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Synthesis of a Fragment of Parathyroid Hormone, bPTH-(28–48): An Inhibitor of Hormone Cleavage in Vivo†

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ABSTRACT: A 21-amino-acid peptide representing a continuous internal region of bovine parathyroid hormone, bPTH-(28–48), was synthesized by the solid-phase method. Within the sequence region 28–48 are located the principal peripheral cleavage sites of native parathyroid hormone in vivo. Therefore, this hormone fragment was selected for synthesis because of the likelihood that it would serve as an acceptable substrate for the enzymes that mediate cleavage of the native hormone. Purification and detailed chemical analysis of the products of synthesis were undertaken as part of the continuing effort to evaluate the quality of product resulting from solid-phase synthesis as different sequences are prepared. The desired peptide, bPTH-(28–48), was purified to homogeneity as assessed by several analytical criteria, including thin-layer electrophoresis, amino acid composition, Edman sequence analysis, and polyacrylamide gel isoelectric focusing. Approximately one-third of the total synthetic product represented peptides of lower molecular weight than the desired product. The lower-molecular-weight side products were separately isolated and later identified as COOH-terminal fragments of the desired product, namely, bPTH-(38–48) and bPTH-(39–48). Early chain termination appears to be the mechanism responsible for generating these fragments; the region 38–39 of the hormone presents synthetic difficulties that may be sequence dependent. The purified hormone fragment bPTH-(28–48) was found to possess the biological property that suggested its synthesis: the synthetic peptide proves to be an effective inhibitor of in vivo cleavage of native hormone when administered simultaneously with the native hormone. This synthetic peptide may therefore prove useful in assessment of the nature and physiological significance of peripheral metabolism of the hormone and the overall heterogeneity of circulating parathyroid hormone, issues that have been extensively studied in recent years.

Parathyroid hormone (bPTH),[1] an 84-amino-acid single-chain peptide, was shown to undergo metabolic conversion in vivo. The principal cleavage positions lie between amino acid residues 33 and 34 and residues 36 and 37 (Segre et al., 1974). The physiologic role, if any, of hormonal cleavage is unknown. However, since structure–activity studies reveal the minimum region necessary for biological activity to be the sequence 2–27 (Tregear et al., 1973),[2] biologically active fragments of PTH may be generated by the cleavage process, or may be involved in expression of PTH action on target organs. Alternatively, the peripheral cleavage of hormone may be principally catabolic, i.e., the pathway of hormone removal from blood and tissue fluids.

Accordingly, a 21-amino-acid sequence of the hormone containing the known sites of cleavage of native bPTH (Figure 1) was selected for synthesis in an attempt to create a hormone fragment that would serve as an alternate substrate for the enzymes that mediate cleavage and therefore competitively inhibit the cleavage of native hormone in vivo.

Synthesis of the hormone fragment bPTH-(28–48) was performed by the solid-phase technique (Merrifield, 1969). Purification and analysis of the products of synthesis revealed generation of a considerable amount of side products. These peptide fragments were obtained despite monitoring of each chain-elongating step in the synthetic process. Separate isolation and identification of the side products of solid-phase synthesis indicated a discrete region, at positions 38 and 39, of difficulty in the synthesis, where peptide chains terminate prematurely.

The desired synthetic peptide, bPTH-(28–48), was purified to homogeneity, as assessed by thin-layer electrophoresis,

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[1] The abbreviations used are: bPTH, bovine parathyroid hormone; *t*-Boc, *tert*-butyloxycarbonyl; DEAE, diethylaminoethyl.

[2] Specialized preparations of canine renal membranes have revealed some biological activity present in sequence regions shorter than 2–27; however, such activity has always been less than 1% that of native PTH.

