Procalcitonin Is a Glycoprotein*

(Received for publication, June 17, 1980)

John W. Jacobs, Pauline K. Lund, John T. Potts, Jr., Norman H. Bell*, and Joel F. Habener§

From the Laboratory of Molecular Endocrinology and Endocrine Unit, Department of Medicine, Massachusetts General Hospital, the Howard Hughes Medical Institute Laboratories at Harvard Medical School, Boston, Massachusetts 02114 and the Departments of Medicine and Pharmacology, Medical University of South Carolina, Veterans Administration Medical Center, Charleston, South Carolina 29403

Messenger RNA extracted from rat medullary carcinoma of the thyroid directs the synthesis in cell-free translation systems of a precursor of calcitonin, M, = 15,000, substantially larger than the mature form of the hormone, M, = 3,500. When translations of the mRNA were carried out in the presence of microsomal membranes prepared from a canine pancreas, a larger product (apparent M, = 17,000) was observed by electrophoresis of the labeled proteins in the translation mixtures on sodium dodecyl sulfate-polyacrylamide gels. This membrane-processed product of M, = 17,000 was specifically immunoprecipitated by an antiserum to synthetic calcitonin and bound to concanavalin A-Sepharose. Incubation of the proteins synthesized in the cell-free translations performed in the presence of microsomal membranes with the glycosidase, endo-β-N-acetylglucosaminidase H, reduced the apparent molecular weight of the membrane-processed precursor from 17,000 to 12,000. In addition, the processed M, = 17,000 calcitonin-related precursor, but not the initial, unprocessed precursor of M, = 15,000, was resistant to proteolytic digestion by a mixture of trypsin and chymotrypsin. These results indicate that the biosynthesis of calcitonin involves the glycosylation and proteolytic cleavage of a newly synthesized precursor along with sequestration of the processed precursor within microsomal vesicles. Thus, the calcitonin precursor undergoes extensive co- and post-translational processing to the smaller, unglycosylated hormone that is secreted.

Calcitonin, a 32-amino acid hormone, is synthesized by the parafollicular or C-cells, of the thyroid and acts as a regulator of calcium homeostasis in mammals and lower vertebrates (1-3). Although the processes involved in the biosynthesis of this hormone are poorly understood, evidence reported previously has shown that calcitonin is derived from a large precursor, M, = 15,000 (4) or 17,500 (5), as determined by immunoprecipitation and SDS-polyacrylamide gel electrophoresis of polypeptides synthesized during cell-free translations of messenger RNAs. Recently, we have determined the primary structure of a large portion of the rat calcitonin precursor by nucleotide sequencing of a cloned DNA complementary in sequence to the mRNA coding for the precursor (6). The structural analysis indicates that the sequence of calcitonin is located within a large precursor flanked at its amino and carboxyl ends by both basic amino acid residues, typical of prohormone cleavage sites, and by extensions consisting of cryptic peptide sequences. Presumably the co- and post-translational processing of calcitonin involves multiple proteolytic cleavages of the precursor, similar to the processing of other prehormones, including preproparathyroid hormone (7), preproinsulin (8), and the common precursor for ACTH, MSH, and the endorphins (9).

Evidence obtained from studies with cell-free systems indicates that certain modifications of protein precursors occur co-translationally on nascent chains. These include the glycosylation of secretory and membrane proteins (10-12), as well as the cleavage from the precursors of N-terminal leader, or signal, sequences (13-15). The functions of leader sequences appear to be in the transport of nascent secretory proteins into the cisternae of the rough endoplasmic reticulum. Cleavages of leader sequences from and glycosylation of, the nascent proteins occur when the chains are approximately 70 amino acids long (11, 16, 17). The biological functions of the carbohydrates attached to the nascent chains are unknown, particularly under circumstances in which certain sugars are cleaved from the carbohydrate complex during further cellular processing and transport of the proteins, as was noted for glycoproteins synthesized in Sindbis virus and vesicular stomatitis virus infected chicken embryo fibroblasts (18).

