in the 5′-direction, and λrcTBG13, 440 nucleotides in the 3′-direction. Since an initiation codon was not found in the nucleotide sequence of λrcTBG12, we searched in six phases for the maximal length of continuous amino acid sequence and found a stretch of 409 amino acid residues that ended with the termination codon TAA. Analysis by the method of von Heijne (1986) predicted a leader peptide

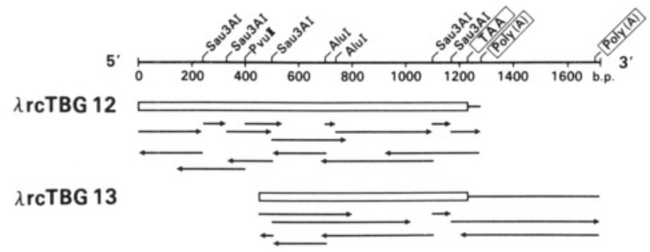

FIGURE 1: Partial restriction map and sequencing strategy for cDNA clones λrcTBG12 and λrcTBG13. TAA and poly(A) represent termination codon and polyadenylation signals, respectively. Length is given in base pairs and restriction sites are indicated. The direction and areas sequenced are shown by the horizontal arrows.

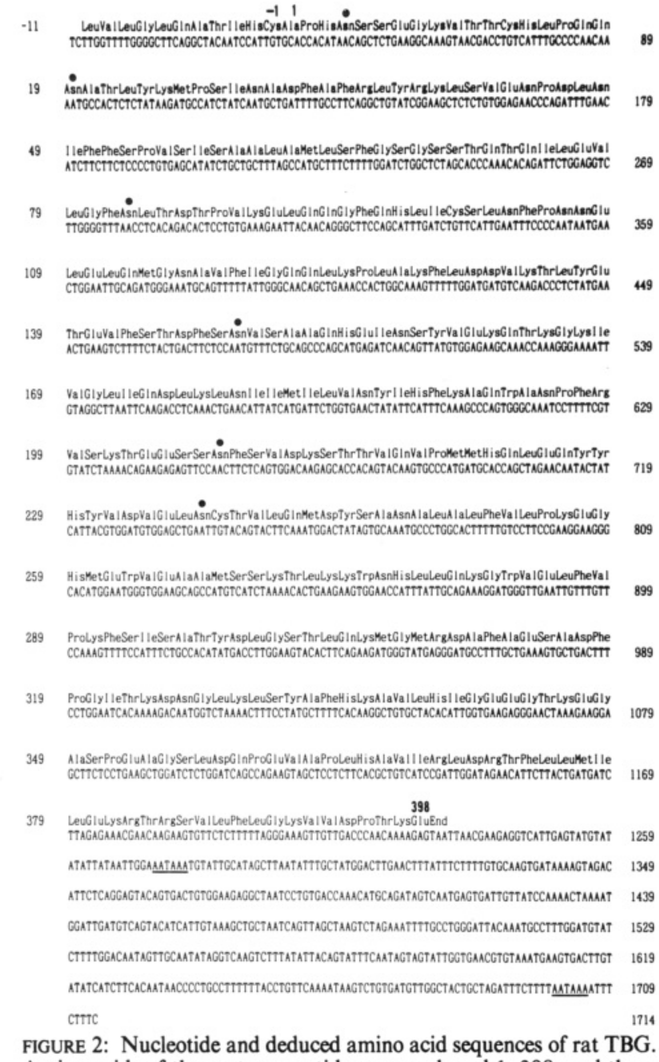

FIGURE 2: Nucleotide and deduced amino acid sequences of rat TBG. Amino acids of the mature peptide are numbered 1–398, and those in the partial signal peptide are −11 to −1. There are six potential N-glycosylation sites (●). The two polyadenylation signals are underlined.

cleavage site located between the 11th and 12th deduced amino acid. The 11 NH_2-terminal amino acids were hydrophobic, suggesting that they were part of a leader peptide. Thus, λrcTBG12 was considered to contain the truncated sequence of the leader peptide, complete nucleotide sequence encoding the mature protein, and a part of the 3′-untranslated region that lacked the polyadenylation signal. λrcTBG13 was considered to contain the nucleotide sequence encoding approximately two-thirds of the mature COOH-terminal portion of the protein and the 3′-untranslated region, including two polyadenylation signals. As shown in Figure 2, alignment of nucleotide sequences from λrcTBG12 and 13 provided a