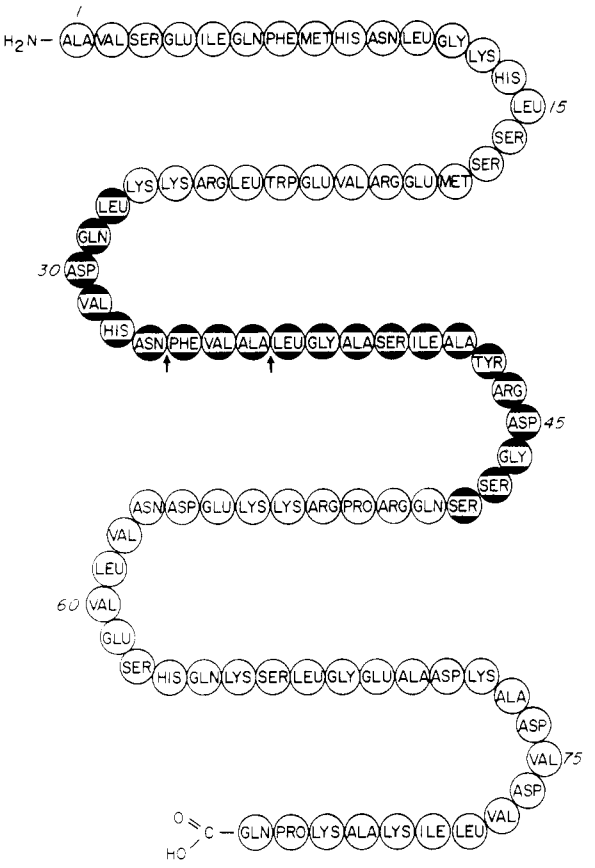

FIGURE 1: The amino acid sequence of bovine parathyroid hormone. Shaded residues depict the sequence of the synthetic hormone fragment bPTH-(28–48). Arrows indicate the principal sites of cleavage of intact hormone in vivo.

amino acid composition, Edman sequence analysis (Edman and Begg, 1967; Tregear, 1975) for detection of deletion-containing or error peptides, and polyacrylamide gel isoelectric focusing. When administered intravenously, the purified synthetic hormone fragment was found to act as an effective inhibitor of metabolic cleavage of intact PTH in vivo when the process was analyzed by methods previously described (Segre et al., 1974). Because the hormone fragment does not include the minimum structural requirements necessary for biological activity, it is devoid of agonist properties and therefore can be administered in large doses. This synthetic fragment may prove useful in investigating the anatomical locale, kinetics, and physiological importance of hormone cleavage in vivo.

Materials and Methods

Synthesis and Purification. A fragment of bovine parathyroid hormone representing the sequence region 28–48 was synthesized by a modification of the Merrifield solid-phase method (Merrifield, 1969; Rosenblatt et al., 1976). Synthesis was performed using a Beckman Model 990 Automated Synthesizer. Merrifield (polystyrene–1% divinylbenzene copolymer) resin, containing 0.75 mequiv of $ClCH_2$/g (Lab Systems, Inc.) served as the insoluble support.

The *tert*-butyloxycarbonyl (*t*-Boc) group was used to protect the α-amino group of each amino acid during coupling, except arginine, which was protected by the amyloxycarbonyl group. Side-function protection was afforded as follows: (a) the hydroxyl group of serine was protected as the *O*-benzyl ether; (b) the hydroxyl group of tyrosine as the *O*-2,6-dichlorobenzyl ether; (c) the carboxyl group of aspartic acid as the benzyl ester; and (d) the imidazole nitrogen of histidine and the guanidine function of arginine were protected by the *p*-toluenesulfonyl group (protected histidine was stored as the dicyclohexylamine salt, and desalted immediately before use). All amino acids were obtained from Beckman Instruments, except *tert*-butyloxycarbonyl-*O*-2,6-dichlorobenzyl-L-tyrosine, which was obtained from Bachem Chemicals.

Esterification of the first amino acid, serine, to the chloromethylated copolymer resin was performed by adding 4.05 mmol of triethylamine (Pierce) to a mixture of 4.5 mmol of *tert*-butyloxycarbonyl-*O*-benzyl-L-serine and 6.0 g (4.5 mequiv of $ClCH_2$) of copolymer resin in 25 mL of absolute ethanol, and refluxing at 80–85 °C for 24 h; 25% of the serine, based on weight analysis of the reacted copolymer resin, was esterified. The *t*-Boc-amino acid–resin complex was washed with ethanol (six times), water (six times), and methylene chloride (six times), and then allowed to swell for 2 h in methylene chloride (freshly distilled over potassium carbonate).

