Conversion of Proparathyroid Hormone to Parathyroid Hormone: Studies in Vitro with Trypsin†

David Goltzman,‡ Edward N. Callahan, Geoffrey W. Tregear,§ and John T. Potts, Jr.*

ABSTRACT: The conversion of proparathyroid hormone to parathyroid hormone (PTH) was studied in vitro employing pancreatic trypsin as a prototype converting enzyme. Digestion of intact radiolabeled bovine prohormone with trypsin (0.1%) (w/w) resulted in release of a peptide comigrating with intact hormone marker in systems resolving both on the basis of charge (urea polyacrylamide gels, pH 4.4) and size (sodium dodecyl sulfate–urea polyacrylamide gels, pH 7.2). Tryptic digestions of a synthetic analogue of bovine prohormone, ProPTH-(−6–+34), consisting of the prohormone hexapeptide covalently bonded to the NH_2 terminus of the active fragment of the hormone, released in high yield the hexapeptide and the intact active hormone fragment before any other smaller fragments. Analyses of digestions were by: (i) thin-layer chromatography and amino acid analysis of digestion products; (ii) comparison of the biological activity of the prohormone substrate and the products of digestion; and (iii) peptide end-group analysis by the Edman method during progressive tryptic hydrolysis over 22 h. The latter experiments demonstrated cleavage of more than 75% of the hexapeptide–hormone peptide bond before cleavage of other trypsin-sensitive sites within the molecule. It is concluded that the specificity of cleavage at the hexapeptide–hormone bond in the process of intracellular hydrolysis of proparathyroid hormone resides primarily in the sequence and/or conformation of the precursor molecule; inasmuch as conversion of prohormone to hormone can be efficiently accomplished by pancreatic trypsin in vitro, there is, therefore, no need to postulate the existence of an intracellular converting enzyme within the parathyroid cell that possesses unique hydrolytic specificity.

The discovery of biosynthetic precursors of several hormonal polypeptides (Chrétien and Li, 1967; Steiner and Oyer, 1967; Noe and Bauer, 1971; Yalow and Berson, 1971; Cohn et al., 1972; Gregory and Tracy, 1972; Kemper et al., 1972; Tager and Steiner, 1973) and nonhormonal polypeptides (Bellamy and Bornstein, 1971; Judah and Nicholls, 1971) has generated interest in the mechanism of conversion of the precursor molecule to the hormone or other product. In many cases, pancreatic trypsin was employed to attempt to convert the precursor to product (Steiner and Oyer, 1967; Chance et al., 1968; Cohn et al., 1972; Gregory and Tracy, 1972; Gewirtz et al., 1974; Quinn et al., 1975). When the conversion process was examined in sufficient detail in certain cases, however, such as in the case of proinsulin, the issue of the degree of specificity of the converting enzyme appeared more complex than was initially envisioned (Chance et al., 1968; Nolan et al., 1971). Thus, no single known enzyme converts proinsulin to insulin in vitro. The process does, however, proceed well with the combined use of two enzymes, trypsin and carboxypeptidase B (Kemmler et al., 1971). This suggested that, if the in vivo converting enzyme for proinsulin is an endopeptidase, it has unique specificity; alternatively, the process may involve an endopeptidase and an exopeptidase operating together to accomplish the conversion (Steiner et al., 1974). Analysis of the structure of proparathyroid hormone (ProPTH)[1] (Hamilton et al., 1974) suggested that this prohormone might be readily converted to parathyroid hormone (PTH) by the action of a trypsin-like enzyme alone. A series of studies was therefore initiated to determine whether pancreatic trypsin, as a convenient prototype, would efficiently cleave PTH from ProPTH without cleaving other trypsin-sensitive bonds within the molecule, thereby simulating the actual enzyme cleavage occurring in the parathyroid cell in vivo.

Materials and Methods

Hormone Preparations. Internally labeled ProPTH and PTH were prepared biosynthetically as previously described (Kemper et al., 1972; Keutmann et al., 1975) by incubation of bovine parathyroid-gland slices with [^{35}S]methionine (12 μCi/ml) or [^{3}H]leucine (50 μCi/ml) (New England Nuclear Corp.) or both at 37 °C in Earle's balanced salt solution with 1.0 mM $CaCl_2$, 0.4 mM $MgCl_2$, and 5% fetal calf serum, equilibrated with a gas phase of 95% air–5% CO_2. Prohormone was extracted from the slices in 0.2 N HCl–8 M urea and precipitated with CCl_3COOH, as previously described. Purification was achieved by gel filtration on Bio-Gel P-100 (Bio-Rad, Riverside, Calif.), equilibrated with 0.1 M NH_4OAc (pH 5.0), followed by ion-exchange chromatography on CM-cellulose (CM-52; Reeve-Angel, Inc., Clifton, N.J.), eluting with a linear gradient of increasing conductivity made with 0.01 M NH_4OAc (pH 5.0, conductivity, 0.8 $m\Omega^{-1}$) and 2.4 M NH_4OAc (pH 6.0, conductivity, 20 $m\Omega^{-1}$). ProPTH eluted at a conductivity of 14 $m\Omega^{-1}$. ProPTH eluting in the prohormone peak was quantitated by a specific radioimmu-

† From the Endocrine Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114. *Received January 22, 1976.* This work was supported by grants from the National Institutes of Health No. AM11794 and AM04501, from the National Aeronautics and Space Administraton No. NAS 9-11011, and from the John A. Hartford Foundation, Inc.

