

# Localization and synthesis of the hormone-binding regions of the human thyrotropin receptor

(thyrotropin/binding site/synthetic peptides)

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**ABSTRACT** Two regions of human thyrotropin (thyroid-stimulating hormone, TSH) receptor (TSHR) (residues 12–44 and 308–364) were selected on the basis that they exhibit no sequence resemblance to luteinizing hormone/chorionic gonadotropin receptor. Five synthetic overlapping peptides (12–30, 24–44, 308–328, 324–344, and 339–364) were studied for their ability to bind <sup>125</sup>I-labeled human TSH (hTSH), its isolated  $\alpha$  and  $\beta$  subunits, bovine TSH, ovine TSH, human luteinizing hormone, and human follicle-stimulating hormone. The human TSHR peptides 12–30 and 324–344 exhibited remarkable binding activity to human, bovine, and ovine TSH and to the  $\beta$  chain of hTSH. Lower binding activity resided in the adjacent overlapping peptides, probably due to the contribution of the shared overlap to the binding. The specificity of TSH binding to these peptides was confirmed by their inability to bind human luteinizing hormone, human follicle-stimulating hormone, and the  $\alpha$  chain of hTSH. Thyrotropins did not bind to bovine serum albumin or to peptide controls unrelated to the TSHR system. Furthermore, the binding of hTSH to TSHR peptides 12–30 and 324–344 was almost completely ( $\approx 90\%$ ) inhibited by rabbit antibodies against hTSH but not by antisera against unrelated proteins. It is concluded that the binding of TSH to its receptor involves extensive contacts and that the TSHR peptides 12–30 and 324–344 contain specific binding regions for TSH that might be either independent sites or two faces (subsites) within a large binding site.

Thyrotropin (thyroid-stimulating hormone, TSH) belongs to a family of closely related glycoprotein hormones that includes lutropin (luteinizing hormone, LH), follicle-stimulating hormone (FSH), and chorionic gonadotropin (CG). The first three (TSH, LH, and FSH) are produced by the anterior pituitary, whereas CG is made in the trophoblast during pregnancy. Each of these hormones consists of two different subunits ( $\alpha$  and  $\beta$ ) that are not covalently linked and, in a given species, the  $\alpha$  subunits are identical for all these hormones (1–4). Although their  $\alpha$  subunit is unchanged, these hormones have different biological activities. TSH stimulates the thyroid gland to secrete thyroxine and triiodothyronine into the circulation, and the latter are required for proper metabolism, differentiation, and development of tissues. The major functions of LH and FSH are in reproductive physiology, whereas CG stimulates the production of progesterone (2). Because they have identical  $\alpha$  chains, the activities of these hormones are clearly determined by their  $\beta$  subunits. The hormones bind to specific receptors on target cells, and the activated receptor stimulates adenylate cyclase through the G protein (2, 5). Recent molecular cloning and cDNA sequencing studies have provided the primary structures of dog TSH receptor (TSHR) (6) and human (h) TSHR (7–9). Also, the primary structures of rat (10) and porcine (11)

LH/CG receptor have been deduced from the respective cDNA sequences. Comparison of the amino acid sequences of the extracellular parts of the LH/CG and the TSH receptors revealed that these G protein-coupled receptors display an extensive sequence similarity. The TSHR, however, possesses two regions that have no sequence similarity to the LH/CG receptor. This fact suggested to us that these two regions might contain the TSH-binding site on the receptor. In the present work, the regions were synthesized, and their specific binding activity for TSH was determined.

