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Biochemistry

Molecular basis of recognition by the glycoprotein hormone-specific *N*-acetylgalactosamine-transferase

(gonadotropin/pituitary/glycosyltransferase/oligosaccharide/peptide)

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ABSTRACT **Lutropin (LH) bears asparagine-linked oligosaccharides terminating with the unique sequence SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα, whereas follitropin (FSH) bears oligosaccharides terminating predominantly with the sequence Siaα-Galβ1-4GlcNAcβ1-2Manα, where Sia is sialic acid. We previously identified a glycoprotein-hormone-specific *N*-acetylgalactosamine-transferase (GalNAc-transferase) that recognizes a peptide-recognition marker(s) present on the common glycoprotein hormone α subunit and β subunits of human chorionic gonadotropin and LH but not on the β subunit of FSH. We have now identified an amino acid sequence motif, Pro-Leu-Arg, that is essential for recognition by the GalNAc-transferase. This tripeptide sequence is found 6–9 residues on the amino-terminal side of a glycosylated asparagine on the α subunit and β subunits of LH and human chorionic gonodatropin but is not present on the β subunit of FSH. The presence of this motif accounts for the differences in LH and FSH oligosaccharide structures. Additional proteins containing this recognition motif have been identified and were determined to bear sulfated oligosaccharides with the same structures as those on the glycoprotein hormones, indicating that these structures are not restricted to the glycoprotein hormones.**

Among the many asparagine-linked oligosaccharides that have been characterized, there are only a small number of clearly unique structures that are found on highly restricted populations of glycoproteins. As a result, the presence of such structures suggests an important biologic function. One example is the sulfated asparagine-linked oligosaccharides found on specific members of the glycoprotein hormone family (1). The glycoprotein hormones lutropin (LH), follitropin (FSH), thyrotropin, and chorionic gonadotropin (CG) are dimeric proteins with highly homologous hormone-specific β subunits and identical α subunits (2, 3). Despite having closely related peptide structures at the primary, secondary, and tertiary levels, their asparagine-linked oligosaccharides are distinct. For example, LH and FSH are synthesized by the same cell, the gonadotroph (4, 5), yet the asparagine-linked oligosaccharides on LH terminate with the sequence SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα whereas those on FSH predominantly terminate with Siaα-Galβ1-4GlcNAcβ1-2Manα, where Sia is sialic acid (1, 6, 7). Few other glycoproteins synthesized in the pituitary terminate with the sequence SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα, indicating this form of posttranslational modification is highly specific. We recently determined that the presence of sulfated as opposed to sialylated oligosaccharides on LH has a major impact on its circulatory half-life (16). This reflects the rapid removal of native bovine (b) LH from the serum by a receptor that is present on hepatic endothelial cells and Kupffer cells and that recognizes oligosaccharides terminating with the sequence SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα (17).

In earlier studies, we demonstrated (8) the presence of a glycoprotein-hormone-specific *N*-acetylgalactosamine-transferase (GalNAc-transferase) in membranes from the anterior pituitary that will transfer GalNAc to the terminal GlcNAc moieties of $GlcNAc_2Man_3GlcNAc_2Asn$ (8). When this oligosaccharide is located on the α subunit or the β subunits of human (h) CG (hCGβ) or LH (hLHβ), the apparent K_m for GalNAc transfer is reduced from 1–2 mM to <15 μM, due to the presence of a peptide-recognition marker (9). We have now identified essential elements of this peptide-recognition marker and determined that it is not present on the β subunit of FSH (FSHβ), accounting for the lack of recognition of this hormone by the GalNAc-transferase. Further, we have identified a similar motif on other glycoproteins and found that they bear the same sulfated oligosaccharide structure. It is, therefore, likely that this unique type of oligosaccharide structure plays an important role in the biologic behavior of a number of glycoproteins that are released into the circulation.