‡ Recipient of a Centennial Fellowship from the Medical Research Council of Canada. Present address: Department of Medicine, McGill University, and Royal Victoria Hospital, Montreal, Canada.

§ Present address: Howard Florey Institute, University of Melbourne, Parkville, 3052, Australia.

[1] Abbreviations used: PTH, parathyroid hormone; ProPTH, proparathyroid hormone; Tos-PheCH_2Cl, tosylamino-2-phenylethyl chloromethyl ketone; SDS, sodium dodecyl sulfate; CM, carboxymethyl; cAMP, cyclic adenosine 3′,5′-monophosphate; MRCU, Medical Research Council Unit (Great Britain).

noassay (Habener et al., 1974). Native bovine parathyroid hormone was extracted (Aurbach, 1959) and purified (Aurbach and Potts, 1964; Keutmann et al., 1971) as previously described.

The biologically active fragment of bovine PTH [PTH-(1-34)[2]], the analogue of bovine ProPTH [ProPTH-(−6-+34)], consisting of the prohormone hexapeptide covalently linked to the NH_2 terminus of the same hormonal active fragment, the hexapeptide ProPTH-(−6-−1), and the analogue of human ProPTH [(human) ProPTH-(−6-+34)] were synthesized by solid-phase procedures and purified as previously described (Potts et al., 1971; Habener et al., 1974; Tregear et al., 1974; Tregear, 1975; Rosenblatt et al., 1976).

Trypsin Digestion. Lyophilized bovine proparathyroid hormone (either native ProPTH-(−6-+84) or the synthetic analogue ProPTH-(−6-+34) or the synthetic hexapeptide ProPTH-(−6-−1) was dissolved in equal amounts in separate aliquots of 10 μl of 0.1 N HOAc, and each aliquot was buffered with 80 μl of 0.2 M NH_4HCO_3 (pH 8.2). Trypsin (Tos-PheCH_2Cl-trypsin, Worthington Biochemicals) was added to each aliquot in 10 μl of 0.2 M NH_4HCO_3, pH 8.2, to a final concentration of 0.1% (w/w), and the reactions were terminated at timed intervals by addition of 2 drops of glacial HOAc. For the experimental control, 2 drops of glacial HOAc was added before addition of trypsin. The reaction mixtures were then quickly frozen and lyophilized before analysis of the digests.

Polyacrylamide Gel Electrophoresis. The timed tryptic digests of biosynthetic ProPTH-(−6-+84) were analyzed by electrophoresis on 10% polyacrylamide gels both in 8 M urea at pH 4.4 (urea gels) and in 0.1% sodium dodecyl sulfate-8 M urea at pH 7.2 (SDS-urea gels) (Kemper et al., 1972). Unlabeled native bovine parathyroid hormone or [^{3}H]leucine-labeled PTH-(1-84) was added to each tryptic digest just before the electrophoresis on urea gels or sodium dodecyl sulfate-urea gels, respectively, to serve as an internal marker. After electrophoresis on urea gels, 1-mm gel slices were each shaken separately with 0.2 ml of 0.05 M Veronal buffer-10% human plasma, pH 8.5, for 24 h at 4 °C. A 10-μl aliquot of each extract was radioimmunoassayed for parathyroid hormone (Kemper et al., 1972), and the remainder was counted for ^{35}S and ^{3}H radioactivity in a dual-channel, liquid-scintillation spectrometer (Packard Tri-Carb). After electrophoresis on sodium dodecyl sulfate-urea gels, 1-mm gel slices were incubated overnight with 1 ml of solubilizer (Amersham/Searle)-water-scintillation fluid (0.4:0.04:0.56) at 37 °C. Radioactivity was then measured as above.

Thin-Layer Chromatography. The products of tryptic digestion of ProPTH-(−6-+34) were resolved by thin-layer chromatography on sheets of silica gel (20 × 20 cm, Eastman Kodak 6060). The sample load was 100 μg in 25 μl of 0.1 N HOAc. Ten micrograms each of ProPTH-(−6-−1), ProPTH-(−6-+34), and PTH-(1-34) were also spotted, each in 25 μl of 0.1 N HOAc, to serve as standards. Plates were developed in a solvent system of 1-butanol-HOAc-pyridine-water (15:3:10:12) for 2 h. The standards were then sprayed with 1% ninhydrin, and the areas of the silica gel containing the tryptic digest and corresponding in position of migration to the peptide standards were scraped from the thin-layer plate (the plate region containing the tryptic peptides having been shielded during spraying). Peptides were eluted from the thin-layer-sheet scrapings by extracting twice with 50% HOAc in tapered centrifuge tubes. HOAC supernatants containing eluted peptide were then lyophilized.