## MATERIALS AND METHODS

**Materials.** The hormones hTSH, bovine (b) TSH, hLH, hFSH, and the  $\alpha$  and  $\beta$  subunits of hTSH were obtained from the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. The hTSHR peptides employed in the present work were as follows: peptide 12–30, HQEDFRVTCCKDIQRIPSL(G); peptide 24–44, IQRIPSLPPSTQTLKLIETHL(G); peptide 308–328, ENLGDSIVGYKEKSKFQDTHN(G); peptide 324–344, ODTHNNAHYVFFEEQEDEII(G); and peptide 339–364, QEDEIIGFGQELKNPQEETLQAFDSH(G). The underlined regions indicate overlaps between consecutive peptides. The C-terminal glycine residues are not part of the hTSHR sequence, but the peptides were synthesized on a glycine resin for convenience. The peptides were prepared by solid-phase synthesis on a benzyloxybenzyl alcohol resin (Vega Biotechnologies) to which 9-fluorenylmethylcarbonyl (Fmoc)-glycine had been coupled. The  $N^{\alpha}$ -Fmoc amino acid derivatives were obtained from Vega or from Peninsula Laboratories. The side-chain protecting groups were as follows: aspartic and glutamic,  $\beta$ - and  $\gamma$ -tert-butyl esters, respectively; cysteine, *S*-tert-butyl; histidine, im-trityl; lysine, *ε*-tert-butoxycarbonyl; serine, threonine, and tyrosine, *O*-tert-butyl; arginine,  $N^{\omega}$ -methoxy-2,3,6-trimethylphenylsulfonyl. Removal of the  $N^{\alpha}$ -Fmoc group before each coupling was done by treatment of the peptide resin with 20% piperidine in dimethylformamide (DMF) for 10 min. This was followed by washing (3 times each, 30 sec) with DMF, methanol, and then DMF. Coupling of consecutive amino acids was done for 2 hr by using 3-molar excess of each of the Fmoc amino acid derivatives, diisopropylcarbodiimide in DMF/CH<sub>2</sub>Cl<sub>2</sub>, 1:1 (vol/vol) and 1-hydroxybenzotriazole. The resin was then washed with DMF and methanol (three times each, 30 sec), followed by two 30-sec washes of CH<sub>2</sub>Cl<sub>2</sub>. The completion of coupling after each residue was monitored by ninhydrin (12), and recoupling was repeated when necessary. After the last cycle and deprotection of the

Abbreviations: TSHR, thyrotropin receptor; TSH, thyrotropin (b, bovine; h, human; o, ovine); CG, chorionic gonadotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; BSA, bovine serum albumin; DMF, dimethylformamide;  $\alpha$  or  $\beta$  in any abbreviation indicates  $\alpha$  or  $\beta$  chain, respectively.

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Fmoc-group, the peptide was cleaved from the resin by treatment (2.5 hr) with 55% trifluoroacetic acid in  $\text{CH}_2\text{Cl}_2$  (13), and the solvent was removed on a rotary evaporator. The peptide was washed three times with cold ether, dissolved in water, and freeze-dried. The products were purified by chromatography on CM-Sephadex  $\text{C}_{50}$  or DEAE-Sephadex  $\text{A}_{50}$ , as appropriate (14, 15). The peptides thus obtained were homogeneous by high-voltage paper electrophoresis and by analytical HPLC on a  $\text{C}_{18}$  column using a gradient of 0.1% trifluoroacetic acid in water/0.1% trifluoroacetic acid in acetonitrile. The amino acid compositions of the peptides were in excellent agreement with those expected from their sequences.

**Binding Studies.** Proteins were labeled with  $^{125}\text{I}$  by the chloramine-T method (16) and were used immediately after labeling. The specific activities of the labeled proteins were as follows: hTSH,  $6.7 \times 10^5$  cpm/pmol; hTSH $\beta$ ,  $3.07 \times 10^5$  cpm/pmol; hTSH $\alpha$ ,  $2.36 \times 10^5$  cpm/pmol; bTSH,  $4.50 \times 10^5$  cpm/pmol; ovine (o) TSH,  $4.77 \times 10^5$  cpm/pmol; hLH,  $3.79 \times 10^5$  cpm/pmol; hFSH,  $4.48 \times 10^5$  cpm/pmol. Proteins and peptides were coupled to CNBr-activated Sepharose CL-4B as described (17). Protein and peptide adsorbents contained packed volumes of  $0.95 \pm 0.06$  mg/ml and  $0.48 \pm 0.04$  mg/ml, respectively. Quantitative solid-phase titration assays were done in phosphate-buffered saline (PBS) (0.15 NaCl in 0.01 M sodium phosphate buffer, pH 7.2) containing 0.1% bovine serum albumin (BSA). Binding studies were carried out in two ways: (i) In one assay, a fixed amount ( $1 \times 10^5$  cpm) of each hormone was titrated with increased amounts of each adsorbent suspension [from 25  $\mu\text{l}$  up to 200  $\mu\text{l}$ , 1:1 (vol/vol)] in a total reaction volume of 260  $\mu\text{l}$ . After reaction (room temperature, 14 hr), the adsorbents were washed five times with PBS, and their radioactivity was counted on a gamma counter. Nonspecific binding was determined by titrating equivalent volumes of uncoupled Sepharose CL-4B and Sepharose coupled to BSA. (ii) Binding studies were also done by using a fixed amount (50  $\mu\text{l}$ ) of adsorbent suspension, 1:1 (vol/vol) in PBS/0.1% BSA and increased amounts of labeled ligands in a total reaction volume of 110  $\mu\text{l}$ . The conditions and manipulations were otherwise exactly as described under *i* above. Nonspecific binding was determined by titrating a fixed amount [50  $\mu\text{l}$ , 1:1 (vol/vol) suspension] of uncoupled Sepharose and BSA-Sepharose with equivalent amounts of labeled hormones. Scatchard analysis (18) of these titrations was used to determine the dissociation constants ( $K_d$ ) of the binding of the hormones to the peptides.

