Copyright 1974. All rights reserved

PEPTIDE HORMONES

×856

Howard S. Tager[1] *and Donald F. Steiner*
Department of Biochemistry, University of Chicago, Chicago, Illinois

Annu. Rev. Biochem. 1974.43:509-538. Downloaded from www.annualreviews.org
by portega@wsgr.com on 12/15/21. For personal use only.

CONTENTS

INTRODUCTION 509
INSULIN 510
NONINSULIN INSULIN-LIKE PROTEINS 514
GROWTH HORMONE, PROLACTIN, AND PLACENTAL LACTOGEN 515
GLYCOPEPTIDE HORMONES 517
GLUCAGON AND RELATED HORMONES 522
GASTRIN AND RELATED HORMONES 525
CORTICOTROPIN, MELANOTROPIN, AND LIPOTROPIN 526
PARATHYROID HORMONE 528
CONCLUDING REMARKS 530

INTRODUCTION

The last comprehensive review in this series on the biochemistry of peptide hormones appeared in 1969 (1). However, various aspects of this subject have been reviewed frequently in the *Annual Reviews of Biochemistry, Physiology, Pharmacology,* and *Medicine,* as well as in other review series including *Recent Progress in Hormone Research, Hormonal Proteins and Peptides,* and *Vitamins and Hormones.* This volume includes a review of peptide hormone binding to cellular constituents and the possible relationship of this phenomenon to their biological effects (2). The *Atlas of Protein Sequence and Structure* by Dayhoff[2] is also a valuable source for comparisons of primary structural relationships among various peptide hormones.

In view of the breadth of this topic, our discussion is limited to structural and biosynthetic studies; peptide hormone secretion and action are not considered. This is necessitated by the abundance of new information and concepts in all these areas. Rapidly accumulating sequence information on peptide hormones has provided interesting new clues to evolutionary and functional interrelationships among many hormones. Thus, several groups of related peptide hormones appear to have evolved from a relatively small number of ancestral proteins. Likewise,

[1] Present Address: Department of Biochemistry, Medical College of Ohio, Toledo, Ohio 43614.

[2] Published by the National Biochemical Research Foundation, Silver Spring, Md. The last comprehensive edition appeared in 1972 (Vol. 5). Supplements are published annually.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

recent biosynthetic studies of a variety of endocrine peptides indicate that the primary gene products differ significantly from the known peptide hormones. In at least six cases these peptides appear to be synthesized from larger precursors, and multiple molecular forms of the same hormone, representing biosynthetic intermediates as well as metabolites, may circulate in the blood. These new findings have altered earlier notions regarding the evolution, biosynthesis, and active forms of peptide hormones.

INSULIN

The last three to four years have seen considerable progress in elucidating the mechanism of insulin biosynthesis and in isolating and characterizing proinsulin (3, 4) and related peptide products from various species. It is now firmly established that proinsulin is synthesized on ribosomes in the rough endoplasmic reticulum of the β cells (5–7) and that the precursor is then transferred via an energy-dependent process to the Golgi apparatus (8–11), where proteolytic conversion to insulin begins (12–15). Conversion continues to about 95% completion over a period of hours within newly formed secretory granules after they have formed by budding from the inner lamallae of the Golgi apparatus (7, 13–15). The process may be terminated at this stage by cocrystallization of the insulin with the residual proinsulin and intermediate products (16), giving rise to the dense crystalline granule inclusion that can be seen by electron microscopy within mature granules (17, 18). This small amount of proinsulin retained within the granules is secreted subsequently with insulin and has been identified in the circulation of man and animals (19–22).

The conversion process has been studied in greater detail in secretion granule fractions isolated from islets prelabeled with radioactive amino acids in vitro (7, 12–15). Conversion in these particles exhibits a relatively sharp pH optimum at or slightly above pH 6.0 (15, 23). At least two kinds of proteolytic activity appear to be required for the conversion of proinsulin to insulin. The first is an endopeptidase with trypsin-like specificity which cleaves on the carboxyl side of the pairs of basic residues that link the connecting polypeptide chain to the termini of the insulin A and B chains (4, 24). The second is an exopeptidase having specificity similar to carboxypeptidase B, which removes the C-terminal basic residues from both insulin and the C peptide[3] (25). Both kinds of activities have been demonstrated in disrupted secretion granule preparations (7, 15), and the endopeptidase activity may be associated with the secretion granule membrane (7). Further support for this localization of the proinsulin converting enzymes within the β cells has been obtained in electron microscopic histochemical studies (26). Sufficient amounts of these enzymes for more detailed chemical characterization

[3] The arrangement of the mammalian proinsulin polypeptide chain is: NH_2-A chain · Arg · Arg · C-peptide · Lys · Arg · B chain-COOH. The C peptide thus becomes that portion of the connecting peptide sequence, aside from the pairs of basic residues at the ends, which is removed in the conversion to insulin (14).