The α-amino group was deprotected by pretreating the peptide–copolymer resin complex (1.5 min, two times) with 30% trifluoroacetic acid (Pierce) in methylene chloride (v/v), followed by treatment for 30 min with the same reagent. The peptide–copolymer resin complex was then washed (six times) with methylene chloride. The resulting trifluoroacetate salt was neutralized by prewashing (1.5 min, two times) followed by treatment (10 min) with 10% triethylamine in methylene chloride (v/v), followed by washing with methylene chloride (four times). The peptide bond was formed by the addition of 4.5 mmol of *t*-Boc-amino acid and 4.5 mmol of dicyclohexylcarbodiimide in methylene chloride to the reaction vessel. After the mixture was stirred (1 h), the peptide–copolymer resin complex was washed to free it of the reagents of coupling with methylene chloride (six times). Asparagine and glutamine were incorporated by the "active ester" method. For these amino acids, 9 mmol of the *p*-nitrophenyl "active ester" in dimethylformamide was added and allowed to react overnight. Completeness of coupling was assessed qualitatively by the fluorescamine test (Felix and Jimenez, 1973). Double coupling was necessary to complete incorporation of isoleucine at position 41 and glutamine at position 29; triple coupling was necessary for histidine at position 32. Because asparagine "active ester" coupling at position 33 yielded a weak positive fluorescamine test despite five coupling attempts, the peptide–resin complex was acetylated by treatment with 2.5 mL of acetic anhydride and 0.75 mL of triethylamine in 25 mL of dimethylformamide (15 min, two times). The acetylated peptide–resin complex yielded a negative fluorescamine test.

The peptide was cleaved from the copolymer resin with simultaneous removal of the side-chain protecting groups using an excess of twice-distilled, anhydrous hydrogen fluoride at 0 °C for 1 h in the presence of 3.5 mL of distilled anisole/2.5 g of peptide–resin complex. Hydrogen fluoride was then removed by distillation under reduced pressure (oil vacuum pump). The peptide–resin mixture was washed with anhydrous ether to remove anisole. The peptide was then extracted by alternate washes of glacial acetic acid and water (four times each). The washes were combined and lyophilized to yield 1.39 g of crude peptide.

Crude peptide was purified initially by gel filtration on a Bio-Gel P-6 (Bio-Rad Laboratories) column (5.0 × 100 cm) and eluted with 1 M acetic acid. After lyophilization, the partially purified peptide was applied to a carboxymethylcellulose (CM-52, Whatman) ion-exchange column (1.2 × 15

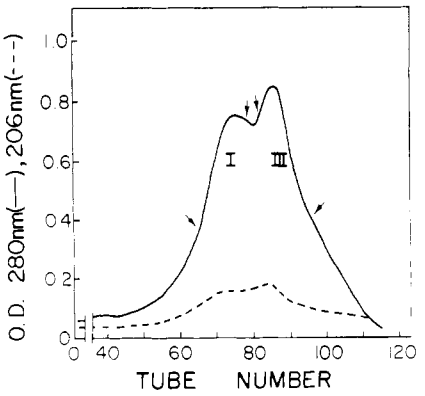

FIGURE 2: Elution profile of bPTH-(28-48) on a polyacrylamide gel (Bio-Gel P-6) column. Chromatography was performed at room temperature, each fraction contained 2.2 mL, and flow rate was 19 mL/h. The early-eluting peak I was taken for further purification by ion-exchange chromatography and represents the desired synthetic product. Peak II contained side products of the synthesis that were separately purified and identified.

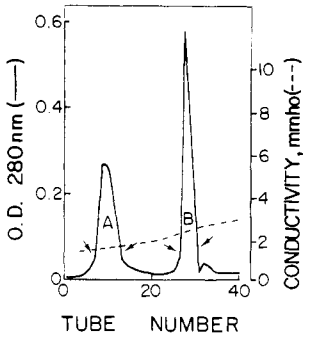

FIGURE 3: Elution profile of bPTH-(28-48) on a carboxymethylcellulose (CM-52) ion-exchange column employing a conductivity gradient of ammonium acetate. Chromatography was performed at room temperature; each fraction contained 2.7 mL and flow rate was 16 mL/h. Peak B contains the desired peptide. Peak A did not adhere to the column and contains low-molecular-weight side products.