**Inhibition of the Binding of hTSH by Anti-hTSH Antiserum.** The specificity of the binding of hTSH to peptides was confirmed by inhibition studies using a rabbit anti-hTSH antiserum. A fixed amount of  $^{125}\text{I}$ -labeled hTSH ( $1 \times 10^5$  cpm in 10  $\mu\text{l}$  of PBS/0.1% BSA) was added to aliquots (50  $\mu\text{l}$ ) of various dilutions (in PBS/0.1% BSA) of rabbit anti-hTSH antiserum. The solutions were mixed gently at room temperature for 14 hr, and then a fixed amount (40  $\mu\text{l}$ ) of peptide-adsorbent suspension, 1:1 (vol/vol) in PBS/0.1% BSA, was added. The total reaction volume was 100  $\mu\text{l}$ . The tubes were subjected to gentle agitation at room temperature for 4 hr, after which they were washed and counted as described above. Control experiments were done using equivalent dilutions of normal rabbit serum and rabbit antiserum against sperm whale myoglobin.

## RESULTS

**Binding of hTSH, bTSH, oTSH, and Control Hormones to hTSHR Peptides.** In quantitative radiometric titrations of a fixed amount of  $^{125}\text{I}$ -labeled hTSH and bTSH with increased amounts of each of the hTSHR peptides (Figs. 1A and 2A1), the peptides 12–30 and 324–344 possessed high binding