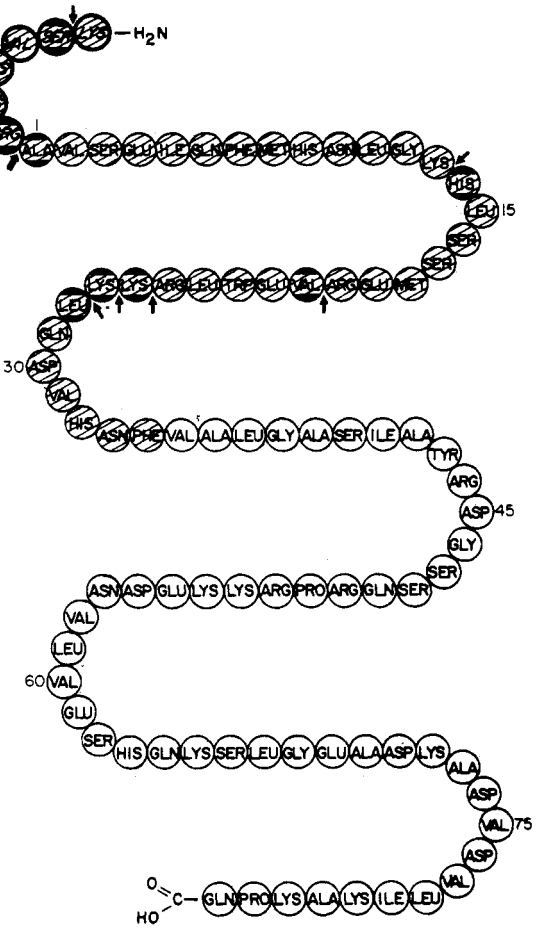

FIGURE 1: Amino acid sequence of the native bovine prohormone, ProPTH-(−6-+84). Shaded circles represent the synthetically prepared fragment of the active sequence of prohormone −6-+34. Heavy circles indicate the prohormone hexapeptide. Arrows indicate the potentially trypsin-sensitive bonds within the sequence −6-+34; residues with darkened borders are those on the carboxyl side of the trypsin-sensitive bonds of this sequence, detectable by end-group analysis.

Amino Acid Analysis. Amino acid analyses were carried out in the Beckman Model 121 automated amino acid analyzer. Acid hydrolyses were performed in 5.7 N HCl containing 1:2000 (v/v) mercaptoethanol (Keutmann and Potts, 1969). Amino acids were normalized from mole fractions to moles per mole of peptide by best fit based upon mean recovery of all stable residues.

Bioassay. Biological activity in vitro was assayed using the canine renal cortical adenylyl cyclase assay system (Peytremann et al., 1975; Goltzman et al., 1976) as previously described (Goltzman et al., 1975). [^{32}P]ATP and [^{3}H]cAMP were purchased from New England Nuclear and Schwarz/Mann, respectively.

[2] The sequence and numbering of the residues adopted in our convention are as follows. The minus sign indicates the nonhormonal residues, and consecutive residues are numbered from −6-−1 to give the complete sequence of the nonhormonal part of ProPTH, which is followed uninterruptedly by the sequence of native PTH-(1-84) (Figure 1). In the hormone sequence, +1 refers to the NH_2-terminal residue (alanine in bovine PTH, and serine in human and porcine PTH). In this convention, there is no position zero. The sequence ProPTH-(−6-−1) is referred to as the prohormone hexapeptide. For purposes of simplification, when native hormone proper or fragments or analogues of native hormone proper exclusive of nonhormonal parts of the prohormone sequence are specified, the sequence positions are given as numbers not preceded by minus or plus.