Preliminary evidence reported previously suggests that large immunoreactive forms of calcitonin, extracted from human medullary thyroid carcinomas, contain carbohydrate (19). Now we report that a membrane-dependent glycosylation of a biosynthetic precursor of calcitonin takes place during cell-free translations of mRNA carried out in the presence of microsomal membranes. In addition, we show that the endoglycosidase, endo-β-N-acetylglucosaminidase H, provides structural information on the nature of the carbohydrate moiety attached to the calcitonin precursor.

EXPERIMENTAL PROCEDURES

Preparation of RNA—Rats of the WAG/Rij strain carrying a calcitonin-secreting medullary carcinoma of the thyroid transplanted beneath the renal capsule were obtained from the Institute for Experimental Gerontology, Rijswijk, The Netherlands (20-22). Small sections of the tumor weighing less than 50 mg were transplanted beneath the left kidney capsule in normal 4-week-old rats of the same strain with the animals under light ether anesthesia. Six months (or more) later, tumors and omental metastases were removed from the rats under light ether anesthesia. The tumor tissue was placed immediately in liquid nitrogen and stored in this manner until used. Polyadenylated RNA was isolated from rat tissue using the procedures described by Kronenberg et al. (23) and Majjoub et al. (24). Approximately 50 μg (1.0 A260 unit) of polyadenylated RNA was obtained from 1 g of the tumor.

* This investigation was supported in part by United States Public Health Service Training Grant AM-07028 and National Science Foundation Grant GB-02114 (J. W. J.) and research funds from the Veterans Administration. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† A Veterans Administration Medical Investigator.

‡ Investigator, Howard Hughes Medical Institute.

§ The abbreviations used are: SDS, sodium dodecyl sulfate; ACTH, adrenocorticotropic hormone; MSH, melanotropin; Con A, concanavalin A.
Cell-free Translations-A heterologous cell-free translation system was prepared from wheat germ (25). RNA (0.1 to 1.0 µg) dissolved in sterile H2O was translated in reaction mixtures (20 to 100 µl) containing 1 µCi/ml of [35S]methionine (800-1,000 Ci/mmol, New England Nuclear, Boston, MA). Translation reactions were terminated by freezing or adding, to the reaction vessels, buffer used for electrophoresis consisting of 0.05 M Tris-HCl, pH 7.0, 1 mM MgCl2, pH 7.0, 0.5% Triton X-100, 1% β-mercaptoethanol, 10% glycerol, and 0.1% bromphenol blue (electrophoresis sample buffer). In experiments involving membrane processing of translation products, 1.0 µl (115 A260 units/µl) of microsomal membranes, prepared by the method of Katz et al. (26) from a dog pancreas, was added directly to the translation mixtures before the addition of the RNA.

Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate—Translation products, dissolved in electrophoresis sample buffer, were heated at 95°C for 2 min and applied to gradient polyacrylamide (10-20%) slab gels containing 0.1% SDS (27). Electrophoresis was performed at a constant 100 V until the dye reached the bottom of the gel. Gel slabs were stained in 0.2% Coomassie brilliant blue (Sigma), destained in 25% methanol, 7% acetic acid, dried in vacuo, and treated with a fluorography enhancer (Enhance, New England Nuclear). Autofluorograms were prepared by exposing the dried gels to Kodak X-ray film for periods of 1 to 14 days. Unlabeled proteins of known molecular weights including bovine parathyroid secretory protein, bovine heart cytochrome c, pl 7.0, 10,300, and parathyroid hormone, pl 5.2, 17,000, were used as molecular weight markers.