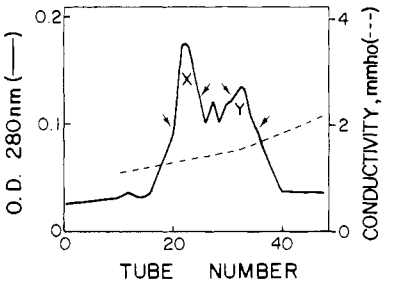

FIGURE 4: Elution profile of late-eluting synthetic side products (Bio-Gel P-6 peak II, Figure 2) on diethylaminoethylcellulose (DE-52) ion-exchange column employing a conductivity gradient. Chromatography was performed at room temperature; each fraction contained 2.7 mL and flow rate was 16 mL/h. Peaks X and Y contain COOH-terminal fragments bPTH-(38-48) and bPTH-(39-48), respectively.

cm). An LKB Ultragrad apparatus created a shallow-sloped conductivity gradient of ammonium acetate from 1.5 (pH 5.1) to 10 mmho (pH 6.2).

Later-eluting, lower-molecular-weight side products separated by gel filtration were separately applied to an identical carboxymethylcellulose ion-exchange column, to which they did not adhere. This material was then applied to an ion-exchange column of diethylaminoethylcellulose (DE-52, Whatman, 1.2 × 8 cm) and eluted using a conductivity gradient of ammonium bicarbonate from 1.0 (pH 8.9) to 10 mmho (pH 7.5).

Bioassay. The synthetic hormone fragment bPTH-(28-48) was assessed for agonist activity in vitro in a modification of the rat renal cortical adenylyl cyclase assay of Marcus and Aurbach (1969, 1971). The fragment was also assessed for antagonist activity in the same assay system by methods previously described for evaluating bPTH antagonists (Goltzman et al., 1975).

Effect of bPTH-(28-48) Administration on in Vivo Cleavage of ^{125}I-Labeled bPTH. In each experiment, native bPTH (126 ± 12 ng), labeled with ^{125}I (63 ± 12 × 10^7 cpm) by a modification of the method of Hunter and Greenwood (1962), was injected rapidly (less than 1 min) through an indwelling right external jugular-vein catheter into awake, male, Sprague-Dawley rats 250 ± 30 g (Charles River Breeding Labs.). Immediately before administration of labeled intact hormone, experimental animals were given 0.53 to 3.82 mg of bPTH-(28-48) dissolved in 250 μL of 0.15 M ammonium acetate-0.04 M urea, pH 4.6, containing 1% outdated blood-bank plasma as carrier. From time 0 to 12 min, experimental animals were infused with synthetic fragment at a rate of 0.13 to 0.89 mg/min in 700 μL of the same diluent. Control animals were subjected to the same protocol, except that diluent alone, without synthetic fragment, was administered. Blood samples, 300 μL, were collected in heparinized syringes at 4, 8, 12, 24, 48, and 96 min through an indwelling left internal carotid-artery cannula. All samples were doubly spun in a Brinkmann Model 3200 centrifuge, and the plasma was stored at −70 °C. Samples were gel filtered on columns (1.2 × 70 cm) of Bio-Gel P-100 (100-200 mesh), equilibrated, and eluted with 0.15 M ammonium acetate-1% plasma, pH 4.6, at 4 °C. To serve as markers for the elution position of intact hormone and salt, trace amounts of ^{131}I-labeled bovine hormone and Na^{131}I were added to each sample before gel filtration. Recovery of ^{125}I, after gel filtration and correction for ^{131}I, was determined for all samples and ranged from 83 to 91%.

The relative quantity of radioiodinated intact hormone and COOH-terminal fragments was calculated by planimetry of the gel-filtration profiles as previously described (Segre et al., 1974, 1976).

Results

Purification of the Synthetic Product. The chromatographic profile of 65 mg of crude synthetic peptide applied to the above-described gel-filtration column is depicted in Figure 2. Peak I contained 17 mg. When this material was further purified by carboxymethylcellulose ion-exchange chromatography, the chromatographic profile of Figure 3 resulted, and 5 mg of purified bPTH-(28-48), peak B, was obtained.