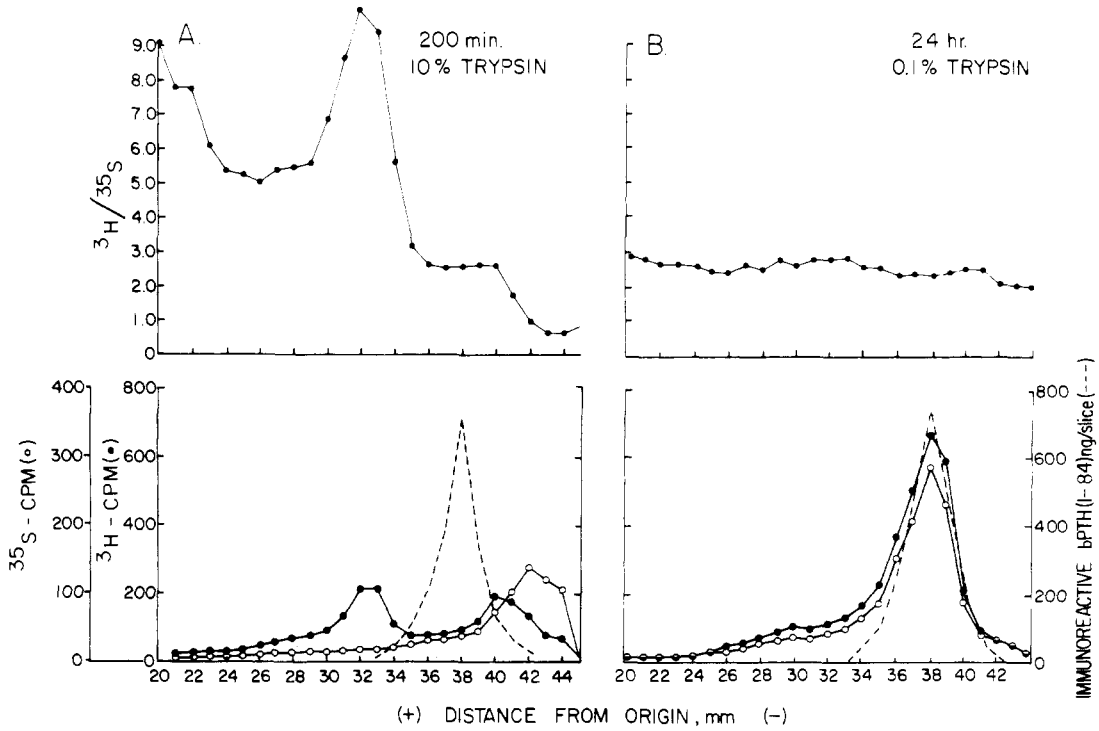

FIGURE 2: Patterns of migration of ^3H cpm and ^{35}S cpm in electrophoresis on urea polyacrylamide gels of products of hydrolysis of biosynthetically doubly labeled ProPTH-(−6-+84) (lower graphs) by either 10% (w/w) trypsin for 200 min at 37 °C (panel A), or 0.1% (w/w) trypsin for 24 h at 37 °C (panel B). Unlabeled PTH-(1-84) was added to the gel as an internal marker for the migration position of intact hormone and measured by radioimmunoassay. The ratio of ^3H cpm to ^{35}S cpm in each gel slice is also shown (upper graphs). Details of the hydrolyses and electrophoretic analyses are given in the text.

End-Group Analysis. During tryptic digestion of the synthetic analogues ProPTH-(−6-+34) and ProPTH-(−6-−1), the NH_2-terminal residues exposed by cleavage were analyzed as a function of time. End groups were analyzed by the technique of Edman (Edman, 1960; Jacobs and Niall, 1975) using [^{35}S]phenyl isothiocyanate (Amersham/Searle, Arlington Heights, Ill.). The phenylthiohydantoin derivatives generated were identified and quantitated by gas-liquid chromatography (Pisano and Bronzert, 1969) and thin-layer chromatography (Edman and Begg, 1967; Jacobs and Niall, 1975). Radioactive phenylthiohydantoins were quantitated by extracting dry silica gel scraped from the appropriate area of the plate as defined by the migration position of phenylthiohydantoin-amino acid standards. The scrapings of silica gel were extracted by gentle agitation in Insta-Gel liquid scintillation fluid (Packard), and the extract was counted for ^{35}S radioactivity.

Results

Tryptic Digestion of ProPTH-(−6-+84). Timed tryptic digestion was performed on labeled intact native ProPTH-(−6-+84) produced biosynthetically. Hormone was labeled uniformly in separate biosynthetic preparations, either with [^{35}S]methionine (present in the NH_2-terminal region only, at sequence positions 8 and 18) or with both [^{35}S]methionine and [^3H]leucine (Figure 1).

Initially, doubly labeled ProPTH-(−6-+84) was incubated with trypsin (10%) (w/w) for 200 min at 37 °C. When the digest was then analyzed on urea polyacrylamide gels (pH 4.4), considerable fragmentation of the prohormone was evidenced, as shown both by the separate patterns of ^3H cpm and ^{35}S cpm and by the pattern of the ratio of ^3H cpm to ^{35}S cpm in each gel slice (Figure 2A).

Reduction of the trypsin concentration to 0.1% (w/w) led to the demonstration of a progressive reduction in the size of the prohormone peak migrating on urea polyacrylamide gels, with increasing length of period of incubation. Although the total quantity of radioactivity added to each timed incubation mixture varied slightly, a corresponding increase (relative to the size of the declining prohormone peak) in the size of a peak comigrating with the intact PTH marker (Figures 2B and 3) was concomitantly observed, with no evidence of other cleavages. The ratio of ^3H cpm to ^{35}S cpm remained virtually the same in the peak comigrating with the PTH marker as it was in the ProPTH peak (Figures 2B and 3), providing evidence for the persistent integrity of hormone generated from prohormone.

Similar results were obtained with timed tryptic digestion of ProPTH-(−6-+34) labeled only with [^{35}S]methionine, and analyzed by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels (Figure 4).

Tryptic Digestion of ProPTH-(−6-+34). A 100-μg aliquot of the synthetic analogue ProPTH-(−6-+34) was digested with 0.1% (w/w) trypsin for 60 min, and the products of the digest were resolved by ascending thin-layer chromatography on silica gel. Three fractions, A, B, and C, corresponding in position of migration to standards of PTH-(1-34), ProPTH-(−6-+34), and ProPTH-(−6-−1), respectively (with R_f's of 0.37, 0.28, and 0.12, respectively), were scraped from the plate, extracted, and lyophilized. Aliquots of each fraction were then subjected to acid hydrolysis and amino acid analysis. Results of the analyses (Table I) were consistent with the identification of fractions A, B, and C as PTH-(1-34), ProPTH-(−6-+34), and ProPTH-(−6-−1), respectively. Recoveries of PTH-(1-34) and ProPTH-(−6-−1) fractions were approximately equimolar.