MATERIALS AND METHODS

GalNAc-Transferase Assays. Assays were performed with GalNAc-transferase (0.79 unit/mg), which had been enriched 165-fold from bovine pituitaries and was stored at 0.2 mg/ml in 50 mM Hepes, pH 7.5/10 mM KCl/20% (vol/vol) glycerol/0.1% Triton X-100 at −80°C. The transferase reactions were incubated at 37°C and contained 4×10^{-3} unit of GalNAc-transferase, 25 mM Hepes, 5 mM KCl, 20% glycerol, 0.1% Triton X-100, 6 mM ATP, 1 μg of leupeptin, 1 μg of antipain, 1 μg of pepstatin, 1 μg of chymostatin, 1.15×10^{-3} trypsin-inhibitory unit of aprotinin, 10 mM $MnCl_2$, 240 μM [^{3}H]UDP-GalNAc (1×10^7 cpm), and 200 pmol of substrate in a final volume of 50 μl. The reactions were terminated by addition of 450 μl of 0.1 M Tris·HCl, pH 8.0/0.02 M $CaCl_2$ containing 1 mg of Pronase (Calbiochem). After a 30-min incubation at 56°C, the samples were boiled for 10 min and 900 μl of Con A buffer [0.02 M Tris·HCl, pH 7.5/0.15 M NaCl/1 mM $MnCl_2$/1 mM $CaCl_2$/bovine serum albumin (1 mg/ml)/0.02% sodium azide] was then added. The digests were clarified by centrifugation and the resultant glycopeptides were isolated by affinity chromatography on Con A-Sepharose (Pharmacia). Each 2-ml column was washed with 35 ml of Con A buffer prior to elution of glycopeptides containing [^{3}H]GalNAc with two 4-ml vol of Con A elution buffer [0.5 M α-methyl mannoside/0.02 M Tris·HCl, pH 7.5/0.15 M NaCl/bovine serum albumin (1 mg/ml)/0.02% sodium azide, heated to 56°C]. Picofluor-30 (Packard) was added (14 ml/

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Abbreviations: LH, lutropin; FSH, follitropin; CG, chorionic gonadotropin; b, bovine; h, human; RCM, reduced and carboxamidomethylated.

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4-ml fraction) and the amount of incorporated [^{3}H]GalNAc was quantitated by scintillation counting.

Preparation of Glycopeptides. Sialic acid and galactose were removed from hCGα, hCGβ, and transferrin enzymatically (8). hCGα and hCGβ were reduced and carboxamidomethylated (RCM) as described (9). hCGα(RCM) (1 mg/ml in 1% NH_4CO_3) was digested for 18 h at 37°C with 5% (wt/wt) sequencing-grade trypsin (Boehringer Mannheim) to obtain glycopeptides hCGα-(52–63) and hCGα-(76–91) and with 5% (wt/wt) sequencing-grade endoprotease Glu-C (Boehringer Mannheim) to obtain glycopeptide hCGα-(35–56). hCGβ-(3–20) was obtained by digestion of hCGβ(RCM) with 0.2% sequencing-grade endoprotease Lys-C (Boehringer Mannheim) and hCGβ-(9–20) was obtained by digestion of hCGβ(RCM) with 5% sequencing-grade trypsin (Boehringer Mannheim). The peptide products were identified and isolated by reverse-phase HPLC on Synchropak RP-P (50 × 4.6 mm, Synchrom) equilibrated in 0.1% trifluoroacetic acid in distilled H_2O. Initial conditions were maintained for 5 min and followed by a gradient of 0–100% of [0.1% trifluoroacetic acid/56% (vol/vol) acetonitrile/14% (vol/vol) isopropanol/30% (vol/vol) distilled H_2O] over 50 min. Fractions containing the desired glycopeptides were pooled, lyophilized, resuspended in 1 ml of 0.1% trifluoroacetic acid, and chromatographed as described above except the gradient was increased at 1%/min. Peptides were identified and quantitated by amino acid analysis. Sequences were confirmed by amino-terminal sequence analysis. The amount of terminal GlcNAc was determined by addition of [^{3}H]galactose using purified galactosyltransferase (Sigma) (9).

