Subunit Structure of Thyrotropin Receptors Expressed on the Cell Surface*

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We studied cell surface thyrotropin receptor (TSHR) by biotinylating proteins on the surface of metabolically labeled, intact cells. In addition to TSHR cleaved into A and B subunits, mature single-chain receptors with complex carbohydrate were also present on the cell surface. A low A/B subunit ratio indicated partial shedding of extracellular A subunits from transmembrane B subunits. TSHR cleavage at upstream site 1 (within amino acid residues 305–316) would generate a B subunit of 51–52 kDa. However, only smaller B subunits (40–46 kDa) were detected, corresponding to N termini from residues ~370 (site 2) extending downstream to the region of B subunit insertion into the plasma membrane. The intervening C peptide region between sites 1 and 2 could not be purified from TSHR epitope-tagged (c-myc) within this region. However, the small proportion of B subunits recovered with a c-myc antibody were larger (45–52 kDa) than the majority of B subunits recovered with a C-terminal antibody. In conclusion, our study provides the first characterization of cell surface TSHR, including their A and B subunits. Single-chain, mature TSHR do exist on the cell surface. The C peptide lost during intramolecular cleavage disintegrates rapidly following cleavage at upstream site 1 of the single-chain TSHR into A and B subunits. N-terminal disintegration of the B subunit pauses at site 2, but then progresses downstream to the vicinity of the plasma membrane, revealing a novel mechanism for A subunit shedding.

Graves’ disease, one of the most common autoimmune diseases affecting humans, is caused by autoantibodies that activate the thyrotropin (TSH) receptor (TSHR) (reviewed in Ref. 1). Remarkably, functional autoantibodies do not arise to the C peptide region between the A and B subunits, while the C peptide itself is shed, at least in vitro (7, 8). Determination of the molecular basis for TSH receptor cleavage and shedding is, therefore, of potential clinical importance in understanding the pathogenesis of Graves’ disease.

Many uncertainties remain regarding the process of TSHR cleavage and shedding, including the enzyme(s) involved (6, 7, 9) and the properties of the shed A subunits (7, 8). The location and number of cleavage sites has also been controversial. Initial evidence for a cleavage site closely upstream of amino acid residue 317, obtained by TSH cross-linking to a TSHR deletion mutant (3), was contradicted by reports that amino acid residues 352–366 lie within the A subunit (10, 11). However, residues 352–366 cannot be part of the A subunit because they are part of a C peptide region excised from the TSHR during intramolecular cleavage (5), an observation recently confirmed (6). Further support for an upstream cleavage site in the TSHR in the region of residues 305–316 was obtained in studies examining the effect of trypsin on subunit structure (12), as well as by the identification in transfected L cells of a minor B subunit component with an N terminus at residue 314 (6).

Less clear, however, is the process by which the C peptide region between the A and B subunits is lost. The observed deletion of this segment (5), taken together with previous TSH cross-linking studies to TSH-luteinizing hormone/chorionic gonadotropin chimeric receptors (13), provided evidence for two separate TSHR cleavage sites, the second (downstream) site being in the vicinity of amino acid residue 370 (14). A recent refinement of this concept is that, rather than being two distinct cleavage sites, cleavage at upstream site 1 (approximately residue 314) is primary and is followed by the sequential excision of the C peptide region, terminating in the region of site 2 (6). However, in this report, there is a discrepancy between the small size of the B subunit polypeptide chains observed and their predicted size based on their N-terminal residues.

Overall, a major problem in evaluating this somewhat confusing compendium of data is that previous studies on the immunodetection or immunopurification of TSHR subunits have been performed on thyroid tissue or cell homogenates that include TSHR products in the degradation and synthetic pathways as well as TSHR on the cell surface (4–6, 10, 15–20). Although in one study, surface-radiolabeled TSHR were examined, subunit forms of the receptor, in particular the B subunit, could not be clearly identified (16). In the present study, we have succeeded in using a cell surface biotinylaton approach to examine the properties of both TSHR subunits expressed exclusively on the cell surface.