Ten milligrams of the later-eluting, lower-molecular-weight synthetic products represented by peak II of gel filtration (Figure 2) was obtained, which did not adhere to carboxymethylcellulose. This material was separately purified by diethylaminoethylcellulose (DEAE), yielding the chromatographic profile (optical density = 280 nm) of Figure 4. The column effluent was also monitored for UV absorption at 206 nm to detect any side products lacking 280-nm absorption; however, significant quantities of such material were not obtained.

Analysis of the Synthetic Products. The synthetic bPTH-(28-48) (carboxymethylcellulose-purification peak B) was demonstrated to be homogeneous by several criteria. Multiple amino acid analyses were performed after acid hy-

TABLE I: Amino Acid Content after Acid Hydrolysis of Synthetic bPTH-(28-48) and Side Products of the Synthesis, bPTH-(38-48) and bPTH-(39-48).[a]

Amino acid	Purified bPTH-(28-48)		Peak X bPTH-(38-48)		Peak Y bPTH-(39-48)	
	expected	obtained	expected	obtained	expected	obtained
His	1	1.0	0	<0.1	0	<0.1
Arg	1	1.0	1	1.2	1	1.0
Asp	3	3.2	1	1.4	1	1.1
Ser	3	2.8	3	2.9	3	2.6
Glu	1	1.1	0	<0.1	0	<0.1
Gly	2	2.1	2	1.7	1	1.2
Ala	3	3.1	2	2.0	2	2.0
Val	2	1.9	0	<0.1	0	<0.1
Ile	1	0.9	1	0.9	1	1.0
Leu	2	2.0	0	<0.1	0	<0.1
Tyr	1	1.0	1	1.0	1	1.1
Phe	1	1.0	0	<0.1	0	<0.1

[a] All values represent the average of three separate aliquots of the peptide after acid hydrolysis for 24 h, expressed as moles of amino acid per mole of peptide.

drolysis in 5.7 N HCl at 110 °C in an evacuated desiccator for 24 h in the presence of 1/2000 (v/v) mercaptoethanol, and amino acid analyses were carried out using a Beckman Model 121M Analyzer. Composition of the synthetic peptide conformed closely to that expected (Table I). Automated Edman sequence analysis was also performed, using a Beckman 890C Sequencer employing the single-coupling, double-cleavage method of Edman and Begg (1967) and other previously described methods (Niall, 1973). Manual Edman degradations were performed as previously described (Niall et al., 1969). Phenylthiohydantoin derivatives of amino acids were identified by thin-layer chromatography on silica-gel plates (Analtech) (Edman and Begg, 1967; Morgan and Henschen, 1969) and by gas-liquid chromatography using a two-column system (10% DC-560 and 1.5% AN-600) (Pisano and Bronzert, 1969). Accumulation of deletion-containing error peptides is readily detected as "preview" because of the amplification effect inherent in the Edman method (Tregear, 1975). Step 1 yielded >99% leucine with <1% preview of the glutamine at position 29. Step 2 yielded 97% glutamine with 2-3% preview of the aspartic acid at position 30. Preview remained at or below this level through cycle 7 of Edman degradation; beyond this point, severe extractive losses prevented further analysis. Hence, the purified synthetic peptide may contain no more than 3% contamination by deletional-error peptides.

The synthetic product appeared as a single band at pH 7.3 during analysis by polyacrylamide gel isoelectric focusing (gel concentration T = 5%, cross-linkage C = 3%; pH 3.5-9.5; ampholine concentration, 2.4% (v/v); LKB Instruments). Thin-layer electrophoresis was conducted on 100-μm cellulose-coated plates (Brinkmann Instruments) in a solvent system of pyridine-acetic acid-water (30:1:270, pH 6.5). The peptide was demonstrated to be homogeneous and had electrophoretic mobility relative to leucine and lysine of 1.27 and 0.59, respectively. Leucine and lysine standards were visualized with ninhydrin; the peptide was visualized with Pauly reagent.

The lower-molecular-weight peptide side products were also analyzed. Edman sequence analysis and amino acid compositional data were used to identify the peptides of peaks X and Y of DEAE-cellulose chromatography (Figure 4). Amino acid analyses are given in Table I. The principal NH_2-terminal sequence of peak X as determined by repetitive Edman degradation for four cycles was Gly-Ala-Ser-Ile. The principal sequence of peak Y from the NH_2 terminus was Ala-Ser-Ile-Ala. Thus, by combining compositional and sequence analysis, peaks X and Y could be identified as the two COOH-terminal fragments bPTH-(38-48), and bPTH-(39-48), respectively. No NH_2-terminal fragments representing complementary portions of the sequence, i.e., 28-37 and 28-38, were detected during gel filtration or any of the ion-exchange chromatographic procedures.