Chemical Modification with 1,2-Cyclohexanedione. Cyclohexanedione-modified hCGα-(35–56) and hCGβ-(3–20) were prepared by dissolving 3 nmol of each glycopeptide in 50 μl 0.3 M NaOH containing 360 nmol of 1,2-cyclohexanedione (Sigma). After 1 h at 25°C in the dark, 30 μl of 30% (vol/vol) acetic acid was added followed by 0.5 ml of distilled H_2O. The cyclohexanedione-modified glycopeptides were eluted as single peaks, which differed in retention time from the unmodified glycopeptides, when isolated by reverse-phase HPLC. After modification, arginine was no longer detected upon amino acid analysis. The "unmodified" glycopeptides used for comparison were treated identically except that 1,2-cyclohexanedione was omitted.

Peptide Synthesis. Peptides were synthesized on a Ramps processor (DuPont) according to the manufacturer's instructions. All peptides were purified sequentially by reverse-phase HPLC, S-Sepharose cation-exchange chromatography, and gel filtration on Sephadex G-10. Peptides were quantitated by amino acid analysis.

RESULTS

A 22-Amino Acid Glycopeptide Is Recognized by the GalNAc-Transferase. Our previous studies established that peptide recognition is required for specific addition of GalNAc to oligosaccharides on hCGβ, LHβ, and the α subunits. GalNAc and sulfate are not present on the oligosaccharides of native hCG and recombinant LH expressed in CHO cells, due to the absence of the GalNAc- and sulfotransferases in placental trophoblasts and CHO cells (8, 10). The sialylated asparagine-linked oligosaccharides on hCG and recombinant LH can be readily converted into substrates for the GalNAc-transferase by removal of terminal sialic acid and galactose moieties. The lack of dependence on tertiary structure for recognition by the GalNAc-transferase indicated that, in contrast to other forms of peptide-dependent recognition, it would be possible to locate and identify the peptide recognition marker by using proteolytic fragments of these hormones as acceptors. We therefore prepared proteolytic glycopeptide fragments from hCGα(RCM) (Fig. 1) and hCGβ(RCM) (Fig. 2) for comparison as GalNAc-transferase substrates. The kinetic parameters determined for hCGα, hCGα(RCM), and hCGα-(35–56), a glycopeptide consisting of amino acids 35–56 of hCGα including the glycosylation site at Asn-52, are summarized in Table 1. As we had determined (9), the apparent K_m for transfer of GalNAc to oligosaccharides on hCGα(RCM) is slightly decreased compared to native hCGα. The apparent K_m of 9.8 μM for transfer of GalNAc to the oligosaccharide acceptor on hCGα-(35–56) does not differ significantly from that for transfer to the oligosaccharides on hCGα(RCM) (Table 1). Therefore, all of the information required for recognition of the α subunit by the GalNAc-transferase resides within this 22-amino acid glycopeptide fragment.

Localization of the Peptide-Recognition Sequence on hCGα. Additional glycopeptides were prepared to further localize the amino acids mediating recognition of hCGα by the GalNAc-transferase and to determine if additional recognition sequences might be present. The glycopeptides shown in Fig. 1 were compared by determining the time course for GalNAc addition at a substrate concentration of 4 μM. hCGα(RCM) and hCGα-(35–56), which have nearly identical catalytic efficiencies for GalNAc addition (Table 1), incorporated GalNAc at identical rates (Fig. 1). The glycopeptide fragments hCGα-(52–63) and hCGα-(35–56) contain the identical asparagine-linked oligosaccharide acceptor at Asn-52, yet hCGα-(52–63) did not incorporate detectable levels of GalNAc until after 100 min of incubation (Fig. 1). We have determined (8) that transferrin does not contain a recognition marker for the GalNAc-transferase. Detectable levels of GalNAc incorporation onto transferrin oligosaccharides, like hCGα-(52–63), were also seen only after 100 min of incubation (Fig. 1). The catalytic efficiency for GalNAc addition to