Equimolar amounts of each fraction were then tested for biological activity in vitro in the renal adenylyl cyclase assay. Synthetic bovine PTH-(1-34) has approximately 30 times the

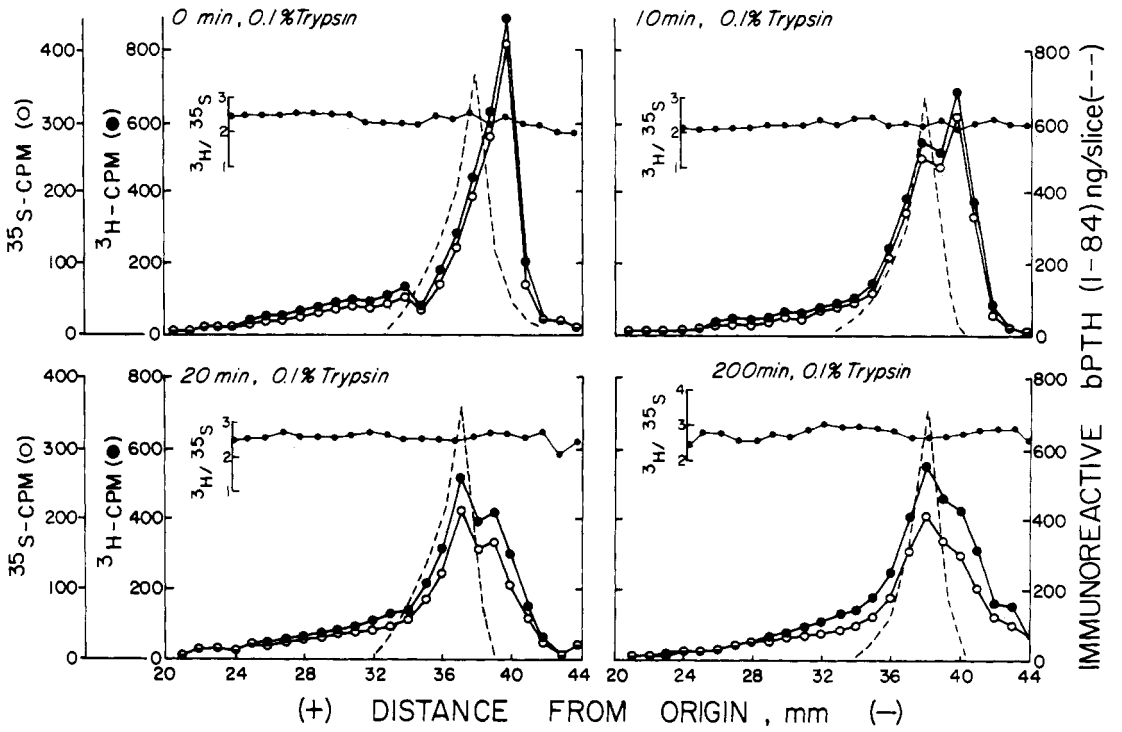

FIGURE 3: Patterns of migration of ^{3}H cpm and ^{35}S cpm in electrophoresis on urea polyacrylamide gels of products of timed hydrolyses of doubly labeled ProPTH-(−6-+84) by 0.1% (w/w) trypsin at 37 °C. Unlabeled PTH-(1-84) was added to the gel as an internal marker for the migration position of intact hormone and was measured by radioimmunoassay. The ratio of ^{3}H cpm to ^{35}S cpm in each gel slice is shown as an inset in each panel. Details of the hydrolyses and electrophoretic analyses are given in the text.