Biological Properties. bPTH-(28-48) demonstrated no agonist activity in vitro at all concentrations tested, to a maximum concentration of 1.35×10^{-4} M. The hormone fragment also failed to inhibit stimulation, by native hormone, of adenylyl cyclase activity in the in vitro assay when tested at multiple concentrations, to a maximum concentration ratio of bPTH-(28-48) to native bPTH of 500 to 1.

Figure 5 shows the disappearance of intact hormone and the appearance and disappearance of the iodinated fragments. The means of the results for the three sham-infused control rats and three rats to whom bPTH-(28-48) was administered are depicted. Infusion of synthetic hormone fragment bPTH-(28-48) effectively inhibited cleavage of intact hormone to the extent that the maximum concentration of fragment in the treated rats was less than 25% of that seen in the control rats. In addition, there was a small but definite prolongation (twofold) in the initial phase (0-25 min) of disappearance of intact hormone.

Discussion

This paper presents the synthesis and chemical evaluation of a fragment of parathyroid hormone, bPTH-(28-48), and establishes the principle that the fragment does significantly inhibit the cleavage of native parathyroid hormone that occurs in vivo after hormone is introduced into the circulation. Although our laboratory has considerable experience in the synthesis and purification of peptides representing the native sequence, fragments, and analogues of the NH_2-terminal sequence of the hormone, this mid region sequence had not been synthesized previously. Therefore, we anticipated that chemical problems different in nature from those previously seen might arise during the synthesis; accordingly, we undertook detailed chemical and analytical evaluation of all the products

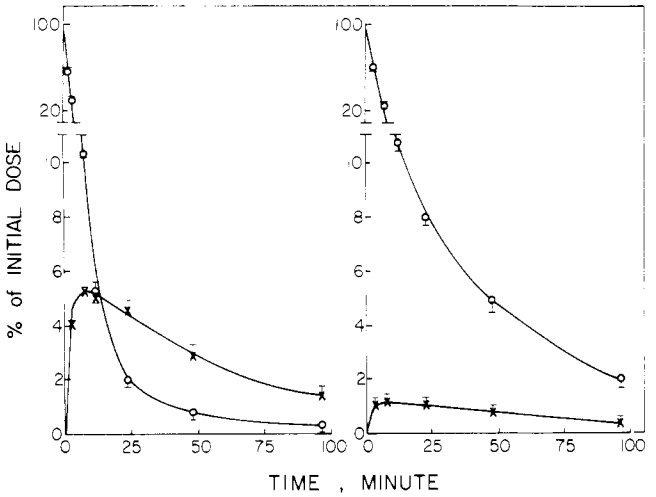

FIGURE 5: Cleavage chromatogram. Disappearance of intact bPTH labeled with ^{125}I (O) and the appearance and disappearance of the ^{125}I-labeled COOH-terminal fragments (X) are shown. A and B represent, respectively, the results in the sham-treated control rats and in the rats treated with bPTH-(28–48). Each value is the mean ± standard error of the mean of results obtained in three independent studies.

of the solid-phase synthesis, not merely the major, desired product, bPTH-(28–48).

The major synthetic product after purification not only yielded the expected theoretical amino acid composition but was found to be homogeneous in several analytical systems of high resolution, namely, thin-layer electrophoresis and polyacrylamide gel isoelectric focusing. Edman sequence analysis was performed to quantitate the presence of contamination by deletion-containing error peptides. Each cycle of Edman degradation quantitates the amino acids present at a given position in the peptides. Should a fraction of the synthetic product contain a deletional error, such an error would be detected by Edman sequence analysis as a "preview" of the amino acid expected at the next cycle of degradation (Tregear, 1975). Although extractive losses prevented analysis of more than the NH_2-terminal one-third of the synthetic peptide, the region examined was at least 97% pure.