Immunoprecipitations—Immunoprecipitations were performed utilizing a double antibody immunoprecipitation procedure. A rabbit antiserum to human calcitonin established previously to cross-react potently with rat calcitonin was used in the experiments. Ten microfilters of the translation mixture were dissolved in 0.1 ml of a solution consisting of 10 mM NaH2PO4, pH 7.6, 1 mM Na2EDTA, and 1% Triton X-100, and incubated overnight at 4°C with 1.0 µl of the antiserum to calcitonin. Then a goat antiserum against rabbit-y-globulin was added. After an 18-h incubation (4°C) with anti-rabbit goat antiserum, the immunoprecipitates were centrifuged at 10,000 rpm for 15 min and washed three times with phosphate-buffered saline, 1% (v/v) Triton X-100. The immunoprecipitates were dissolved in sample electrophoresis buffer, heated to 37°C for 1 h, and boiled for 2 min before electrophoresis on polyacrylamide-SDS gels.

Limited Proteolysis Experiments—Aliquots of reaction mixtures from cell-free translations carried out with or without the addition of microsomal membranes were incubated for 60 min at 0°C with a mixture of trypsin and chymotrypsin at a final concentration of 50 µg/µl of each of the enzymes. To one-half of each reaction mixture, Triton X-100 was added to a final concentration of 1% before addition of the proteases. Proteolytic digestions were terminated by addition of Trasylol (FBA Pharmaceuticals) to a final concentration of 1,000 units/µl.

Digestion with Endoglycosidase H—Aliquots of reaction mixtures from cell-free translations were adjusted to 100 mM sodium citrate, pH 6.0, 0.1 M 2-mercaptoethanol, 0.8% SDS, and heated for 2 min at 100°C. After cooling, approximately 3 µg of endo-H-acetylgalcosaminidase H (a generous gift of Dr. H. Green, Massachusetts Institute of Technology) was added, and the mixture was incubated for 16 h at 37°C. The reaction was terminated by the addition of electrophoresis sample buffer and boiled at 100°C for 2 min.

Concanavalin A Affinity Binding—Concanavalin A (Con A) coupled to agarose (Pharmacia) was extensively washed by alternating centrifugation (15 min, 12,000 rpm) and resuspension in buffer containing 1 mM MgCl2, 1 mM MnCl2, 1 mM CaCl2, 200 mM NaCl, and 50 mM Tris, pH 7.4 (Con A buffer). To the washed Con A-Sepharose (approximately 100 µl of packed volume in a 0.5 ml of microfuge tube) was added 25 µl of a cell-free translation mixture containing 1% Triton X-100. After gentle mixing for 30 min at 4°C the Con A-Sepharose was washed with Con A buffer containing 1% Triton X-100. The material bound to the lectin was removed by either elution with Con A buffer containing 1% Triton and 0.2 M α-methylmannoside (12) or by boiling the Con A-Sepharose in electrophoresis sample buffer.

Results

Polyadenylated RNA prepared from the rat medullary carcinoma of the thyroid directs the synthesis of two major polypeptides (M, = 15,000 and 13,000) in wheat germ cell-free translation assays (Fig. 1, lane 4). The M, = 15,000 product was shown previously to be a biosynthetic precursor of calcitonin (4). As shown in Fig. 1, the M, = 15,000 product is specifically immunoprecipitated by an antisera against synthetic calcitonin (lane 3), and the immunoprecipitation is inhibited by the addition to the immunoprecipitation reaction of 10 µg of synthetic human calcitonin (lane 2).

To characterize further the cell-free product, the translations were performed in the presence of microsomal membranes. These membranes process secretory proteins by way of cleavages of leader, or signal sequences from the NH2 terminus of these proteins, and, in some instances, by glycosylation of the nascent polypeptides (10-17). Addition of microsomal membranes to the translation assay significantly altered the mobility of several of the RNA-directed translation products (Fig. 1, lane 5). The protein of M, = 15,000, as visualized by autofluorography of the SDS-polyacrylamide gels, is greatly diminished concomitant with the appearance of several new polypeptides (M, = 17,000, 12,000, and 10,000). When antiserum to synthetic human calcitonin was added to the membrane-reacted translation products, the M, = 17,000 protein is specifically immunoprecipitated (lanes 6, 9, and 10). Two minor products of M, = 15,000 and 12,000 also appear in the immunoprecipitated products (Fig. 1, lane 6) and most probably represent small amounts of the unprocessed calcitonin precursor (M, = 15,000) and of the calcitonin precursor from which a leader peptide of M, = 3,000 has been cleaved but has escaped glycosylation (M, = 12,000).