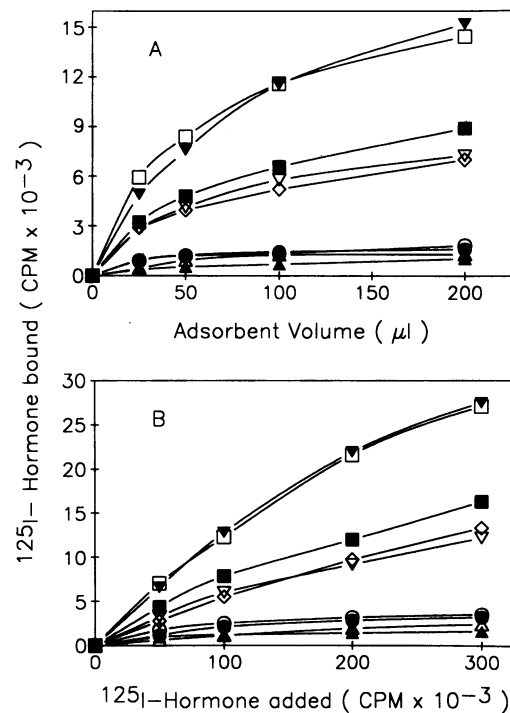


FIG. 1. Binding of  $^{125}\text{I}$ -labeled hTSH and control hormones (hLH and hFSH) to adsorbents of the hTSHR peptides and of controls. (A) Titration of fixed amounts ( $1 \times 10^5$  cpm) of labeled hormones with increased amounts of adsorbents [given in aliquots of 1:1 suspension (vol/vol)]. (B) Titration of fixed amounts (50  $\mu\text{l}$ ) of suspension, 1:1 (vol/vol) of each adsorbent with increased amounts of labeled hormones. For experimental details, see text.  $\nabla$ , Binding of hTSH to peptide 324–344;  $\square$ , binding of hTSH to peptide 12–30;  $\blacksquare$ , binding of hTSH to peptide 24–44;  $\diamond$ , binding of hTSH to peptide 308–328;  $\diamond$ , binding of hTSH to peptide 339–364. The following controls are shown:  $\Delta$ , binding of hLH to all peptides;  $\blacktriangle$ , binding of hFSH to all peptides;  $\circ$ , binding of all hormones (hTSH, hLH, hFSH) to BSA;  $\bullet$ , binding of all hormones to uncoupled Sepharose CL-4B. Results are given in cpm without correction for nonspecific binding by controls.

activity with both TSH hormones. The amounts of a given TSH bound by these two peptides were essentially identical throughout the titration curves. Peptides 24–44, 308–328, and 339–364 showed, with a given TSH, comparable binding activities that were lower (about half in the plateau) than the activities of peptides 12–30 or 324–344. Both hormones showed an insignificant level of nonspecific binding (1–1.5% of total label added) to uncoupled Sepharose CL-4B and to BSA-Sepharose. Control hormones hLH and hFSH did not bind to any of the hTSHR peptides, further confirming the specificity of the TSH binding. In titrations of fixed amounts of adsorbents [50  $\mu\text{l}$ , 1:1 (vol/vol) suspension] with increased amounts of  $^{125}\text{I}$ -labeled hTSH and bTSH (Figs. 1B and 2B1), peptides 12–30 and 324–344 exhibited strong binding activities and, with a given hormone, their binding curves were virtually superimposable. The peptides 12–44, 339–364, and 308–328 showed significant, but considerably lower, binding activities. Nonspecific binding of  $^{125}\text{I}$ -labeled hTSH and bTSH to uncoupled Sepharose or to BSA-Sepharose was insignificant. Control hormones hLH and hFSH did not bind to any of the hTSHR peptides, thus providing further evidence for the specificity of the hTSH and bTSH binding to the peptides.

The binding activity of the peptides for oTSH was much lower (about half) (Fig. 2A2 and B2) than their corresponding activities for hTSH or bTSH. With oTSH, only three peptides exhibited significant binding activities, which were in decreasing order, peptide 324–344, peptide 12–30, and peptide