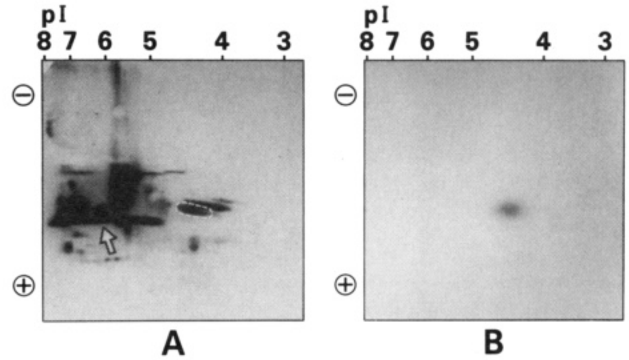

FIGURE 3: Protein stain (A) and autoradiography (B) of 2DGE. After partial purification of rat TBG by thyroxine affinity chromatography, an aliquot was resolved by 2DGE and electroblotted to a PVDF membrane. The membrane was stained with Coomassie blue (panel A). Another aliquot, incubated with ^{125}I-thyroxine, was subjected to 2DGE under the same conditions. The gel was autoradiographed on Kodak X-AR film for 1 day (panel B). A group of proteins on the PVDF membrane (circled by broken line in panel A), which were clearly separated as several dots by further destaining, corresponded to the spot on the autoradiogram. Those proteins were excised and subjected to direct protein analysis. Arrow in panel A indicates albumin.

Table I: NH_2-Terminal Amino Acid Sequences Deduced from cDNA and Identified by Direct Analysis[a]

From cDNA	A P H N S S E G K V T T C H L P Q Q N A T L Y K M P
Direct Analysis	A P H N - S E G K V T - - H - P Q Q - A - - - K M P

[a] NH_2-terminal amino acid sequence deduced from the cDNA sequence was compared with that determined by direct protein microsequencing. Amino acids are presented as a single letter, and a hyphen indicates an ambiguous result.

cDNA sequence of 1714 nucleotides. This cDNA showed 70% homology with the human TBG cDNA for the overlapped 1526 nucleotides.

To confirm that the cloned cDNA truly encodes rat TBG, we purified the protein from rat serum and determined its NH_2-terminal sequence. The rat TBG partially purified by thyroxine affinity chromatography was subjected to 2DGE, followed by electroblotting onto a PVDF membrane. Proteins transferred to the PVDF membrane were visualized by Coomassie blue staining (Figure 3A) and by autoradiography following exposure to ^{125}I-thyroxine (Figure 3B). The single ^{125}I-thyroxine-binding spot was used to localize the proteins (circled by broken lines in Figure 3A). The TBG isoforms migrated at p*I*s ranging from 4.2 to 4.6, a slightly cathodal position from albumin as previously described (Davis et al., 1970; Vranckx et al., 1986). The area on the membrane corresponding to the oval spot on the autoradiogram was excised and subjected to NH_2-terminal amino acid sequence analysis. Eighteen amino acid residues were identified after 26 cycles of direct microsequencing. As shown in Table I, the NH_2-terminal amino acid sequence was identical with that deduced from the nucleotide sequence, indicating that the clones sequenced were those of rat TBG cDNA.

Having identified the signal peptide cleavage site, analysis of the cDNA sequence indicated that rat TBG consists of 398 amino acids of molecular weight 44 607. Compared to human TBG, there are three additional amino acids at the NH_2 terminus (Figure 4). The overall homology between the mature forms of rat and human TBG was 76% for the overlapped 395 residues. Rat TBG was found to have six potential N-glycosylation sites, four of which were located in the same relative positions as in human TBG. Rat TBG contained only three cysteines, all of which were also located in identical

FIGURE 4: Comparison of the amino acid sequence of rat TBG to that of human TBG. Hyphens indicate identical amino acids. Potential N-glycosylation sites (●) and cysteine residues (▲) in rat and human TBG are also indicated.

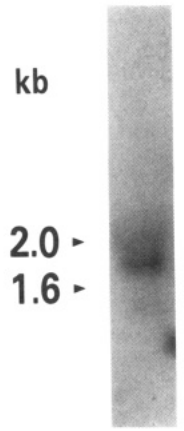

FIGURE 5: Northern blot analysis of TBG mRNA from rat liver. Total liver RNA (10 μg) was used for analysis. The positions of denatured DNA molecular size standards are shown on the left of the figure.

positions relative to human TBG.