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The abbreviations used are: TSH, thyrotropin; TSHR, thyrotropin receptor; CHO, Chinese hamster ovary; PBS, phosphate-buffered saline; mAb, monoclonal antibody; FCS, fetal calf serum.
**Cell Surface TSH Receptor Subunits**

**Materials and Methods**

**Cell Lines and Culture**

TSHR-10,000 is a Chinese hamster ovary (CHO) cell line overexpressing the human TSHR (~2 × 10^6 receptors/cell) (20). Overexpression was attained by transgene amplification using a dihydrofolate reductase minigene approach. The TSHRmyc-10,000 CHO cell line was generated by the same method. To accomplish this, we transferred the cDNA for an epitope-tagged TSHR (TSHR amino acids 338–349) replaced with the human c-myc peptide EKQKLISEEDL (21) into the plasmid pSV2-DHFR-ECE (22). Cells were propagated in Ham’s F-12 medium supplemented with 10% fetal calf serum (FCS), penicillin (100 units/ml), gentamicin (50 μg/ml), and amphotericin B (2.5 μg/ml).

**Biotinylation and Extraction of Metabolically Labeled TSHR on the Cell Surface**

TSHR-10,000 or TSHRmyc-10,000 cells in 60-mm diameter culture dishes were pulsed (1 h at 37 °C) with 0.2 mM[35]S)methionine/cysteine in Dulbecco’s modified Eagle’s, high glucose (4500 mg/ml), methionine- and cysteine-free medium containing 5% heat-inactivated FCS, as described previously (20). The cell monolayers were washed twice with ice-cold PBS, pH 8.0, and the biotin cross-linker sodium sulfosuccinimidyl-2-(biotinamide) ethyl-1,3-dithiopropionate (Pierce; 0.5 mg/ml in PBS, pH 8.0) was added for 20 min on ice. The solution was replaced and the cross-linking procedure was repeated once. After aspiration, remaining reactive sulfosuccinimidyl-2-(biotinamide) ethyl-1,3-dithiopropionate was blocked by addition of 50 mM NaN₃ in PBS, pH 8.0, for 10 min on ice with occasional agitation.

Extraction of TSHR was as described previously (20). In brief, cells were washed twice with PBS and scraped into 1 ml of ice-cold buffer A (20 mM Hepes, pH 7.2, 150 mM NaCl, and 100 μg/ml phenylmethylsulfonyl fluoride and 1 μl/mg leupeptin (both from Sigma)). The cells were pelleted, rinsed, and resuspended in buffer A containing 1% Triton X-100. After 2 h at 4 °C with occasional vortexing, the mixture was centrifuged for 1 h at 100,000 x g and the supernatant containing non-biotinylated TSHR was biotinylated. Cells were harvested by scraping, and TSHR were extracted with detergent and immunoprecipitated with a monoclonal antibody to the A subunit (A9; epitope between amino acid residues 147 and 229) (24). TSHR were then separated with Sepharose-streptavidin beads into streptavidin-adherent (biotinylated) and non-adherent (non-biotinylated) pools. Aliquots of each were enzymatically deglycosylated using Endo F (4) and endoglycosidase H (Endo H). Panel B, biotinylated TSHR. Panel C, non-biotinylated TSHR. Autoradiography was for 18 days.

**Attempts to Purify the TSHR C Peptide Region Released into the Culture Medium**

**Metabolic Labeling and Immunoprecipitation**—TSHRmyc-10,000 cells near confluence in 60-mm diameter culture dishes were metabolically labeled as described above, with the following modifications. Cells were pulsed for 3 h at 37 °C in 2 ml of Earle’s balanced salt solution with 5% dialyzed FCS and 5 μCi/ml l-[35]S)methionine/cysteine mixture (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom). After rinsing, cells were cultured in standard F12 medium with 10% FCS. The medium was harvested after 16 h, and 9E10 mouse monoclonal antibody to the c-myc epitope was added, as described above.

**Affinity Purification**—23 mg of purified 9E10 IgG was added to 2 g of pre-washed CNBr-Sepharose 4B (Amersham Pharmacia Biotech) and tumbled for 2 h at room temperature. The beads were then washed four times with buffer B and once with PBS and resuspended in Laemmli sample buffer with 0.7 μg β-mercaptoethanol (30 min at 50 °C) to release the bound, biotinylated TSHR. Aliquots of both biotinylated and non-biotinylated TSHR were electrophoresed on 10% SDS-polyacrylamide gels, and electrophoretically transferred to poly(vinylidene difluoride) membranes (Bio-Rad). Membranes were incubated overnight (4 °C) with 9E10 (final dilution 1:1500). After rinsing, the membranes were incubated for 1 h at room temperature with alkaline phosphatase-conjugated goat anti-mouse IgG (1:400) (Cappel, Durham, NC). The signal was developed with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate in 100 mM Tris-HCl buffer, pH 9.5, containing 100 mM NaCl and 5 mM MgCl₂.