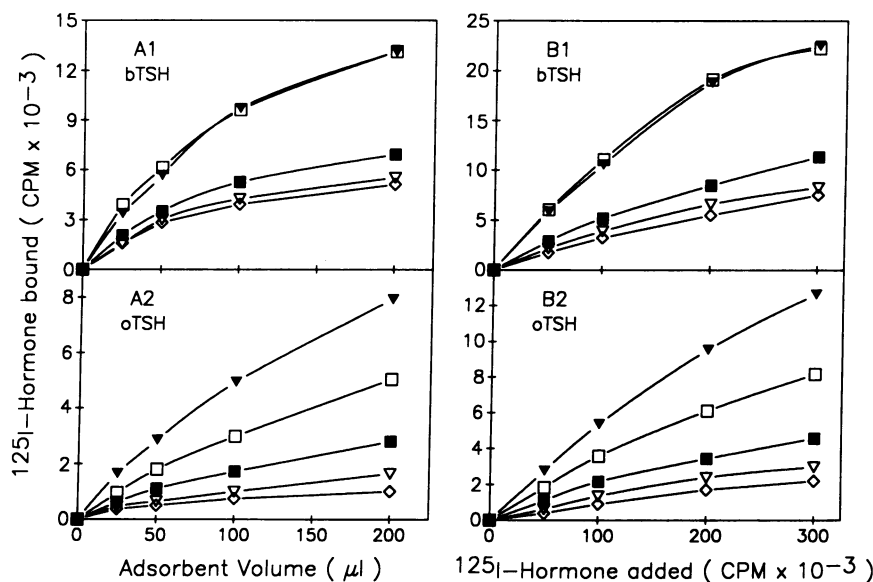


FIG. 2. Binding of <sup>125</sup>I-labeled bTSH (A1, B1) and oTSH (A2, B2) to adsorbents of the hTSHR peptides. (A1 and A2) Titration of fixed amounts ( $1 \times 10^5$  cpm) of labeled hormones with increased amounts of adsorbents [given in aliquots of 1:1 suspension (vol/vol)]. (B1 and B2) Titration of fixed amounts (50  $\mu$ l) of suspension, 1:1 (vol/vol) of each adsorbent with increased amounts of labeled hormones. For experimental details, see text. Binding to peptide 324–344 ( $\blacktriangledown$ ); peptide 12–30 ( $\square$ ); peptide 24–44 ( $\blacksquare$ ); peptide 308–328 ( $\triangledown$ ); peptide 339–364 ( $\diamond$ ). Controls were the same as those given in Fig. 1. Results have not been corrected for nonspecific binding to controls.

24–44. The binding activities of the other two peptides (308–328 and 339–364) were only very slightly higher than nonspecific binding to control adsorbents (uncoupled Sepharose and BSA-Sepharose). Nonspecific binding to these control adsorbents was 0.5–1.0% of total label added.

**Binding of hTSH Subunits to hTSHR Peptides.** Because the hormones hTSH, hLH, and hFSH possess identical  $\alpha$  subunits, then binding studies on the isolated subunits will determine the roles of the  $\alpha$  and  $\beta$  subunits in the binding of hTSH to its receptor. In titrations of a fixed amount of <sup>125</sup>I-labeled hTSH  $\alpha$  subunit ( $\alpha$ ) and hTSH  $\beta$  subunit ( $\beta$ ) with increased amounts of peptide adsorbents, and of a fixed amount of adsorbents with increased amounts of labeled subunits (Fig. 3 A and B), peptides 12–30 and 324–344 displayed strong and essentially equal binding activities. It should also be noted that, in either assay, the amounts of hTSH $\beta$  and hTSH bound by these two peptides were quite comparable (compare Figs. 1 and 3 and also see Fig. 4). As found with the whole TSH hormones, peptides 24–44, 308–328, and 339–364 showed lower, but significant, binding activities toward hTSH $\beta$ . Control adsorbents (uncoupled Sepharose and BSA-Sepharose) showed very low binding of hTSH $\beta$  (Fig. 3). Finally, <sup>125</sup>I-labeled hTSH $\alpha$  did not bind to any of the hTSHR peptides, strongly indicating that the results obtained with hTSH $\beta$  reflected specific binding.

Fig. 4 summarizes the results when equal amounts of each of the hormones (hTSH, bTSH, oTSH, hLH, and hFSH) and the subunits (hTSH $\alpha$  and hTSH $\beta$ ) are allowed to bind to a fixed volume of each peptide adsorbent. Peptides 12–30 and 324–344 bind equal amounts of hTSH and hTSH $\beta$ , whereas their binding of bTSH is  $\approx$ 20% lower. With oTSH, peptide 324–344 loses  $\approx$ 55% of its binding, relative to hTSH, whereas the activity of peptide 12–30 is decreased by  $\approx$ 72%. None of the hTSHR peptides bound hTSH $\alpha$ , hLH, or hFSH, again confirming the binding specificity of TSH and its  $\beta$  subunit.

**Measurement of Dissociation Constants.** The results of titrations of fixed amounts of hTSHR peptide adsorbents with various amounts of the three <sup>125</sup>I-labeled TSH variants and of hTSH $\beta$  were subjected to Scatchard analysis (18). The  $K_d$  values (Table 1) of the binding of hTSH to peptides 12–30 and 324–344 were essentially equal but smaller than the respective values for hTSH $\beta$  and oTSH by factors of  $\approx$ 2 and 3, respectively. The  $K_d$  values for bTSH binding to these two peptides were only slightly larger than the corresponding values of hTSH.  $K_d$  values of the binding of these labeled ligands to the other three hTSHR peptides were about an

order of magnitude higher than the values to the aforementioned two peptides.