Northern blot analysis of total RNA from thyroidectomized rat liver, using radiolabeled rat TBG cDNA as a probe, revealed an approximately 1.8-kb band (Figure 5). The change in hepatic TBG mRNA abundance was studied in rats 4 weeks after thyroidectomy and on the 5th day of thyroxine administration in varying doses. As shown in Figure 6, dot-blot analysis failed to detect TBG mRNA in control sham-operated rats (C group) but showed markedly increased amounts in nontreated thyroidectomized rats (Tx group). Administration of thyroxine reduced significantly the abundance of TBG mRNA after 5 days of treatment with 2.25 nmol of thyroxine day^{-1} (100 g of body wt)$^{-1}$ (MT_4 group; 37% of Tx) and 4.5 nmol of thyroxine day^{-1} (100 g of body wt)$^{-1}$ (HT_4 group; 18% of Tx).

Discussion

The nucleotide sequence of rat TBG cDNA, cloned from a rat liver cDNA library, showed 70% overall homology in comparison to human TBG cDNA: 81% homology in the coding sequence and 30% homology in the 3′-untranslated region. The NH_2-terminal amino acid sequence determined by direct analysis was identical with that deduced from the nucleotide sequence and started with the predicted residue, confirming that the cDNA encoded rat TBG. The mature protein consists of 398 amino acids (M_r = 44 607), three more at its NH_2 terminus than human TBG, with which it shares 76% sequence identity. As expected for a member of the serine protease inhibitors/hormone transport proteins superfamily

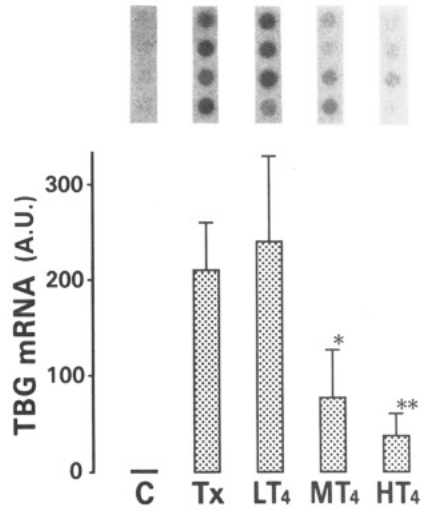

FIGURE 6: Changes in the abundance of TBG mRNA in liver after thyroidectomy and thyroxine replacement. The amount of TBG mRNA was determined by dot blot hybridization, followed by autoradiographic analysis. After exposure to an imaging plate for 2 days, the signal was visualized by using a bioimage analyzer. On the upper part of the figure, each spot represents the hepatic TBG mRNA from individual rats (n = 4) treated as follows: C, sham-operated control; Tx, thyroidectomized rats given vehicle injections; LT_4, thyroidectomized rats replaced with 1.15 nmol of thyroxine day^{-1} (100 g of body wt)$^{-1}$; MT_4, thyroidectomized rats replaced with 2.25 nmol of thyroxine day^{-1} (100 g of body wt)$^{-1}$; HT_4, thyroidectomized rats replaced with 4.5 nmol of thyroxine day^{-1} (100 g of body wt)$^{-1}$. Signal changes are shown in the bar graph in arbitrary units (AU). Statistical analysis was performed by unpaired t test compared to the Tx group. (*) and (**) indicate significant differences, $p < 0.01$ and $p < 0.005$, respectively. TBG mRNA in the C group of rats was not detectable. The abundance of TBG mRNA in the LT_4 group was not significantly different from that of Tx animals ($p > 0.05$).

(Flink et al., 1986; Hammond et al., 1987), rat TBG shows moderate homology in amino acid sequence with these proteins of both rat and human: α_1-antitrypsin, 43% and 40%, respectively; cortisol-binding globulin, 40% and 42%, respectively; and human α_1-antichymotrypsin, 39%.

The microheterogeneity of rat TBG (Vranckx et al., 1986) suggested indirectly its glycoprotein nature, but it remains unknown how many carbohydrate chains it contains. We identified six potential N-linked glycosylation sites at positions 4, 19, 82, 148, 207, and 236 of the amino acid sequence. Four of the five potential N-glycosylation sites of human TBG (Flink et al., 1986) are conserved in rat TBG. They are located at asparagines 16, 79, 145, and 233 and correspond to asparagine positions 19, 82, 148, and 236 of rat TBG (Figure 4). The four carbohydrate chains of human TBG (Zinn et al., 1978) are most likely located on these conserved asparagines since the consensus sequence Asn-Pro-Thr at Asn-391 of human TBG is unlikely to be glycosylated because of the intervening proline (Bause, 1983). It is likely that the four conserved N-glycosylation sites in the rat TBG are also glycosylated.