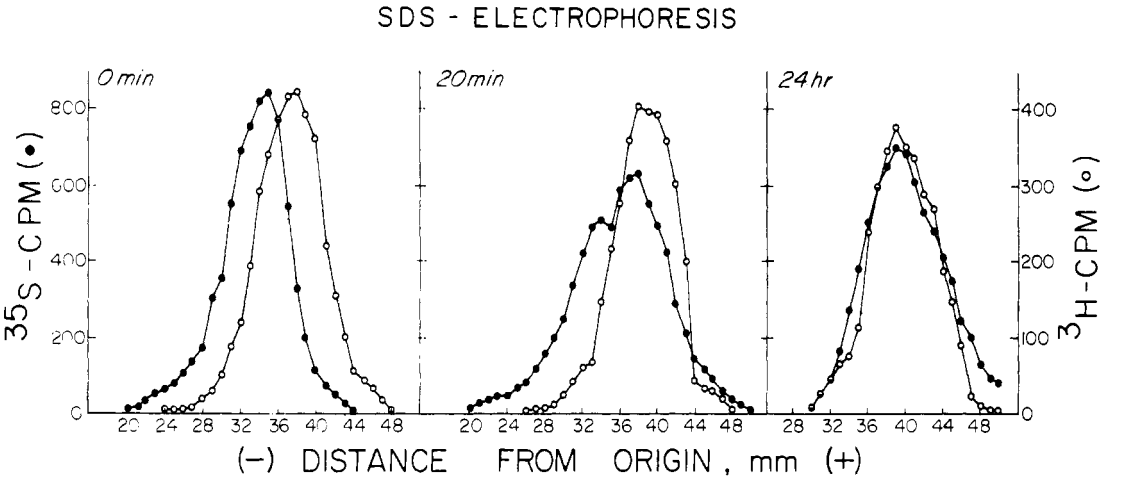

FIGURE 4: Patterns of migration in electrophoresis on sodium dodecyl sulfate-urea polyacrylamide gels of ^{35}S-labeled products of timed hydrolyses of [^{35}S]ProPTH-(−6-+84) with 0.1% (w/w) trypsin at 37 °C. Biosynthetic [^{3}H]PTH-(1-84) was added to the gels as an internal marker for the migration position of intact hormone. Details of the hydrolyses and electrophoretic analyses are given in the text.

activity per mole of bovine ProPTH-(−6-+34) in this assay system (6800 and 230 MRCU/mg, respectively). A considerably higher activity was seen in fraction A (1710 pmol of cAMP generated per mg of protein per 10 min) than in fraction B (282 pmol of cAMP generated per mg of protein per 10 min; basal activity was 164 pmol of cAMP per mg of protein per 10 min), consistent with conversion of prohormone fragment ProPTH-(−6-+34) to hormone fragment PTH-(1-34). The actual activity found in fraction A was less than theoretically expected; this lower potency presumably reflects oxidation of methionine, which lowers activity in PTH (Rosenblatt et al., 1976) during thin-layer chromatography.

The considerable increase in biological activity seems to provide powerful evidence against tryptic cleavage at potential internal sites within the 1-34 fragment (Figure 1), inasmuch as it is known that biological activity requires the integrity of the continuous peptide sequence from sequence positions 2 through 27, and activity, therefore, would be lost with internal cleavages (Tregear et al., 1973).

A specific chemical approach was used to test more rigorously for internal cleavages. End-group analysis by one-step Edman degradation was performed on the mixture of peptides generated during progressive tryptic digestion of ProPTH-(−6-+34) to determine the relative sensitivities of the various internal bonds (Figure 1) to tryptic hydrolysis. Trypsin-to-peptide ratio was 0.1% w/w or 1:5000 mol/mol. A progressive increase with time in the recovery of the phenylthiohydantoin derivative of alanine (on gas-liquid chromatography) was

TABLE I: Amino Acid Composition of Products of Tryptic Digestion of ProPTH-(−6-+34).[a]

	Fraction A PTH-(1-34)	Fraction B ProPTH-(−6-+34)	Fraction C ProPTH-(−6-−1)
Asp	2.91 (3)	3.27 (3)	
Ser	2.41 (3)	3.87 (4)	1.15 (1)
Glu	5.20 (5)	4.55 (5)	
Gly	1.92 (1)	1.82 (1)	
Ala	1.15 (1)	1.32 (1)	
Val	2.48 (3)	3.31 (4)	1.01 (1)
Met	1.34 (2)	1.36 (2)	
Ile	0.99 (1)	0.81 (1)	
Leu	3.80 (4)	4.28 (4)	
Phe	1.84 (2)	1.73 (2)	
Lys	2.63 (3)	5.68 (6)	2.52 (3)
His	2.43 (3)	3.05 (3)	
Arg	2.65 (2)	2.84 (3)	0.85 (1)
Total nmol recovered	4.38	3.27	3.83
% recovered	57.3	42.7	50.1

[a] Each datum is the mean amino acid recovery (in residues per mole) from separate analyses of two acid-hydrolyzed samples of each fraction. Tryptophan was not determined. Numbers in parentheses are the theoretical numbers of residues per mole, assuming the peptide content of each fraction to be solely the respective peptide indicated. Percent recoveries show the appearance of ProPTH-(−6-−1) and PTH-(1-34) to be approximately equimolar and stoichiometric with the amount of disappearance of ProPTH-(−6-+34).

found before the appearance of significant quantities of other phenylthiohydantoin-amino acids (Figure 5). When the trypsin/substrate ratio was increased from 1:5000 to 1:50, a general increase in the rate of hydrolysis of other bonds could be demonstrated. Yields of NH_2-terminal leucine and valine greater than 50% of the yield of alanine were rapidly detected precisely consistent (Figure 1) with the cleavage of internal trypsin-sensitive sites.

The relative rates of exposure of newly NH_2-terminal amino acids in digestion of ProPTH-(−6-+34) with 0.1% trypsin, as determined by recoveries on gas-liquid chromatography and thin-layer chromatography of products of one-step Edman degradations, are summarized in Figure 5; the results indicate the markedly greater sensitivity of the hexapeptide-hormone bond, viz., the bond arginine(−1)-alanine(+1), to cleavage by modest quantities of trypsin. A total yield of 75% of theoretical maximum of newly accessible NH_2-terminal alanine was accomplished (at 100 min; Figure 5, upper left panel) before the detected exposure of any other residue.