**Results**

**Cell Surface TSH Receptors Identified with an Antibody to the A Subunit**—In order to examine the feasibility of distinguishing between cell surface and intracellular TSHR, we first metabolically labeled CHO cells with [35]S)methionine and cysteine and then biotinylated proteins on the surface of these intact cells in monolayer culture. TSHR in detergent extracts of these cells were immunoprecipitated with an antibody to the extracellular A subunit and were then enzymatically deglycosylated was performed according to the protocol of the manufacturer (New England Biolabs). N-Glycosidase F digestion (100 units for 2 h at 37 °C) was in 50 mM sodium citrate, pH 7.5, 1% Triton X-100. Endoglycosidase H digestion (50 units for 2 h at 37 °C) was in 50 mM sodium citrate, pH 5.5. Samples were then subjected to SDS-polyacrylamide gel electrophoresis as described above.

**Enzymatic Deglycosylation of TSHR Protein**

Biotinylated TSHR bound to streptavidin-agarose or TSHR not bound to the streptavidin-agarose beads were incubated (10 min, 100 °C) in denaturing buffer containing 0.5% SDS, 1% β-mercaptoeth-
an antibody to the A subunit would not, therefore, co-isolate B subunits

... continues downstream to the vicinity of the ultimate Cys-408 or even to the plasma membrane (Fig. 3B). Loss of Cys-408 would lead to A subunit shedding. An additional observation was that the intensity of the B subunit band relative to the A subunit band was greater when B subunits were isolated with the B subunit antibody as opposed to the A subunit antibody. Although the intensity of precursor-labeled A and B subunit bands must be interpreted in the light of a nearly 2:1 excess of methionine and cysteine residues in the B versus the A subunit, these data are consistent with the existence on the cell surface of some B subunits without attached A subunits, i.e. A subunit shedding.

**Detection of “Big” B Subunits—**Before the C peptide region is lost, TSHR cleavage at upstream site 1 would create a big B subunit still containing the C peptide region with a predicted size of 51–52 kDa (Fig. 3B). No B subunits of this size were detected in the preceding experiments using cells expressing the wild type TSHR. In order to facilitate study of the TSHR C peptide region, we generated a cell line overexpressing the TSHR with a c-myc epitope at amino acid residues 338–349, within the C peptide region. Clear differences were observed between cell surface TSHR subunit forms immunoprecipitated from the same precursor-labeled material using monoclonal antibodies (mAb) to the c-myc epitope (9E10) and to the B subunit (T3–365; epitope in the B subunit C-terminal region, amino acid residues 640–764) (Fig. 3C). First, much less (4-fold) radioactivity was recovered with 9E10 than with the mAb to the B subunit. Second, consistent with previous observations on an unamplified cell line expressing the same receptor, loss of the c-myc epitope-containing C peptide region resulted in proportionately greater recovery of single-chain versus two-subunit forms of the TSHR (5). Finally, by normalizing the radioactivity applied to each lane to compensate for the lesser recovery of radioactivity with the anti-myc mAb, we were able for the first time to visualize B subunits recognized with this antibody. This small proportion of B subunits expressed on the surface of intact TSHRmyc-10,000 cells were, in most part, larger than the B subunits isolated with the mAb to a more downstream epitope, extending (as predicted) up to ~51 kDa in size (Fig. 3C).

**Inability to Purify an Intact C Peptide from Culture Medium—**We also used the TSHRmyc-10,000 cell line in an attempt to purify a C peptide from culture medium. Conditioned medium harvested from this cell line (3 days, 1,200 ml) was applied to an affinity column containing mAb 9E10 to the c-myc epitope. Eluted material was subjected to polyacrylamide gel electrophoresis and examined both by Coomassie Blue staining and by immunoblotting using monoclonal antibody 9E10. No polypeptide fragments were observed (data not shown). In separate experiments, no C peptide could be immunoprecipitated from medium harvested from TSHRmyc-10,000 cells metabolically labeled with $^{13}$C-labeled amino acids (there are no cysteine or methionine residues within the C peptide region) (data not shown). Taken together, these data support the concept that the C peptide region is not excised as a single fragment.