To test the possibility that the M, = 17,000 represents a glycosylated form of procalcitonin, membrane-reacted translation products were incubated with concanavalin A-Sepharose, a lectin which specifically binds glycoproteins (28). As shown in Figs. 1, lane 7, the M, = 17,000 product binds to Con A, whereas a majority of the other membrane-reacted translation products do not (lane 8). No calcitonin-related translation products bound to Con A when translations were performed in the absence of membranes (data not shown). These results suggest that the larger (M, = 17,000) calcitonin-related polypeptide is glycosylated.

Fig. 1. Autofluorogram of a gradient SDS-polyacrylamide gel (10-20%) showing [35S]methionine-labeled products of the translation of medullary carcinoma mRNA synthesized in a wheat germ cell-free system with and without the addition of microsomal membranes prepared from a canine pancreas. Details of methods for cell-free translations, immunoprecipitations, and Con A-Sepharose binding are described under "Experimental Procedures." Condition of translations were as follows: lane 1, products (no membranes) precipitated with nonimmune serum; lane 2, products (no membranes) precipitated by an antisera to calcitonin in the presence of 10 µg of human calcitonin; lane 3, immunoprecipitate (no membranes) from an antisera to calcitonin; lane 4, translation products (without added membranes); lane 5, translation products (with membranes added); lane 6, translation products (with membranes immunoprecipitated by an antisera to calcitonin; lane 7, translation products (with membranes) specifically bound by concanavalin A-Sepharose; lane 8, translation products (with membranes) precipitated with nonimmune serum, AATROPE point to the M, = 17,000 and 15,000 translation products.
To further test the possibility that the $M_r = 17,000$ calcitonin-related translation product is a glycoprotein, we treated the translation products with the endoglycosidase, endo-$\beta$-N-acetylglucosaminidase H (endoglycosidase H). This enzyme cleaves from glycoproteins the di-N-acetyllchitobiose sugar moiety linked to asparagine residues. In addition, for full cleavage from glycoproteins the di-N-acetylchitobiose sugar residues linked to the di-N-acetylchitobiose correspond to the intensity of labeling of a protein of $M_r = 12,000$. This protein corresponds to the predicted migration of the nonglycosylated calcitonin precursor from which a leader sequence of $M_r = 3,000$ was removed. Treatment, with endoglycosidase H, of the translation reactions performed in the absence of microsomal membranes had no effect on the mobility of any of the labeled products (Fig. 2, lanes 1 and 2). Immunoprecipitation analyses using an antiserum against synthetic human calcitonin show that the protein of $M_r = 17,000$, are related to calcitonin (Fig. 2, lanes 6-9). The presence of a small amount of the $M_r = 15,000$, unprocessed calcitonin precursor in lanes 6 and 7 suggests that in this particular experiment, cleavage and/or glycosylation of the precursor was incomplete.

Evidence presently available indicates that glycosylation and cleavage of NH$_2$-terminal leader sequences from nascent polypeptides occur cotranslationally during growth of the nascent chains (10-12). The glycoproteins are sequestered within microsomal vesicles (14-16). Such sequestration of glycoproteins can be tested for experimentally by digestion of the products of the cell-free translation with proteolytic enzymes. Sequestered proteins are resistant to digestion by mixtures of trypsin and chymotrypsin, whereas products that lie outside the microsomal vesicles are hydrolyzed by the proteases. As shown in Fig. 3, both the $M_r = 17,000$ and $M_r = 12,000$ calcitonin-related translation products synthesized in cell-free systems containing membranes are fully protected from proteolytic digestion (lane 5) but are almost completely digested when Triton X-100 is added (1% final concentration) to disrupt the microsomal membranes (lane 4). As expected, translation products synthesized in the absence of membranes are susceptible to digestion (lane 1). These results demonstrate processing and sequestration of procalcitonin by microsomal membranes, and are consistent with the co-translational glycosylation and cleavage of leader sequences by these membranes as reported previously for other secreted proteins (11, 16).