**Inhibition of the Binding of hTSH to the Peptides by Antibodies Against hTSH.** Specificity of hTSH binding to hTSHR peptides was further confirmed by inhibition studies with rabbit anti-hTSH antisera. The binding of hTSH to peptides 12–30 and 324–344 was almost completely (90%) inhibited by rabbit antibodies against hTSHR (Fig. 5). The inhibitor (IgG antibody) concentrations at 50% inhibition ( $IC_{50}$ ) were as

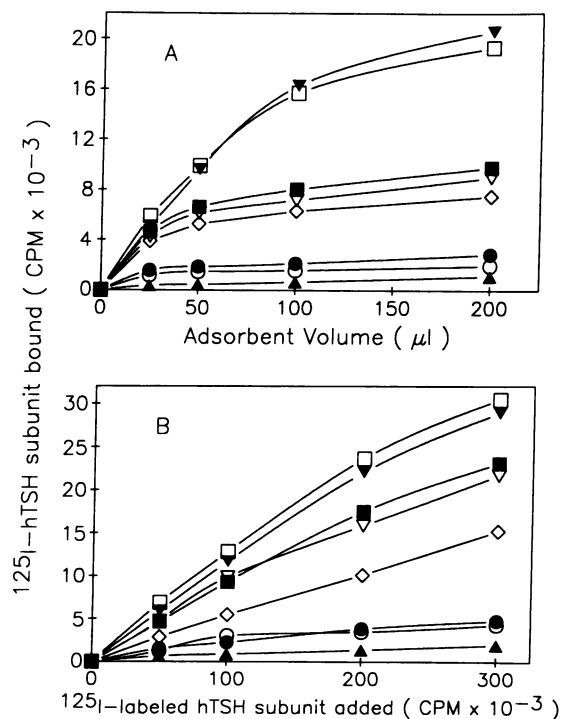


FIG. 3. Binding of <sup>125</sup>I-labeled  $\alpha$  and  $\beta$  subunits of hTSH to adsorbents of hTSHR peptides and controls. (A) Titration of fixed amounts ( $1 \times 10^5$  cpm) of labeled subunits with increased amounts of suspension, 1:1 (vol/vol) of each adsorbent. (B) Titration of fixed amounts (50  $\mu$ l) of suspension, 1:1 (vol/vol) of each adsorbent with increased amounts of labeled subunits. Binding of hTSH $\beta$  to peptide 324–344 ( $\blacktriangledown$ ); peptide 12–30 ( $\square$ ); peptide 24–44 ( $\blacksquare$ ); peptide 308–328 ( $\triangledown$ ); peptide 339–364 ( $\diamond$ ).  $\blacktriangle$ , Binding of hTSH $\alpha$  to all peptides;  $\circ$ , binding of either subunit (hTSH $\alpha$  or hTSH $\beta$ ) to BSA;  $\bullet$ , binding of either subunit to Sepharose CL-4B. Results are given in cpm without correction for nonspecific binding by controls.

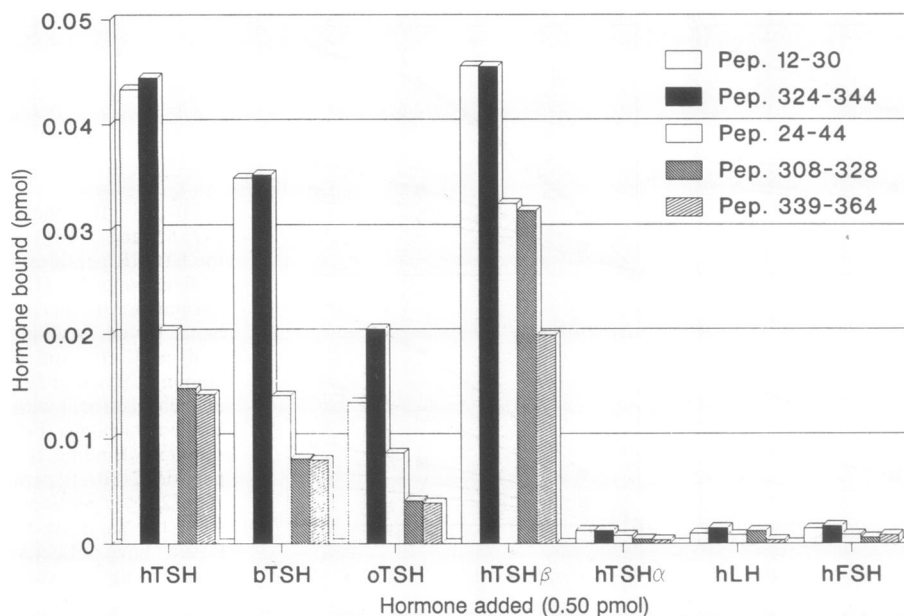


FIG. 4. Comparison of amounts of  $^{125}\text{I}$ -labeled hormones (or subunits) bound when equivalent amounts (0.5 pmol) of each hormone and equal amounts (25  $\mu\text{l}$ , packed volume) of each peptide adsorbent are mixed in a total reaction volume of 110  $\mu\text{l}$ . The procedure for binding studies is described in text.