**Influence of TSH Receptor Extraction Procedure on B Subunit Structure—**A wide spectrum of B subunit sizes has been identified in TSHR extracted from thyroid tissue or transfected mammalian cells (4–6, 10, 11, 17, 25). In all of these studies, TSHR extraction has involved tissue or cell homogenization, commonly after freezing and thawing. However, the TSHR is a

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**Cell Surface TSH Receptor Subunits**

![Diagram of TSHR Subunits](image-url)

**Fig. 2. Schematic representation of cysteine residues involved in A subunit linkage to the B subunit.** Although it is not known precisely which cysteines are paired, available evidence suggests a critical role for Cys-283 and Cys-284 on the A subunit and Cys-398 and Cys-408 on the B subunit (reviewed in Ref. 1). Cys-301 and Cys-390 contribute to the stability of the cleaved receptor, but their mutation does not lead to loss of the A subunit (28). N-terminal degradation of the A subunit leading to loss of Cys-408 would be incompatible with retention of the B subunit. Immunoprecipitation of the cleaved TSHR with an antibody to the A subunit would not, therefore, co-isolate B subunits degraded downstream of Cys-408.

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**Polyacrylamide Gel Electrophoresis under Reducing Condition Revealed Single-Chain, Uncleaved TSHR with Cysteine Linkage to the B Subunit.** Although it is not known precisely which cysteines are paired, available evidence suggests a critical role for Cys-283 and Cys-284 on the A subunit and Cys-398 and Cys-408 on the B subunit (reviewed in Ref. 1). Cys-301 and Cys-390 contribute to the stability of the cleaved receptor, but their mutation does not lead to loss of the A subunit (28). N-terminal degradation of the A subunit leading to loss of Cys-408 would be incompatible with retention of the B subunit. Immunoprecipitation of the cleaved TSHR with an antibody to the A subunit would not, therefore, co-isolate B subunits degraded downstream of Cys-408.

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**Separated on the Basis of Their Binding, or Lack of Binding, to Streptavidin.** Polyacrylamide gel electrophoresis under reducing condition revealed single-chain, uncleaved TSHR of 120 kDa with complex carbohydrate (endoglycosidase H-resistant) only in the biotinylated pool of receptors (Fig. 1B). In a reciprocal manner, single-chain TSHR of ~100 kDa with immature, high mannose glycan (endoglycosidase H-sensitive) were detected only in the non-biotinylated receptor pool (Fig. 1C). These data indicate the efficiency and selectivity of biotinylation of cell surface versus intracellular TSHR. Further, they establish that single-chain, uncleaved TSHR with mature, complex glycan do occur on the cell surface.

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**In Addition to the Single-Chain Receptors, Dissociated Cell Surface A and B Subunits were Also Evident Under the Same Reducing Conditions.** The A subunits, derived from the single-chain receptors (17), contain complex carbohydrate whereas the largely transmembrane or intracellular B subunits are non-glycosylated (panel A). Only trace amounts of non-biotinylated, cleaved receptor subunits are present within the cell (panel B). The size of the deglycosylated polypeptide core of the cell surface A subunit (approximately 34 kDa) is consistent with an upstream intramolecular cleavage site (“site 1”) in the vicinity of amino acid residues 305–316 (3, 5, 6, 12). In contrast to the more sharply defined A subunit polypeptide core revealed by endoglycosidase F treatment, non-glycosylated B subunits on the cell surface isolated with an antibody to the A subunit were evident as a diffuse band of approximately 40–46 kDa (Fig. 1). In most experiments (as in Fig. 1), this diffuse band was a poorly defined doublet.

**Cell Surface TSH Receptors Identified with an Antibody to the B Subunit—**Using an antibody to the A subunit, it is not possible to detect B subunit cleavage or degradation downstream of amino acid residue 408. This is because there are only three cysteine residues (Cys-390, -398, and -408) on the B subunit prior to its predicted insertion into the plasma membrane at residue 418 (Fig. 2). Which of these cysteines are involved in B subunit tethering to the A subunit is not definitively established (reviewed in Ref. 1), but loss of the ultimate B subunit Cys-408 would certainly be incompatible with a retained A subunit (Fig. 2). On the other hand, immunoprecipitation with an antibody to the B subunit would determine the size(s) of B subunits regardless of their attachment to the A subunit.