Partial support to this hypothesis was obtained from direct microsequencing of rat TBG. The identification of Asn-4 but not the asparagine located at position 19 suggests that Asn-19 but not Asn-4 is glycosylated. We have no information concerning the other nonconserved site of glycosylation (Asn-207) in rat TBG. Thus, rat TBG may contain four or five carbohydrate chains.

The precise structural requirements for biological activity of TBG remain unknown. Tabachnick and Perret (1987) used chemical cross-linking of thyroxine to determine its binding site on human TBG. They identified residues 249–252 and 283–286 as possible binding regions. The amino acid se-

quences of the corresponding regions in rat TBG (252–255 and 286–289) are fully conserved. Huber and Carrel (1989) singled out the importance of residues 238 and 256 at the entrance of a pocket formed by a barrel of β-strands as a putative ligand-binding site. These residues, threonine and lysine, respectively, are conserved in the rat and human TBGs but are different in cortisol-binding globulin and α_1-antitrypsin. Finally, the positions of the only three cysteines in rat TBG are also conserved in human TBG, which contains two additional cysteines. Also, the two regions surrounding Cys-100 and -237 are well conserved (Figure 4). If these two cysteines formed a disulfide bond, these conserved regions may play an important role in imparting the specific hormone-binding function. Indeed, mutations of amino acids 96 and 227 of human TBG (corresponding to residues 99 and 230 of rat TBG) have been shown to virtually abolish thyroxine binding (Mori et al., 1989, 1990).

Glu-264 and -342 and Lys-290 and -387 of human α_1-antitrypsin are thought to have an important role in the stabilization and secretion of protein by making internal salt bridges, between Glu-264 and Lys-387 and between Lys-290 and Glu-342 (Loebermann et al., 1984; Huber & Carrell, 1989; Crystal, 1990). The positions of these residues are fully conserved in rat TBG (Glu-264 and -342 and Lys-290 and -391). However, the reactive center, Met-358 and Ser-359 of human α_1-antitrypsin, as a target region for serine proteases is not conserved in rat TBG.

Using a human TBG cDNA probe, Vranckx et al. (1990b) found two TBG mRNAs, of approximately 1.8 and 2.4 kb, in adult thyroidectomized rat liver. They correspond to the sizes of the two TBG mRNAs in human liver of approximately 1.8 and 2.0 kb (Kambe et al., 1988). In the latter case the 3′-untranslated region spans 297 bp with polyadenylation signals 228 bp apart, in agreement with the 0.2-kb difference between the two mRNAs. In rat TBG the 3′-untranslated region spans 485 bp with the two polyadenylation signals 426 bp apart, predicting two mRNAs differing by approximately 0.4 kb, or 1.8 and 2.2 kb. Since this difference cannot be affected by the size of the 5′-untranslated region, either the estimation of the larger rat TBG mRNA by Vranckx et al. (1990b) is off by 0.2 kb or there is a third polyadenylation signal, a possibility we cannot exclude since the our λrcTBG13 clone did not contain a poly(A) tail. Our failure to detect a larger TBG mRNA may be related to its relatively low abundance.

Vranckx et al. (1990b) also reported that TBG mRNA abundance in rat liver increased after thyroidectomy. The same result was obtained in the present study by using a rat TBG cDNA probe. Furthermore, we show that the marked increase of TBG mRNA following thyroidectomy was significantly reduced by the administration of both physiologial and supraphysiological doses of thyroxine. This indicates that expression of the rat TBG gene in liver is negatively regulated by thyroxine. We are currently searching for a thyroid hormone regulatory element in genomic rat TBG clones.

Serum TBG levels in the rat also increase in response to various manipulaations other than the thyroid hormone state. These include fasting (Young et al., 1985), vitamin A deficient diet (Garcin & Higueret, 1980), and low-protein, high-carbohydrate diet (Young et al., 1982). A transient increase during postnatal development has been also reported (Savu et al., 1987; Young et al., 1988). The precise mechanisms involved in these changes remain unknown and may be clarified by measurement of their influence on TBG transcription and translation.