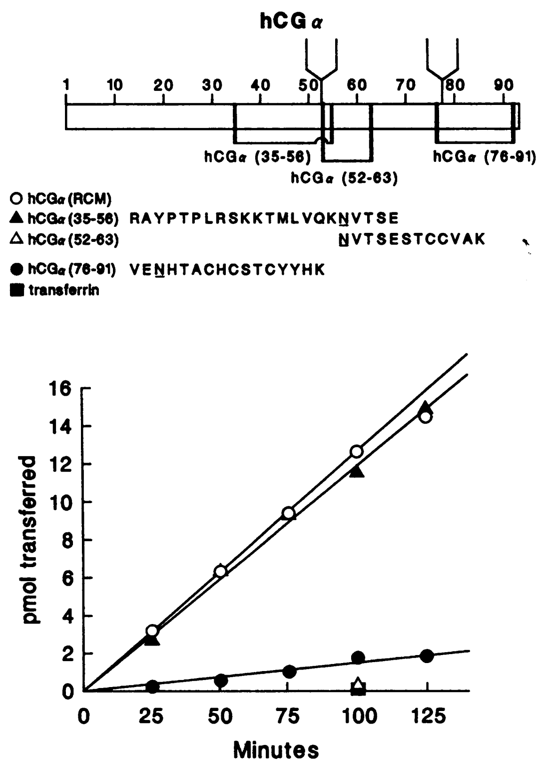

FIG. 1. Comparison of proteolytic fragments generated from hCGα(RCM) as substrates for the glycoprotein-hormone-specific GalNAc-transferase. Assays, containing 4 × 10^{-3} unit of partially purified GalNAc-transferase and 240 μM UDPGalNAc, were incubated for the indicated time at 37°C with hCGα(RCM), hCGα-(35–56), hCGα-(52–63), hCGα-(76–91), or native human transferrin at a concentration of 4 μM. Amino acid sequences are shown in single-letter code, with the glycosylated asparagines underlined.

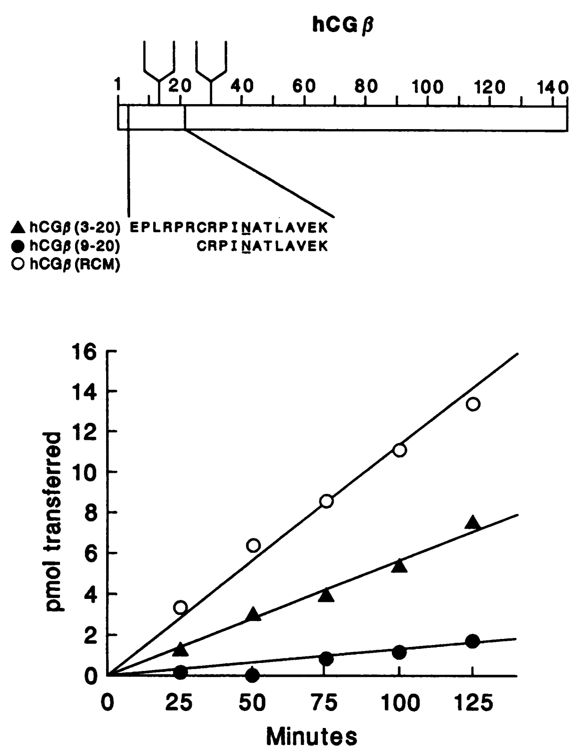

FIG. 2. Comparison of proteolytic fragments generated from hCGβ(RCM) as substrates for the glycoprotein-hormone-specific GalNAc-transferase. Assays containing 4×10^{-3} units of partially purified GalNAc-transferase, 240 μM UDPGalNAc, and hCGβ(RCM), hCGβ-(3–20), or hCGβ-(9–20) at a concentration of 4 μM. The amino acid sequences of hCGβ-(3–20) and hCGβ-(9–20) are indicated by the single-letter code. The glycosylated asparagine at position 13 is underlined.