We, therefore, compared the sizes of B subunits of TSHR on the surface of intact cells as detected by immunoprecipitation with antibodies to the TSHR A and to the B subunits (Fig. 3A). The size range of B subunits was similar regardless of whether they were isolated with an antibody to the A or B subunit. The lower limit of the B subunit smear (approximately 40 kDa) indicates that B subunit cleavage does continue downstream to the vicinity of the ultimate Cys-408 or even to the plasma membrane (Fig. 3B). Loss of Cys-408 would lead to A subunit shedding. An additional observation was that the intensity of the B subunit band relative to the A subunit band was greater when B subunits were isolated with the B subunit antibody as opposed to the A subunit antibody. Although the intensity of precursor-labeled A and B subunit bands must be interpreted in the light of a nearly 2:1 excess of methionine and cysteine residues in the B versus the A subunit, these data are consistent with the existence on the cell surface of some B subunits without attached A subunits, i.e. A subunit shedding.

**Figure 2. Schematic representation of cysteine residues involved in A subunit linkage to the B subunit.** Although it is not known precisely which cysteines are paired, available evidence suggests a critical role for Cys-283 and Cys-284 on the A subunit and Cys-398 and Cys-408 on the B subunit (reviewed in Ref. 1). Cys-301 and Cys-390 contribute to the stability of the cleaved receptor, but their mutation does not lead to loss of the A subunit (28). N-terminal degradation of the A subunit leading to loss of Cys-408 would be incompatible with retention of the A subunit. Immunoprecipitation of the cleaved TSHR with an antibody to the A subunit would not, therefore, co-isolate B subunits degraded downstream of Cys-408.
labile protein and the method used for TSHR preparation markedly affects its ligand binding properties. Thus, only a small fraction of the TSH binding capacity on intact cells is recovered after cells are scraped off the culture dish, a reduction not prevented by conventional protease inhibitors. Recently, direct detergent extraction of cell monolayers (without scraping, freezing, or homogenization) was found to greatly enhance recovery of TSHR capable of ligand binding (23). We, therefore, examined the effect of cell scraping on the size of TSHR B subunits expressed on the surface of stably transfected CHO cells.

After metabolic labeling and biotinylation of TSHR-10,000 cell surface proteins, the cells were processed in two different manners prior to TSHR immunopurification with an antibody to the B subunit and selection of cell surface receptors with streptavidin: (i) our conventional method of first harvesting the cells by scraping before detergent extraction, and (ii) direct application of detergent to CHO cell monolayers without other manipulation of the cells. With direct solubilization, in some (four of six) experiments, the 40–46-kDa B subunit smear was biased toward the higher molecular mass form (Fig. 4). Conversely, after cell scraping, the B subunits were either uniformly distributed between the higher and lower molecular weights, or were biased toward the lower size. However, in other experiments, direct detergent solubilization did not bias toward recovery of larger B subunits (see, for example, the TSHRmyc-10,000 cells in Fig. 3C).

**DISCUSSION**

The present data are the first to examine the properties of TSHR, including their A and B subunits, expressed on the surface of intact cells. Analysis of TSHR carbohydrate moieties establishes the efficacy of the cell surface biotinylation approach that we used to distinguish between cell surface and intracellular receptors. Previous studies on TSH cross-linking to TSHR on the surface of intact cells (for example, Refs. 2 and 3) could distinguish between single-chain receptors and the A subunits of cleaved receptors, but the ligand-receptor com-
plexes were not amenable to fine resolution of their sizes and the non-ligand binding B subunit could not be detected. Immunopurification of TSHR does provide more detailed information (for example, Ref. 4). However, such studies have been performed on cell and thyroid homogenates, which include mixtures of cell surface receptors and intracellular receptors at different stages of the synthetic and degradative pathways. Intracellular products are reported to be particularly abundant in transfected cells expressing the recombinant TSHR (17).