To eliminate the possibility that enzyme denaturation or product inhibition, rather than bond selectivity, was influencing the results seen, an identical digestion with 0.1% (w/w) trypsin was carried out with 20 nmol of synthetic bovine ProPTH-(−6-+34), but, at 22 h, 20 nmol of synthetic human ProPTH-(−6-+34) was added to the digest mixture (at point indicated by arrow, Figure 5, lower left panel), and the incubation was continued for an additional 160 min. A sharp rise in the amount of NH_2-terminal serine found in one-cycle Edman degradation of the digest at the end of this second phase of incubation indicated rapid cleavage of the bond arginine-(−1)-serine(+1) in the human molecule and demonstrated the continued activity of the originally added small quantity of trypsin. This supported the thesis that the rapid rate of cleavage of the prohormone-hormone bond relative to rate of cleavage of other trypsin-sensitive sites within the hormone, when the quantity of trypsin is rate limiting, reflected true substrate or sequence specificities rather than a failure of the other susceptible bonds to cleave because trypsin had been inactivated or inhibited.

When similar studies were performed on digests of ProPTH-(−6-−1) (20 nmol) employing the same low ratio of trypsin to substrate, minimal cleavage of this peptide was seen during the first 200 min, the period during which virtually complete hydrolysis of the hexapeptide-hormone bond had been accomplished, further illustrating that cleavage of the hexapeptide-hormone bond is highly preferred over other cleavages, including potential cleavages within the hexapeptide itself.

Discussion

The present studies have defined several aspects of the conversion of proparathyroid hormone to parathyroid hormone. The sequence and/or conformation of proparathyroid hormone is such that cleavage of the hexapeptide-hormone bond can be accomplished by a single endopeptidase with trypsin-like specificity. Although most of these studies were performed with bovine proparathyroid hormone, containing alanine at sequence position +1, the rapidity of the cleavage of the corresponding arginine(−1)-serine(+1) bond of the synthetic human prohormone analogue used in control studies (Figure 5) suggests that the human prohormone is as sensitive to cleavage by dilute trypsin as the bovine prohormone. The sequence of the hexapeptide extension of ProPTH has now been shown to be identical in two species (bovine and human) (Cohn et al., 1974; Hamilton et al., 1974; Jacobs et al., 1974) and similar in one other (porcine) (Chu et al., 1975), all structures containing arginine at sequence position −1. The NH_2-terminal residue of the hormone sequence in the three species is either alanine or serine. Furthermore, all three known proparathyroid hormones contain the sequence -lysyllysylarginyl-X- (where X is either alanine or serine); therefore, on the basis of this similarity, it seems reasonable to suggest that conclusions based on the detailed studies with bovine ProPTH are applicable also to ProPTH of human and porcine origin.

Although the specificity of the cleavage of the prohormone-hormone bond obviates the need to postulate the existence of a unique converting enzyme with hitherto-unknown specificity in conversion of ProPTH to PTH in vivo, the prohormone connecting enzyme within the parathyroid cell may well differ in kinetic properties from pancreatic trypsin. The rate and efficiency of conversion of bovine ProPTH are higher in the intact cell (based on the kinetics of conversion in tissues slices in vitro demonstrated in pulse-chase experiments (Cohn et al., 1972; Kemper et al., 1975)) than is the conversion of the prohormone to hormone by pancreatic trypsin in aqueous solution in vitro. If the in vivo converting enzyme, for example, is bound to particulate elements of the cytoskeleton along the route of vectorial transport of prohormone to the hormone storage sites at the cell periphery, the true kinetics and efficiency of conversion may be only very imperfectly demonstrated in cell homogenates or in simple aqueous solutions of enzymes.

These studies are also of interest with respect to the general topic of prohormone-to-hormone (or other peptide precursor-to-product) conversion, in two particular respects. First, in those cells that synthesize precursors with both NH_2- and COOH-terminal extensions, such as the pancreatic β cell, which synthesizes proinsulin, the converting mechanism seems to require two enzymes, one with trypsin-like and another with