oligosaccharides on hCGα(RCM) is more than 100-fold greater than for addition to the same oligosaccharide structures on either hCGα-(52–63) or transferrin (9). In control experiments using bovine β-1,4-galactosyltransferase, an enzyme that requires only terminal GlcNAc for transfer and is not influenced by the underlying protein structure (11), equal amounts of galactose were added to the oligosaccharides on hCGα-(35–56) and hCGα-(52–63) confirming that the same amount of $GlcNAc_2Man_3GlcNAc_2Asn$ was present on both glycopeptides. The amino acids mediating recognition by the GalNAc-transferase must, therefore, be located between amino acids 35 and 51 of the human α subunit.

hCGα has a second glycosylation site at Asn-78 (Fig. 1). The peptide sequence surrounding this site does not resemble that surrounding Asn-52. A glycopeptide, hCGα-(76–91), containing this glycosylation site was also tested as substrate for the GalNAc-transferase (Fig. 1). GalNAc was transferred to hCGα-(76–91) at <10% the rate of transfer to either hCGα-(35–56) or hCGα(RCM), indicating that hCGα-(76–91) does not have the peptide-recognition marker found on hCGα-(35–56).

Table 1. Kinetic parameters for addition of GalNAc to asparagine-linked oligosaccharide acceptors on the hormone α subunit

Substrate	Apparent K_m, μM	Apparent V_{max}, pmol per h per μg of protein	Catalytic efficiency (V_{max}/K_m)
hCGα	14.6	18.26	1.25
hCGα(RCM)	8.1	6.06	0.75
hCGα-(35–56)	9.8	5.19	0.53

Native hCGα, hCGα(RCM), and hCGα-(35–56) were characterized as substrates for the glycoprotein-hormone-specific GalNAc-transferase. The apparent K_m and V_{max} values were determined for each substrate from secondary plots of Lineweaver–Burk plots at multiple concentrations of UDPGalNAc.

Localization of the Peptide-Recognition Sequence on hCGβ. GalNAc is transferred to oligosaccharides on native hCGβ and hCGβ(RCM) with apparent K_m values of 6.2 μM and 9.3 μM, respectively (9). The catalytic efficiencies for transfer of GalNAc to hCGβ(RCM) and hCGα(RCM) are nearly identical (9), as are the rates of transfer seen in Figs. 1 and 2. GalNAc was transferred to the Asn-linked oligosaccharide on Asn-13 of the proteolytic fragment hCGβ-(3–20) at half the rate of transfer to hCGβ(RCM) but 50-fold greater than the rate of transfer to transferrin. The marked decrease in the rate of GalNAc transfer when residues 3–8 were removed to produce hCGβ-(9–20) (Fig. 2) was particularly striking. The GalNAc-transferase adds GalNAc to the oligosaccharide of hCGβ-(9–20) at 20% the rate of transfer to the same oligosaccharide on hCGβ-(3–20), indicating that the amino-terminal 6 residues of hCGβ-(3–20) are required for efficient recognition of hCGβ.

GalNAc-Transferase Substrates Contain the Sequence Pro-Leu-Arg. hCGβ, bLHβ, and hCGα contain a peptide-recognition marker for the glycoprotein-hormone-specific GalNAc-transferase that is not present on hFSHβ (9). Alignment of the amino acid sequences of the glycoprotein-hormone subunits in the regions of their glycosylation sites (Fig. 3) reveals a number of features that may account for the pattern of recognition of the hormone subunits and the glycopeptides described above. hCGβ and bLHβ contain the sequence Pro-Leu-Arg 6 residues on the amino-terminal side of an asparagine glycosylation site. The same tripeptide is found 9 residues on the amino-terminal side of the glycosylated Asn-52 of hCGα. In contrast, the Pro-Leu-Arg tripeptide is not present on hFSHβ due to an amino-terminal truncation of FSHβ compared to LHβ and CGβ. The Pro-Leu-Arg tripeptide is included within the amino-terminal 6 residues, Ser-Lys-Glu-Pro-Leu-Arg, of hCGβ-(3–20) that are required for efficient recognition by the GalNAc-transferase. hCGα-(52–63) and hCGα-(76–91) do not contain a Pro-Leu-Arg sequence and are not recognized by the glycoprotein-hormone-specific GalNAc-transferase. Therefore, the presence of the tripeptide Pro-Leu-Arg 6–9 residues on the amino-terminal side of an asparagine glycosylation site appears to be necessary for recognition by the glycoprotein-hormone-specific GalNAc-transferase.