**Fig. 2.** Autoradiogram of a gradient SDS-polyacrylamide gel (10-20%) showing the effects of endoglycosidase H on the electrophoretic mobilities of $[^{35}S]$methionine-labeled products of cell-free translations. Preparation of translation products and immunoprecipitates is described under "Experimental Procedures" Lane 1, translation products (without added membranes); lane 2, translation products (without added membranes) treated with endoglycosidase H; lane 3, translation products (with added membranes); lane 4, translation products (with added membranes) treated with endoglycosidase H; lane 5, immunoprecipitation of translation products shown in lane 1; lane 6, immunoprecipitation of translation products shown in lane 3; lane 7, immunoprecipitation of translation products shown in lane 4; lane 8, translation products shown in lane 3 treated with nonimmune rabbit serum; lane 9, translation products shown in lane 4 treated with nonimmune serum. Arrows point to the $M_r = 17,000$, 15,000, and 12,000 translation products.

**Fig. 3.** Autoradiogram of a gradient SDS-polyacrylamide gel (10-20%) of $[^{35}S]$-labeled cell-free translation products incubated with proteolytic enzymes. Samples were prepared as described under "Experimental Procedures." Lane 1, translation products (without added membranes) treated with enzymes; lane 2, translation products (without membranes); lane 3, translation products (with added membranes); lane 4, translation products (with added membranes) plus Triton X-100 and treated with enzymes; lane 5, translation products (with added membranes) treated with enzymes. Arrows point to the $M_r = 17,000$ and 15,000 translation products.

**DISCUSSION**

In these studies, we show that polyadenylated RNA prepared from a rat medullary carcinoma of the thyroid directs the synthesis in cell-free translations supplemented with microsomal membranes of a glycosylated precursor of calcitonin of apparent $M_r = 17,000$. The $M_r = 17,000$ translation product was shown to be structurally related to calcitonin by immunoprecipitation of the precursor with antisera directed against synthetic calcitonin. Inasmuch as the antiserum used in these studies was raised against synthetic calcitonin, it is highly unlikely that antibodies present in the antiserum recognize proteins that are not related to calcitonin. In addition, the immunoprecipitation of the 17,000 $M_r$ protein was specifically inhibited by the presence of unlabeled, homogeneous synthetic calcitonin. Recently, we have obtained additional information about the structure of the calcitonin precursor by analysis of the nucleotide sequence of a cloned cDNA (6). The cDNA was shown to contain a nucleotide sequence coding for the complete amino acid sequence of calcitonin and to specifically hybridize with the mRNA coding for the 15,000 $M_r$, and the processed 17,000 $M_r$, proteins shown in Figs. 1-3. These studies confirm that the cell-free translation products immunoprecipitated by antisera to calcitonin are precursors of calcitonin.

Two distinct methods were used to determine that the $M_r = 17,000$ calcitonin precursor contained carbohydrate; adsorption to concanavalin A-Sepharose and sensitivity of the oligosaccharides of the precursor to cleavage by the endoglycosidase, endo-$\beta$-N-acetylglucosaminidase H. The $M_r = 17,000$ product (glycosylated procalcitonin) bound to concanavalin A whereas other forms of the precursor did not including the $M_r = 15,000$ polypeptide (unglycosylated preprocalcitonin) and the $M_r = 12,000$ polypeptide (unglycosylated procalcitonin). Studies with endoglycosidase H reveal that the mobility of the $M_r = 17,000$ precursor of calcitonin is reduced to a mobility of $M_r = 12,000$ after treatment with the enzyme, consistent with the cleavage of carbohydrate from the precursor. In addition, the glycosylated calcitonin precursor as judged by its resistance to proteolytic digestion was shown to be sequestered within microsomal vesicles after its synthesis, a finding consistent with the membrane-dependent processing and translocation of this glycoprotein into the vesicles.