Annu. Rev. Biochem. 1974.43:509-538. Downloaded from www.annualreviews.org
by portega@wsgr.com on 12/15/21. For personal use only.

DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

have not been obtained from β granules, and studies with a variety of proteolytic inhibitors have been inconclusive in establishing their relationships to other known trypsin-like enzymes or carboxypeptidases (15). A further complication arises from the recent finding that a chymotrypsin-like cleavage occurs in rat connecting peptides during their proteolytic excision in incubated whole rat islets (27). These results suggest that the secretory granules may contain low levels of several kinds of proteolytic enzyme activities. Specificity of cleavage thus may be determined as much by tertiary structural features of the substrates as by special adaptations of the proteases. An enzyme that converts proinsulin has been isolated from whole pancreas, but it has not been fully characterized with respect to either its origin in the islet tissue of the pancreas or its cleavage specificity and mechanism of action (28).

In view of a recent report that immunoglobulin chains having extended amino-terminal regions are synthesized during in vitro translation of myeloma cell mRNA fractions (29), one might inquire whether insulin precursors larger than proinsulin also exist. However, aside from some evidence for the expected role of an N-terminal residue of methionine in the initiation of proinsulin synthesis in fetal calf pancreas (30), no convincing indications of larger precursors have been found. Islet polysomes active in proinsulin synthesis appear to be mainly trisomes, a size consistent with the expected mRNA length of about 258 nucleotides required to encode the 86-residue proinsulin polypeptide (31).

Comparative studies of insulin biosynthesis in the cod (32) and angler fish (33), as well as in such primitive vertebrates as cyclostomes (34), indicate the formation and cleavage of a proinsulin similar in size to the mammalian proteins. A requirement for trypsin-like cleavage has been demonstrated for both of the fish proinsulins, and an interesting intermediate cleavage form, having an N-terminal tripeptide A-chain extension, has been isolated from angler fish islets by Yamaji et al (35). A number of reports have appeared on the biosynthesis, isolation, and characterization of intermediate forms of mammalian proinsulins in various species (15, 24, 27, 36–40).

The proinsulin C peptide, somewhat analogous to the activation peptide in some zymogen proteins, also has become a focus of attention. Due to localization of the conversion process within secretion granules, the C peptide accumulates with insulin in equimolar amounts (41) and is secreted along with the hormone by exocytosis of the granule contents (42). C peptides from nine mammalian and one avian species have been isolated and sequenced (41, 43–50). A high rate of mutation acceptance—much higher than the rate for insulin and approaching that for the fibrinopeptides (49)—as well as the appearance of deletions in more than one region of the C-peptide sequence, suggest that structural requirements in this portion of proinsulin are less stringent than in the hormonally active portion of the molecule. Whether or not biologically important functions other than efficient peptide chain folding, sulfhydryl oxidation, and specific enzymic cleavage are also encoded in this peptide remains unanswered.

Synthesis of several mammalian C peptides has been accomplished recently by classical fragment condensation (51–56). The synthetic porcine C peptide, containing all four terminal basic residues, was tested for its ability to promote the

Annu. Rev. Biochem. 1974.43:509-538. Downloaded from www.annualreviews.org by portega@wsgr.com on 12/15/21. For personal use only.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

recombination of insulin A and B chains in vitro, but it failed to influence the yield (57). Synthetic porcine and bovine C peptides cross-react well with antibodies directed against the corresponding natural proinsulins or C peptides, and fragments of these peptides have been successfully utilized to study the antigenic determinants in this region of the proinsulin molecule (53, 54, 58).

Both proinsulin and the C peptide have been detected in the circulation of man and other species by means of specific immunoassays (for a review see 59). The level of proinsulin rises slowly after a glucose load in normal subjects but does not exceed 20% of the total insulin-like immunoreactive material. Although abnormal proportions or absolute concentrations of proinsulin have been found in obesity, chronic renal failure, and patients with severe hypokalemia, the major diagnostic significance of elevated proinsulin levels has been in detecting patients with β cell tumors (60). The immunoassayable C-peptide levels have been shown by Rubenstein and co-workers to mirror changes in insulin levels and thus provide a means for evaluating endogenous insulin production in diabetic individuals in whom insulin antibodies and administered animal insulins invalidate direct insulin measurements (61, 62). The handling of proinsulin and C peptide in vivo differs significantly from that of insulin (63, 64). This factor must be taken into consideration when interpreting changes in peripheral blood levels of immunoreactive insulin and in estimating the relative biological potency of proinsulin by means of in vivo blood glucose-lowering assays. The biological activity of proinsulin on fat cells (65) and muscle tissue (66) in vitro is about 3–5% that of insulin. Higher activity is usually found in vivo, ranging from 20–30%, a result attributable to the slower turnover rate of proinsulin rather than to any proteolytic conversion of proinsulin to insulin in the circulation or tissues (59). The difference in turnover rates of proinsulin and insulin is due largely to the relatively greater uptake and degradation of insulin by the liver, a major site of insulin destruction in the intact organism (67). The precise enzymic mechanism of insulin degradation remains controversial despite a recent renewal of interest in this problem (68–71). The studies of Varandani and co-workers have brought forth new evidence implicating an initial step of reductive cleavage in this process (71–73).