Chemical Modification of Arginine Destroys the Recognition Marker. To directly implicate the Pro-Leu-Arg sequence as an essential element of the GalNAc-transferase recognition marker, we chemically modified the arginine residues present in hCGα-(35–56) and hCGβ-(3–20) using 1,2-cyclohexanedione. The conditions used for chemical modification of arginine were chosen to produce a stable neutral product, thus destroying the cationic character of the arginine residue (12). The rate of GalNAc transfer to hCGα-(35–56) with modified arginine residues at positions 35 and 42 was reduced to 10%

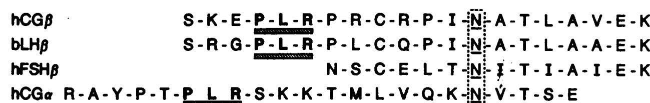

FIG. 3. Alignment of peptides known to be recognized by the glycoprotein-hormone-specific GalNAc-transferase and comparison with the amino-terminal sequence of hFSHβ. The amino acid sequences represented in the single-letter code are aligned with respect to glycosylated asparagine residues (dashed box) and the conserved cysteine of the β subunits. The Pro-Leu-Arg motif required for recognition by the GalNAc-transferase is underlined. The sequences correspond to amino acids 1–20 for hCGβ, 1–20 for bLHβ, 1–14 for hFSHβ, and 35–56 for the human α subunit (2).

that of unmodified hCGα-(35–56) (Fig. 4A). Likewise modification of the three arginine residues in hCGβ-(3–20) reduced the rate of GalNAc transfer to 10% that of unmodified hCGβ-(3–20) (Fig. 4B). In contrast, treatment of either hCGα-(35–56) or hCGβ-(3–20) with 1,2-cyclohexanedione under conditions that result in a cationic product (13) did not significantly alter recognition by the GalNAc-transferase (data not shown). Thus, it appears to be the cationic nature of arginine that is essential for recognition by the GalNAc-transferase. Since the only arginine residues at homologous positions in hCGα-(35–56) and hCGβ-(3–20) are both a part of the Pro-Leu-Arg motif, the loss of recognition after modification strongly implicates these specific arginine residues as an essential part of the recognition marker used by the GalNAc-transferase.

Inhibition of the GalNAc-Transferase by Synthetic Peptides. The presence of the peptide recognition marker on glycopeptide substrates reduces the apparent K_m for the oligosaccharide acceptor but is not required for transfer of GalNAc (8), suggesting the peptide marker and the oligosaccharide are recognized independently. If this is the case, peptides containing the recognition marker but no oligosaccharide should act as competitive inhibitors of the GalNAc-transferase. Since hCGα-(35–56) contains all of the necessary information required for recognition by the GalNAc-transferase, we prepared the identical nonglycosylated peptide synthetically. A peptide with the same sequence as hCGα-(35–56) showed a level of inhibition significantly better than a peptide containing the same amino acids in a random order (Fig. 5). The level of inhibition seen with the scrambled peptide was similar to that obtained with a number of other unrelated peptides of similar size but differing composition. Digestion with Pronase completely abolished inhibition by either the peptide with the same sequence as hCGα-(35–56) or the scrambled peptide (data not shown), indicating that in both cases inhibition is due to peptide and not to nonpeptide contaminants. Replacement of the Pro-Leu-Arg sequence of the peptide representing hCGα-(35–56) with Ala-Ala-Ala significantly reduced its ability to inhibit the GalNAc-transferase (Fig. 5). Therefore, inhibition of GalNAc transfer by synthetic peptides is sequence-specific, requiring the Pro-Leu-Arg motif for maximal inhibition.