Studies from a number of laboratories indicate that the glycosylation of proteins occurs through the transfer of carbohydrate to nascent polypeptide chains via a dolichol-oligosaccharide intermediate localized in microsomal membranes (30-34). The carbohydrate transferred is a di-N-acetyllchitobiose-mannose unit that can subsequently extended by the terminal addition of other sugar residues (35, 36). In the present studies, the nature of carbohydrate moiety attached to the calcitonin precursor was probed with a well character-
ized enzyme, endo-β-N-acetylglucosaminidase H, that requires for its enzymatic activity the presence of a di-N-acetylchitobiose unit containing at least 4 mannose residues (29). In addition, the attachment of the dolichol sugar unit to the peptide chain requires an amino acid sequence of the form Asn-X-Ser (Thr), with asparagine serving as the carbohydrate acceptor (37, 38). From the above studies, a minimum structure of the carbohydrate moiety attached to the calcitonin precursor can be deduced, consisting of: mannose (greater than 3 residues) → N-acetylglucosamine → N-acetylglucosamine → asparagine.

Although the complete structure of the calcitonin precursor is not yet available, the sequence of 80 of the approximately 130 amino acids has been deduced from the nucleotide sequence of a cloned cDNA (6). Based on this sequence, the only potential acceptor site for N-linked glycosylation of the form -Asn-X-Ser (Thr) - is found in the sequence of calcitonin (43). In addition, the attachment of the dolichol sugar unit to the peptide chain requires an amino acid sequence of the form Asn-X-Ser (Thr), with asparagine serving as the carbohydrate acceptor (37, 38). From the above studies, a minimum structure of the carbohydrate moiety attached to the calcitonin precursor can be deduced, consisting of: mannose (greater than 3 residues) → N-acetylglucosamine → N-acetylglucosamine → asparagine.

The finding of a glycosylated precursor provides further incentive for a systematic analysis of the processing of this hormone in intact cells.

Acknowledgments—We thank Louise Fred and Jeanne Sullivan for their assistance in the preparation of this manuscript. We thank Joseph Majzoub for the gift of the microsomal membranes.

REFERENCES
1. Potts, J. T., Jr., and Ullrich, A. G. (1976) in Handbook of Physiology (Greep, R. O., Astwood, E. B., Aurbach, G. D., and Geiger, S. R., eds) Sec. 7, Vol. 7, pp. 433-430, The American Physiological Society, Washington, D. C.
2. Jacobs, J. W., Potts, J. T., Jr., Bell, N. H., and Habener, J. F. (1979) J. Biol. Chem. 254, 10600-10603
3. Amara, S. G., Rosenfeld, M. G., Birnbaumer, R. S., and Roos, B. A. (1980) J. Biol. Chem. 255, 2645-2649
4. Jacobs, J. W., Potts, J. T., Jr., Bell, N. H., and Habener, J. F. (1981) in Calcitonin, 1980, (Pecile, A., ed) Excerpta Medica, Amsterdam, in press
5. Habener, J. F., and Potts, J. T., Jr. (1978) N. Engl. J. Med. 299, 580-585, 635-643
6. Chan, S. J., Keim, P., and Steiner, D. F. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1964-1968
7. Epper, B. A., and Mains, R. E. (1978) J. Biol. Chem. 253, 5732-5744
8. Kiely, M. L., McKnight, G. S., and Schimke, R. T. (1976) J. Biol. Chem. 251, 5490-5495
9. Rothman, J. E., and Lodish, H. F. (1979) Nature 283, 775-789
10. Bidlinska, M., and Boime, I. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 1768-1772
11. Szczena, E., and Boime, I. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1179-1181
12. Lingappa, V. R., Devillers-Thiery, A., and Blobel, G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 2432-2436
13. Shields, D., and Blobel, G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 2059-2063
14. Boime, I., Szczena, E., and Smith, D. (1977) Eur. J. Biochem. 73, 515-520
15. Palmiter, R. D., Gagnon, J., Ericsson, L. H., and Walsh, K. A. (1977) J. Biol. Chem. 252, 6386-6393
16. Robbins, P. W., Hubbard, S. C., Turco, S. J., and Wirth, D. F. (1977) Cell 12, 893-900
17. Baylin, S. R., Wieman, K. C., O'Neil, J. A., and Roos, B. A. (1979) Program of the 61st Annual Meeting of the Endocrine Society, Abstr. 61
18. Boorman, G. A., and Hollander, C. F. (1976) Am. J. Pathol. 83, 237-240
19. Panter, E., Queueer, S. F., and Bell, N. H. (1978) Clin. Res. 26, 72A
20. Roos, B. A., Yoon, M. J., Freilinger, A. L., Pensky, A. E., Birnbaum, R. S., and Lamburt, P. W. (1979) Endocrinology 105, 27-32
21. Kronenberg, H. M., Roberts, B. E., Habener, J. F., Potts, J. T., Jr., and Rich, A. (1977) Nature 267, 804-807
22. Majzoub, J. A., Kronenberg, H. M., Potts, J. T., Jr., Rich, A., and Habener, J. F. (1979) J. Biol. Chem. 254, 749-745
23. Roberts, B. E., and Patterson, B. M. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2330-2334
24. Katz, F. N., Rothman, J. E., Lingappa, V. R., Blobel, G., and Lodish, H. F. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3278-3282
25. Lasemali, U. K. (1979) Nature (Lond.) 227, 680-685
Procalcitonin Is a Glycoprotein