The recent elucidation of the three-dimensional structure of insulin, initially at a resolution of 2.8 Å (74, 75) and with recent refinements at 1.9 Å (76) by Hodgkin and her co-workers, represents an important breakthrough in the study of peptide hormone structure. It is beyond the scope of this review to describe this structure in detail or the growing literature on the chemical and immunological properties and their structural correlations in normal, modified, or synthetic insulin molecules. Several recent reviews are cited (76–80). Modifications include the introduction of various substituent groups on the amino, histidyl, or carboxyl groups of insulin (76, 81–86), selective reduction and substitution of disulfide bonds (87), and the introduction of intramolecular crosslinks between the A1 and B1, or A1 and B29 (ε-lysine) amino groups (79, 88, 89). The latter group of derivatives, especially the series linked between A1 and B29 by dicarboxylic acids (79), are of added interest, since these bridges simulate the naturally occurring connecting polypeptide in proinsulin. Adipoylinsulin, in which adipic acid serves to crosslink the amino

Annu. Rev. Biochem. 1974.43:509-538. Downloaded from www.annualreviews.org by portega@wsgr.com on 12/15/21. For personal use only.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

groups of residues A1 and B29, has been reduced and reoxidized in vitro under conditions suitable for proinsulin reoxidation with comparable yields ranging up to 75% (90). These results indicate that the role of the connecting peptide in promoting correct pairing of half-cystine residues in proinsulin can be played by a non-peptide molecular prosthesis.

None of the insulin derivatives prepared to date have exhibited higher biological activity than insulin itself. As with proinsulin, assays often indicate higher activity in vivo than in vitro (79), suggesting that many of these analogs may accumulate to a greater extent in the blood as a result of their decreased susceptibility to degradation. This cannot be the case with the identical turkey and chicken insulins which exhibit 2–4 times higher activity than bovine insulin in several in vitro bioassay systems (91). Weitzel and co-workers suggest that this heightened activity may result from enhanced receptor binding due to the substitutions of histidine for alanine and asparagine for serine at positions A8 and A9, respectively. On the other hand, substitution of the B5 histidine by alanine in a synthetic bovine insulin led to lower biological activity (92). Duck insulin may help to clarify the role of substitutions in the A8–10 region in enhancing biological activity of chicken and turkey insulins. This avian species has glutamic acid at position A8 and proline at A10, and the B chain differs only at position 30 where threonine replaces alanine (93). Bioassay results have not yet been reported.

In contrast, guinea pig insulin, which differs from porcine insulin at 17 positions (49), displays significantly lower biological activity in other mammals as well as in guinea pigs (94). In addition to altered molecular topography due to the amino acid changes, guinea pig insulin does not bind zinc and does not form dimers or higher polymers in solution (94). Associated with this rather drastic change in properties is the replacement by asparagine of the B10 histidine residue, which coordinates with zinc in two zinc porcine insulin crystals (74). By contrast, the two insulins in the mouse, an old-world rodent, closely resemble other mammalian insulins and are identical to those of the rat (95).

Another insulin of considerable interest has been isolated from the islet organs of a primitive jawless vertebrate, the Atlantic hagfish (34, 96), an animal belonging to one of two extant orders of the cyclostomes, which are believed to have diverged from the gnathostomes about 600 million years ago (97, 98). Hagfish insulin has about 10% of the activity of bovine insulin in mammalian systems (96). About 40% of its amino acid residues differ from those found in mammalian insulins, including replacement of the zinc-binding B10 histidine residue by aspartic acid (99). Nevertheless, it forms large tetragonal crystals at pH 6.0 in the absence of zinc (34). Almost all of the invariant residues in the known gnathostomian insulins (49), including the half-cystines, are conserved in hagfish insulin.

Studies with selectively degraded or synthetic insulins indicate that the absence of the A1 glycine amino group (100) or the A1 glycine residue (79, 81) results in a loss of biological activity. In the absence of the A1–4 tetrapeptide sequence (101) or the C-terminal A21 asparagine residue (102, 103), biological activity is either absent or extremely low. Although C-terminal shortening of the B chain up to residue 27 does not affect the biological activity (104, 105), further deletions toward

Annu. Rev. Biochem. 1974.43:509-538. Downloaded from www.annualreviews.org by portega@wsgr.com on 12/15/21. For personal use only.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.

WHAT WILL YOU BUILD? | sales@docketalarm.com | 1-866-77-FASTCASE

fastcase® Smarter legal research.