DISCUSSION

Based on the evidence presented here, we conclude that the tripeptide sequence Pro-Leu-Arg is the basis for recognition

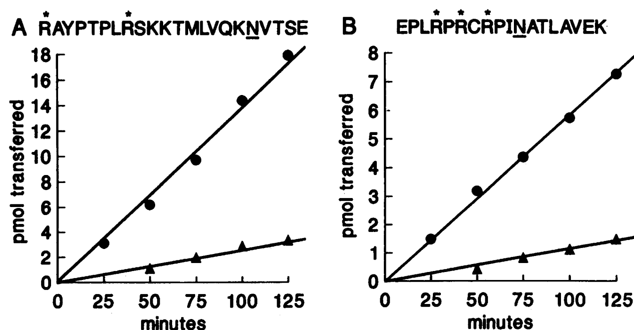

FIG. 4. Recognition of hCGα-(35–56) and hCGβ-(3–20) by the glycoprotein-hormone-specific GalNAc-transferase is disrupted by chemical modification of arginine residues. Time courses comparing the addition of GalNAc to oligosaccharides on unmodified (circles) and 1,2-cyclohexanedione-modified (triangles) hCGα-(35–56) (A) and hCGβ-(3–20) (B). Assays contained 4 × 10^{-3} unit of partially purified GalNAc-transferase, 240 μM UDPGalNAc, and the indicated glycopeptide at 4 μM. The amino acid sequence of hCGα-(35–56) and hCGβ-(3–20) is indicated in the single-letter code. Modified arginine residues are indicated (*), and glycosylated asparagines are underlined.

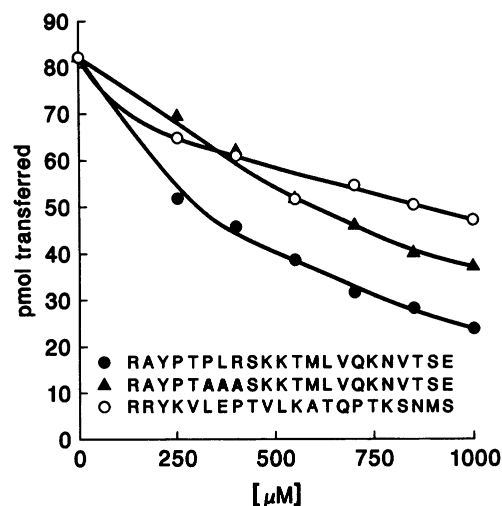

FIG. 5. Inhibition of GalNAc transfer to hCGα oligosaccharides by synthetic peptides. Synthetic peptides corresponding to amino acids 35–56 of hCGα (solid circles), amino acids 35–56 of hCGα with residues 40–42 (Pro-Leu-Arg) replaced with Ala-Ala-Ala (solid triangles), and the same amino acids in a random sequence (open circles) were compared as inhibitors of GalNAc addition to hCGα. Assays containing 4 × 10^{-3} unit of partially purified GalNAc-transferase, 100 μM UDPGalNAc, 4 μM hCGα, and the indicated concentration of synthetic peptide were incubated at 37°C for 90 min. The amino acid sequences are shown in single-letter code.

of hCGα and hCGβ by the glycoprotein-hormone-specific GalNAc-transferase. This 3-amino acid sequence is located 6–9 residues on the amino-terminal side of the asparagine glycosylation sites on hCGβ and hCGα, respectively. A comparison of the sequences residing between this tripeptide motif and the glycosylation site suggests that considerable variation will be tolerated in this region by the GalNAc-transferase. For most animal species other than humans, the α subunit contains the sequence Pro-Ala-Arg rather than Pro-Leu-Arg (2), suggesting there is also flexibility in the identity of the middle amino acid of the tripeptide motif; however, the spectrum of acceptable substituents at this position remains unclear. Based on homologies with other GalNAc-transferase substrates (see below), this position may be restricted to hydrophobic amino acids. Since chemical modification of the arginine only results in a loss of recognition when its cationic character is destroyed, it is likely that lysine can be substituted for arginine. We, therefore, propose that recognition by the GalNAc-transferase requires the presence of a Pro-Xaa-Arg/Lys motif (where Xaa is most likely a hydrophobic residue) located 6–9 residues on the amino-terminal side of an asparagine glycosylation site.