follows: peptide 12–30,  $7.8 \times 10^{-9}$  M; peptide 324–344,  $2.8 \times 10^{-8}$  M. Nonimmune rabbit IgG and rabbit antibodies against an unrelated protein (sperm whale myoglobin) did not inhibit hTSH binding to hTSHR peptides.

## DISCUSSION

Comparison of the amino acid sequences of the extracellular domains of dog and human TSHR with those of rat and porcine LH/CG receptor revealed that these G protein-coupled receptors contain extensive regions of homology. However, the TSHR molecule contains two particular regions (residues 12–44 and 308–364) that have no homology with the LH/CG receptor. In fact, regions 18–25 and 313–364 have no counterpart in LH/CG receptor. We, therefore, reasoned that the regions specific to TSHR might be responsible for its binding to TSH. The two regions of hTSHR were thus synthesized using an overlapping peptide strategy (19) to protect against the inadvertent scission of a binding region between two consecutive peptides that might result in destruction of binding activity (19). The overlapping peptide approach has enabled localization of protein-binding sites of diverse activities (19–21).

The finding that the TSHR peptides 12–30 and 324–344 possessed considerable binding activity for TSH and did not bind LH or FSH confirmed that these two peptides contain specific binding regions for TSH. The specificity of TSH binding to the peptides was also indicated from their remarkable binding activity for the  $\beta$  subunit of hTSH and complete lack of binding for its  $\alpha$  subunit. Finally, the ability of

anti-hTSH antibodies to completely inhibit the binding of hTSH to peptides 12–30 and 324–344 strongly confirmed the specificity of this binding. The small binding activity of peptide 24–44 for TSH and its  $\beta$  subunit is probably due to the 7-residue overlap it shares with peptide 12–30. Similarly, the low activities of peptides 308–323 and 339–364 are probably derived from their overlaps with peptide 324–344. Note that the overlapping peptide strategy is not designed to give the exact boundaries of binding sites but rather to localize the maximal continuous regions within which these sites would reside (19–21). Further studies of other appropriately designed synthetic peptides spanning these areas are required to define the boundaries of the sites.

The  $\alpha$  subunit is identical in TSH, LH, FSH, and CG of a given species. The binding site on the hormone for the receptor has been proposed to comprise parts of both the  $\alpha$  and  $\beta$  subunits of a given hormone (1, 2). Our finding that the isolated  $\beta$  subunit of hTSH binds to the synthetic binding regions of hTSHR and that it does so with an affinity only slightly smaller than that of the  $\alpha\beta$  dimeric hTSH indicates, at least in TSH, that no contact residues are contributed by the  $\alpha$  chain to the binding site. Furthermore, the  $\alpha$  chain may play only a minimal role in inducing the  $\beta$  chain to achieve appropriate folding that will produce the correct conformation of its binding site because a major role would have been reflected in large affinity differences between hTSH and its  $\beta$  subunit for the peptides. The role of the  $\alpha$  subunit is to be internalized (after binding of the  $\beta$  subunit to the receptor) to stimulate adenylate cyclase (1, 2).