28. Liu, H., and Sharon, N. (1973) Ann. Rev. Biochem. 42, 541–574
29. Tai, T., Yamashita, K., and Kobata, A. (1977) Biochem. Biophys. Res. Commun. 78, 434–441
30. Kronquist, K. E., and Lennarz, W. J. (1978) J. Supermol. Struct. 8, 51–65
31. Waeber, C., and Lennarz, W. J. (1976) Ann. Rev. Biochem. 45, 95–112
32. Spiro, M. J., Spiro, R. G., and Bhooryo, V. D. (1976) J. Biol. Chem. 251, 6400–6408
33. Chen, W. W., and Lennarz, W. J. (1978) J. Biol. Chem. 253, 5774–5779
34. Parodi, A. J., Behrens, N. H., Leloir, L. F., and Carminatti, H. (1972) Proc. Natl. Acad. Sci. U. S. A. 74, 4411–4414
35. Choi, Y. S., Knopf, P. M., and Lennox, E. S. (1971) Biochemistry 10, 659–667
36. Hunt, L. A., and Summers, D. F. (1976) J. Virol. 20, 637–645
37. Pless, D. D., and Lennarz, W. J. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 134–138
38. Struck, D. K., Lennarz, W. J., and Brew, K. (1978) J. Biol. Chem. 253, 5786–5794
39. Raulais, D., Hagaman, J., Ontjes, D. A., Lundblad, R. L., and Kingdon, H. S. (1970) Eur. J. Biochem. 64, 607–611
40. Singer, F. R., and Habener, J. F. (1974) Biochem. Biophys. Res. Commun. 61, 710–716
41. Sizemore, G. W., and Heath, H. (1975) J. Clin. Invest. 55, 1111–1118
42. Heath, H., and Sizemore, G. W. (1979) J. Lab. Clin. Med. 93, 390–401
43. Nakayama, S., Inoue, A., Keta, T., Nakamura, M., Chang, A. C. Y., Cohen, S., and Numa, S. (1979) Nature 278, 423–427
44. Brownstein, M. J., Russell, J. T., and Gainer, H. (1980) Science 207, 373–378
45. Pizzari, T., Tager, H. S., Carrol, R. J., and Steiner, D. F. (1979) Nature 282, 260–266
46. Lund, P. K., Goodman, R. H., Jacobs, J. W., and Habener, J. F. (1980) Diabetes 29, 583–585
47. Goodman, R. H., Lund, P. K., Jacobs, J. W., and Habener, J. F. (1980) J. Biol. Chem. 255, 6549–6552