Identification of the tripeptide sequence recognized by the GalNAc-transferase provides a convincing explanation for the presence of SO_4-GalNAc on specific glycoprotein hormones. In addition to the α subunit, the sequence Pro-Xaa-Arg/Lys is found on LHβ and CGβ but is not present on FSHβ due to a truncation of its amino terminus relative to LHβ and CGβ at the gene level. This correlates well with the presence of sulfated oligosaccharides on the LH dimer and on uncombined α subunits synthesized in the pituitary and their absence on the FSH dimer (1, 6, 7). In previous studies we determined (9) that combination of FSHβ with the α subunit results in masking of the recognition sequence on the α subunit. The degree to which the Pro-Leu-Arg recognition sequence on the α subunit is masked most likely accounts for the extent to which oligosaccharides on FSH dimers bear SO_4-4GalNAc. hCGβ and hCGα contain the recognition sequence and the hCG dimer is a substrate for the GalNAc-transferase *in vitro*; however, hCG synthesized by placental

trophoblasts does not bear sulfated oligosaccharides because neither the glycoprotein-hormone-specific GalNAc-transferase nor the sulfotransferase is expressed in placenta (8).

We have searched the NBRF protein data base for additional glycoproteins containing a Pro-Xaa-Arg/Lys sequence located 6–9 residues on the amino-terminal side of an Asn-Xaa-Ser/Thr. Among the glycoproteins identified by these criteria, two have been reported to contain sulfated asparagine-linked oligosaccharides. Rat proopiomelanocortin and the glycosylated form of its cleavage product corticotropin contain sulfated asparagine-linked oligosaccharides (14). A tripeptide sequence, Pro-Val-Lys, is located 7 residues on the amino-terminal side of the asparagine glycosylation site found within corticotropin. Lipoprotein-associated coagulation inhibitor, synthesized by human umbilical vein endothelial cells, can be metabolically labeled on its asparagine-linked oligosaccharides with $[^{35}S]SO_4$ (G. J. Broze, personal communication). Lipoprotein-associated coagulation inhibitor has the tripeptide sequence Pro-Phe-Lys located 9 residues on the amino-terminal side of one of its glycosylation sites (15). We have characterized the asparagine-linked oligosaccharides on corticotropin synthesized in AtT-20 cells (T. P. Skelton, S. Kumar, P.L.S., and J.U.B., unpublished data) and on lipoprotein-associated coagulation inhibitor synthesized in 293 cells (P.L.S., T. P. Skelton, M. C. Beranek, G. J. Broze, and J.U.B., unpublished data) and found that >70% of their asparagine-linked oligosaccharides terminate with the sequence SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα. Thus, the sulfated oligosaccharide structures that we originally identified on the pituitary glycoprotein hormones are also present on other glycoproteins that contain the recognition motif Pro-Xaa-Arg/Lys in close proximity to an asparagine-linked oligosaccharide.

Synthesis of the unique sulfated oligosaccharides found on the glycoprotein hormones is highly specific and tightly regulated. The presence of such unique sulfated oligosaccharides suggested that they play an important role in the biologic behavior of glycoprotein such as LH. The sulfated oligosaccharides found on LH do not affect its bioactivity at the receptor level but do have a marked impact on circulatory half-life of LH and, as a result, on its *in vivo* potency (16). We have identified a receptor in hepatic reticuloendothelial cells that is specific for oligosaccharides with the terminal sequence SO_4-4GalNAcβ1-4GlcNAcβ1-2Manα and can account for the rapid clearance of LH and other glycoproteins bearing these structures (17). The presence of the Pro-Leu-Arg motif and sulfated oligosaccharide structures on other glycoproteins of diverse function and origin suggests that these structures may be of biologic significance for a number of glycoproteins found in the circulation.

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