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Site-Specific Incorporation of Nonnatural Residues during In Vitro Protein Biosynthesis with Semisynthetic Aminoacyl-tRNAs†

J. D. Bain,‡ Edward S. Diala,§ Charles G. Glabe,‖ Dean A. Wacker,‡ Matthew H. Lyttle,⊥ Thomas A. Dix,# and A. Richard Chamberlin*,‡

Departments of Biological Chemistry, Chemistry, and Molecular Biology and Biochemistry, University of California at Irvine, Irvine, California 92717, Department of Pediatrics, University of California at San Diego, School of Medicine, La Jolla, California 92093, and Milligen/Biosearch, Division of Millipore, Incorporated, 81 Digital Drive, Novato, California 94949

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ABSTRACT: A method is presented for the incorporation of nonnatural amino acids into proteins during in vitro cell-free translation. A combination of chemical synthesis and run-off transcription was employed to prepare a semisynthetic, nonhypermodified $tRNA^{Gly}$ nonsense suppressor acylated with L-3-[^{125}I]iodotyrosine. The presence of this synthetic tRNA during in vitro translation of mRNA containing a nonsense suppression site (e.g., a UAG termination codon) results in the incorporation of the nonnatural amino acid L-3-iodotyrosine into the polypeptide exclusively at the position corresponding to that site. Incorporation of the nonnatural amino acid L-3-[^{125}I]iodotyrosine into the model polypeptide was assessed by quantitative and unambiguous determination of suppression efficiency, read-through, and site specificity of incorporation. Minor modifications of the method employed in this initial experiment also allow the rapid analysis of unlabeled acylated tRNA analogues. Under optimum conditions, the unlabeled amino acid L-3-iodotyrosine was found to be incorporated with a suppression efficiency of 65%. Other nonnatural residues, including *N*-methylphenylalanine, D-phenylalanine, and phenyllactic acid, were tested in the assay under these same conditions. Suppression efficiencies for this series ranged from 0 to 72% depending on the structure of the residue incorporated. Several other aspects of this methodology, such as tRNA structure and context effects, are briefly discussed.

Site-specific mutagenesis is one of the most important experimental tools available for protein research, but the methodology suffers from the limitation that amino acid substitutions are restricted to the 20 primary amino acids. This drawback precludes the direct site-specific introduction of "designer" amino acids (e.g., detection, catalytic, linking, or cleaving residues) that could modify the function or activity of a protein in a novel way. One method to circumvent this problem has been to modify a specific residue in a protein posttranslationally. The exceptionally high degree of chemoselectivity required to modify a protein in this manner has limited the scope of this approach, although a few notable successes exist, as exemplified by work of Kaiser and coworkers (Hilvert et al., 1988). It seems likely, however, that a truly general method would require intervention during protein biosynthesis, i.e., during translation, if the innate selectivity problems associated with posttranslational chemical modification are to be avoided.

Methods to manipulate protein structure during biosynthesis have centered around incorporation of residues via an exogenous source of a "misacylated" tRNA or tRNA analogue, which have been prepared by various combinations of chemical and enzymatic methods. One of the earliest experiments in this area was the insertion of alanine at a cysteine codon by conversion of cysteinyl-$tRNA^{Cys}$ to alanyl-$tRNA^{Cys}$ through reductive desulfhydration with Raney nickel (Chapeville et al., 1962). This experiment was the first direct test of the "adaptor hypothesis" (Crick, 1958) and clearly established that recognition of each aminoacyl-tRNA by the ribosome is not dependent upon the amino acid itself, but rather upon structural elements of the tRNA to which it is attached, specifically the anticodon. Thus, a reasonable strategy for the introduction of nonnatural residues into proteins can be envisioned whereby a tRNA acylated with the desired residue is introduced into a protein expression system.

One successful approach for the formation of aminoacyl-tRNAs comes from the many reports from Hecht and coworkers, who have pioneered the area of "chemically misacylated" tRNAs (Pezzuto & Hecht, 1980; Heckler et al.,

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* To whom correspondence should be addressed.
‡ Department of Chemistry, University of California at Irvine.
§ Department of Pediatrics, University of California at San Diego.
‖ Department of Molecular Biology and Biochemistry, University of California at Irvine.
⊥ Milligen/Biosearch.
Departments of Chemistry and Biological Chemistry, University of California at Irvine.

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