The two hormones hTSH and bTSH possessed comparable affinities in their binding to hTSH peptides 12–30 and 324–

Table 1. Dissociation constants ( $K_d$ ) of the binding of hTSH, hTSH $\beta$ , bTSH, and oTSH to hTSHR peptides

Labeled ligand	$K_d$ of binding to human TSHR peptides, M				
	Peptide 12–30	Peptide 324–344	Peptide 24–44	Peptide 308–328	Peptide 339–364
hTSH	$3.8 \times 10^{-9}$	$3.9 \times 10^{-9}$	$1.0 \times 10^{-8}$	$1.1 \times 10^{-8}$	$1.6 \times 10^{-8}$
hTSH $\beta$	$7.5 \times 10^{-9}$	$7.2 \times 10^{-9}$	$1.2 \times 10^{-8}$	$1.3 \times 10^{-8}$	$1.5 \times 10^{-8}$
bTSH	$5.4 \times 10^{-9}$	$4.8 \times 10^{-9}$	$2.0 \times 10^{-8}$	$2.2 \times 10^{-8}$	$4.0 \times 10^{-8}$
oTSH	$1.1 \times 10^{-8}$	$9.8 \times 10^{-9}$	$5.5 \times 10^{-8}$	NB	NB

NB, no binding or very low binding of oTSH to these peptides was obtained (see Fig. 2).

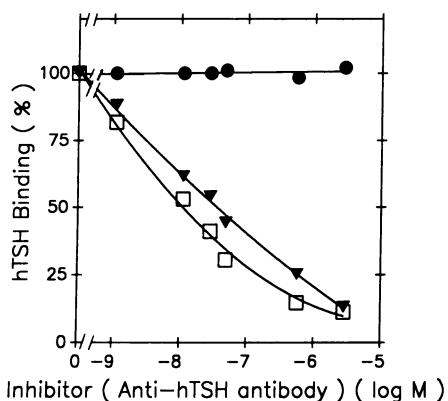


FIG. 5. Inhibition of hTSH binding to the two most active hTSHR peptides by increased amounts of rabbit anti-hTSH antibodies. hTSH binding is given in % hormone bound in the presence of increased molar concentrations of the IgG antibodies at various dilutions of antiserum. ▼, Binding to peptide 324–344; □, binding to peptide 12–30; ●, binding to either peptides in the presence of nonimmune IgG or antibodies against an unrelated protein (sperm whale myoglobin). Amounts of <sup>125</sup>I-labeled hTSH bound to the two peptides in the absence of any inhibitors were 12,136 ± 185 cpm.

344. There are 12 amino differences between the β subunits of hTSH and bTSH (Table 2). Except for the substitution at position 69 (Leu → Arg), these replacements are conservative, and they clearly do not cause significant differences in the binding properties of the two hormones. On the other hand, oTSH showed a lower binding activity to any given hTSHR peptide than that of the other two hormones. This result must be caused by the amino acid differences between the β subunit of oTSH and the consensus sequence for the subunit in hTSH and bTSH. The amino acid sequence of the β subunit of oTSH has not been determined.

Humans can mount autoimmune responses against self-hTSHR. Some autoantibodies are stimulatory and cause Graves disease, one of the most frequent causes of hyperthyroidism (23). It is, therefore, important to determine whether the present peptides bind to anti-hTSHR autoantibodies.

We conclude that the TSH-binding regions on TSHR reside within, but may not necessarily include all of, areas 12–30 and 324–344. The two binding regions of TSHR might carry separate binding sites or might constitute different faces (subsites) within a single binding site, and each of these subsites possesses sufficient binding energy to give a fruitful complex. For example, the binding of α-neurotoxins to acetylcholine receptor involves several contact regions on the receptor (21, 24, 25) and on the neurotoxin (26). It has recently been shown (27) that the binding regions on acetylcholine receptor form a single cavity within which the toxin fits. In addition to the main binding regions within hTSHR peptides 12–30 and 324–344, the lower activities in peptides

Table 2. Sequence differences between hTSHβ and bTSHβ

	Residue											
	8	11	14	26	36	58	60	62	69	72	81	113
hTSHβ	T	I	R	I	I	I	R	V	L	A	L	
bTSHβ	M	V	K	V	V	M	K	A	R	T	I	M

Sequence (in one-letter code) information for bTSHβ and hTSHβ was obtained from refs. 1 and 22, respectively. hTSHβ terminates at Tyr-112.

24–44, 308–328, and 339–364 may indicate a smaller contribution by these regions to the architecture of the site and its binding energy. Alternatively, the activities of these three peptides may stem from the fact that they share overlaps with the two main active regions within 12–30 and 324–344. Our results do not rule out that other parts of the receptor molecule that come in close proximity to these two regions in the three-dimensional structure might also contribute to the architecture of the site.

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