The data on cell surface TSHR expression clarify a number of puzzling or controversial issues. One issue that has been the subject of debate for many years is whether or not mature, single-chain TSHR exist on the cell surface. This possibility was raised by the detection of single-chain TSHR on TSH cross-linking to intact cells (2, 3). Disulfide-linked, two-subunit receptors were well recognized (4, 26). However, high affinity TSH binding to the single-chain TSHR comparable to the cleaved receptor led to the suggestion that the former were physiological (3). This concept has been vigorously disputed, and these observations have been considered an artifact of transfected cells (4, 6, 17, 27). It is proposed that single-chain TSHR precursors with immature carbohydrate have been “mistaken for the mature receptor” (6) and that such precursors are readily detected in transfected cells because of their abnormal abundance consequent to TSHR overexpression overwhelming the mechanism for glycan maturation and TSHR processing (17, 27). The present data clearly indicate that single-chain TSHR on the cell surface have mature, complex carbohydrate and are consistent with the previous observation of high affinity TSH binding to single-chain TSHR on the cell surface (3). Moreover, single-chain receptors capable of TSH binding are not confined to transfected cells, but are also observed in a well differentiated rat thyroid cell line (2). Finally, the same proportion of single-chain versus cleaved TSHR on the cell surface is observed over a 100-fold range in the level of expression in transfected cells (20), excluding the suggested limitation in synthetic capacity.

Turning to the process of TSHR cleavage, the impressive accomplishment of de Bernard et al. (6) in affinity-purifying large quantities of TSHR B subunits from thyroid tissue and from transfected mouse L cells confirms previous findings that an epitope within a C peptide segment is excised from the TSHR (5) with an upstream cleavage site in the vicinity of amino acid residues 305–316 (3, 12). Our inability to purify an intact C peptide, similar to the experience of this group (6), and transfected mouse L cells reveal multiple N termini, the dominant ones being between amino acid residues 366 and 378, the calculated B subunit size is 45 kDa. It is unclear why no 45-kDa B subunits were observed by de Bernard et al. (6). Indeed, the majority of the B subunit fragments on which they were able to determine N-terminal sequence (see above) were 38–39 kDa in size. In contrast, our data on cell surface B subunit size do conform with the expected sizes of B subunits with N termini at site 1 and site 2. Possible explanations for the discrepancy between N-terminal sequences and B subunit sizes in the de Bernard study include inaccurate molecular size markers and B subunit degradation. Because B subunits were purified from cell or tissue homogenates, it cannot be certain that N-terminal sequence analysis was on cell surface B subunits. Indeed, in transfected mouse L cells, there is a large excess of immature TSHR molecules that undergo rapid degradation (17).

In our study on cell surface receptors, a B subunit “smear” between site 1 (52 kDa) and site 2 (45 kDa) was evident only in a small proportion of B subunits purified with the anti-myct antibody whose epitope is in the C peptide region. The majority of B subunits were evident as a broad biphasic band of 40–46 kDa. These observations indicate that after cleavage at upstream site 1, N-terminal removal of the C peptide region is not a gradual and progressive process. Rather, the C peptide region appears to disintegrate rapidly. This disintegration pauses downstream at site 2. The lower part of the biphasic B subunit band (~40 kDa) suggests that, after slowing at site 2, N-terminal clipping or degradation of the B subunit re-accelerates to the vicinity of the plasma membrane. Continued degradation would lead to A subunit destabilization (with decreased ligand binding) (23) and, ultimately, A subunit shedding (Fig. 2), as is evident from the greater proportion of B subunits immunoprecipitated using the B subunit antibody versus the A subunit antibody (Fig. 3A). Shedding of the A subunit may, therefore, not require protein disulfide isomerase (9). Whether continued B subunit N-terminal degradation beyond site 2 to its membrane insertion is physiological or an artifact is uncertain. The TSHR is a labile protein (8, 23). Further, in some (but not all experiments) in which TSHR were extracted directly with detergent without first scraping the cells, there was a lesser amount of the smaller B subunit component. Conversely, culture of cells in serum-poor medium, a condition previously used to study the mechanism of A subunit shedding (7, 9), shifts the size of TSHR B subunits toward their lower molecular weight forms (8).

In conclusion, the present study characterizes TSHR (including their A and B subunits) on the cell surface. Single-chain TSHR with mature carbohydrate do exist on the cell surface. The C peptide region lost from the TSHR during intramolecular cleavage is not released intact, but disintegrates rapidly following cleavage of the single-chain TSHR into A and B subunits at upstream site 1. This N-terminal disintegration pauses at site 2, but then progresses further downstream to the vicinity of the plasma membrane, revealing a novel mechanism for shedding of the A subunit.

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