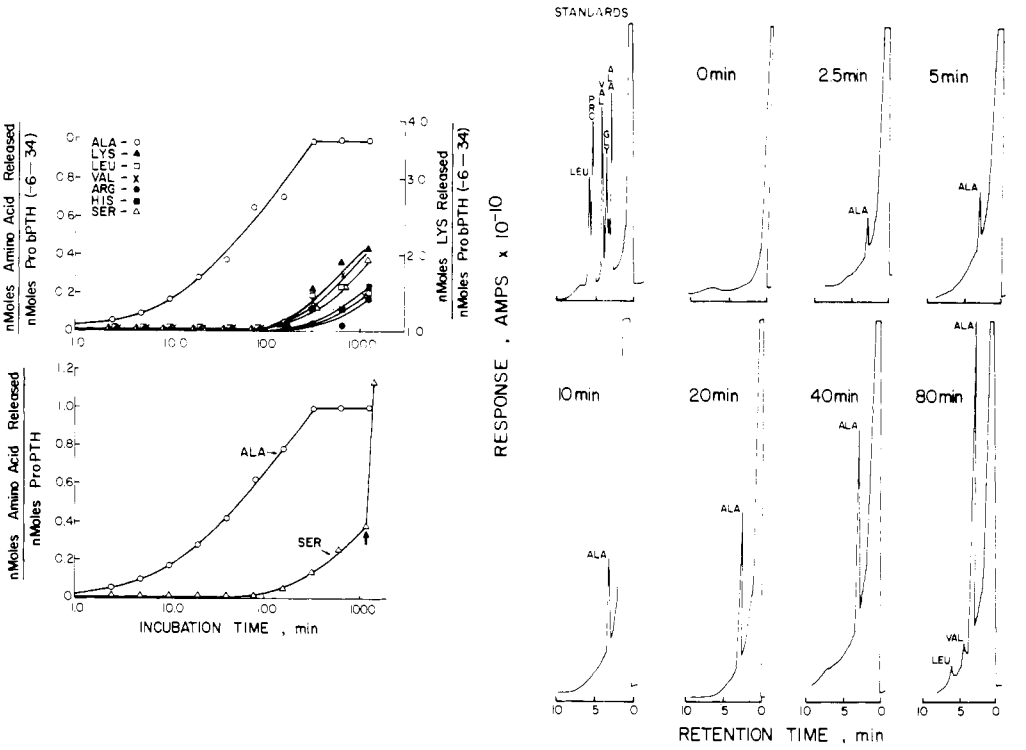

FIGURE 5: (Upper Left Panel): Rate of exposure of NH_2-terminal amino acids by the action of 0.1% trypsin (w/w) on 20 nmol of ProPTH-(−6-+34). NH_2-terminal amino acids were identified and quantitated as their [^{35}S]phenylthiohydantoin derivatives, as described in the text. Amounts (nmol) of end groups, estimated by this method, are expressed as a fraction of the amount (nmol) of ProPTH-(−6-+34) hydrolyzed; lysine, the NH_2-terminal residue of intact ProPTH-(−6-+34), is found at 1 mol per mol of peptide at zero time. (Lower Left Panel): Rate of exposure of alanine and serine by the action of trypsin on bovine ProPTH-(−6-+34). After incubation of 20 nmol of bovine ProPTH-(−6-+34) with 0.1% trypsin (w/w) for 22 h (as above), 20 nmol of human ProPTH-(−6-+34) was added (arrow), and the incubation was continued for another 160 min. Details of the incubation are given in the text. Amino acids were identified and quantitated as their [^{35}S]phenylthiohydantoin derivatives, as described in the text. (Right Panel): Gas–liquid chromatograms of the phenylthiohydantoin-amino acids obtained after one cycle of Edman degradation following timed digestions of ProPTH-(−6-+34) by 0.1% trypsin (w/w) for up to 80 min.

carboxypeptidase-B-like activity (operating together efficiently in a sequential manner). In contrast, the converting mechanism in cells synthesizing peptides with only NH_2-terminal extensions seems to require only an endopeptidase with trypsin-like specificity. This conclusion is based on the findings reported here for proparathyroid hormone, as well as on reported findings for proalbumin (Russell and Geller, 1975), in which the precursor extension at the NH_2 terminus is similar to that in the proparathyroid hormone. Similar conclusions may be pertinent for "big gastrin" (Gregory and Tracy, 1972, 1975). Alternatively, if the conjunction of trypsin-like and carboxypeptidase-B-like activities represents a general mechanism present in all cells involved in synthesis and secretion of polypeptides (perhaps bound in a spatially precise configuration in the cytoskeleton), then the presence of carboxypeptidase-B-like activity in close association with a trypsin-like endopeptidase may be found only when further studies of the fate of the precursor extension peptide itself, e.g., the proparathyroid hexapeptide, are made. If the basic residues at the site of endopeptidase cleavage are attached to the COOH terminus of the product polypeptide, as in the case of insulin, the subsequent action of an exopeptidase on the hormone is evident; with proparathyroid hormone, in which the basic residues at the site of initial endopeptidase cleavage are at the COOH terminus of the liberated precursor-specific peptide, exopeptidase activity on the hormone itself would not be detected.

Secondly, the studies reported here may, by analogy, suggest a chemical basis for the apparently efficient conversion by low concentrations of trypsin of other peptides such as "big, big gastrin" or "big ACTH," to their hormonal products (Yalow and Berson, 1973; Yalow and Wu, 1973; Gewirtz et al., 1974). In these cases, the precursor role of the larger peptides has not, as yet, been definitely established, and their sequences have not been elucidated, thereby precluding careful chemical documentation of the susceptibility of these larger peptides to specific conversion to hormonal forms by low concentrations of trypsin. The documentation presented here for proparathyroid hormone in a chemically defined system, however, lends indirect support to the thesis that "big, big gastrin" and "big ACTH" do represent larger molecular forms efficiently convertible by trypsin to authentic gastrin and ACTH, respectively. These detailed studies with proparathyroid hormone may, therefore, serve as a useful model for the study of conversion of precursor to product for a variety of polypeptide molecules.

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