A point of particular interest, however, was the finding that approximately one-third of the product of synthesis represented peptides of lower molecular weight than the desired bPTH-(28–48), a result not found during synthesis of the NH_2-terminal region of the hormone (Tregear, 1975). These side products were readily isolated in the same purification systems and identified by amino acid and Edman sequence analyses as COOH-terminal fragments of bPTH-(28–48): bPTH-(38–48) and bPTH-(39–48). The possibility that such fragments arise at the time of hydrogen fluoride cleavage of the peptide from the copolymer resin seems unlikely because complementary NH_2-terminal peptide fragments were not found. More likely, these side products result from premature termination of a population of peptide chains for physical or chemical reasons during synthesis (Erickson and Merrifield, 1976; Hancock et al., 1973).

Although each step of amino acid incorporation was monitored for completeness of addition by the fluorescamine test, the early chain termination in the sequence region 38–39, as expected, escaped detection. Fluorescamine testing can only detect one type of synthetic failure, namely, incomplete coupling of an amino acid to the free NH_2-terminal amino function of the growing peptide chain. Peptide chains having a particular length or conformation that makes them sterically inaccessible, or chains whose NH_2 terminus cannot be successfully deprotected, are not detected by fluorescamine monitoring. This type of premature chain termination has not been found to any great degree in previous syntheses of other regions of parathyroid hormone (Tregear, 1975) and thus appears to be sequence dependent. The discovery and identification of these peptide side products emphasize the need to purify and thoroughly chemically evaluate by multiple techniques a synthetic peptide, especially a hormone or an enzyme inhibitor, before it is used in a biological application, such as the one discussed below.

Synthetic bPTH-(28–48) appears to make possible an extremely useful means of studying the biological significance of cleavage of parathyroid hormone in vivo. Although parathyroid hormone is an 84-amino-acid peptide, all the structural requirements necessary for full biological potency in several assay systems reside within the NH_2-terminal one-third of the molecule (Potts et al., 1971; Tregear et al., 1973). Studies of the metabolism of intact PTH reveal that similar NH_2-terminal fragments, containing the structural requirements for biological activity, may be generated by cleavage of the hormone after its release from the parathyroid glands, and that the process is augmented by hypocalcemia (Canterbury et al., 1973, 1975). However, the site, nature, and physiological significance of such cleavage have yet to be elucidated. The cleavage process may simply reflect the first step in metabolic degradation and eventual clearance of the hormone. Alternatively, if biologically active fragments are produced in vivo by cleavage, and if cleavage occurs in proximity to membrane receptors in target tissues such as kidney and bone, the hormone fragments could constitute the predominant, if not the sole, mediator of all or of certain PTH actions; the intact hormone may not represent the active molecular species for expression of all or even any biological action of the hormone. Cleavage causing activation of the hormone might then represent a potentially important control point in the biological expression of one or more PTH actions, and perhaps an abnormally functioning control point in disorders of parathyroid regulation. Studies in our laboratory indicate that intact hormone need not be cleaved in vitro in order to simulate adenylyl cyclase activity in renal cortical membrane preparations (Goltzman et al., 1976), but the in vitro systems need not adequately reflect the order of events (cleavage, receptor binding, stimulation) or other details of specificity that operate in vivo. Thus, the deliberate alteration of hormone metabolism in vivo may still provide information critical to understanding the mode of action of PTH.

One approach to investigating the possible physiological or pathophysiological significance of the peripheral cleavage of hormone is to inhibit the cleavage process and then to search for any resulting alterations in biological response to parathyroid hormone in vivo. To inhibit the enzymes responsible for cleavage, we selected for synthesis a sequence region of the hormone molecule likely to be an acceptable substrate for the cleavage enzymes, but lacking inherent hormonal activity, so that the substrate could be administered in large quantity. A similar strategy was applied successfully to the synthesis of inhibitors of renin-mediated cleavage of angiotensinogen to angiotensin I (Skeggs et al., 1964, 1967; Poulsen et al., 1973). bPTH-(28–48) lacks the minimum sequence region necessary for biological activity, amino acids 2–27, but includes all the known cleavage positions.

We have demonstrated that when administered intravenously bPTH-(28–48) inhibits cleavage of radioactively labeled native hormone: formation of hormone fragments is

decreased by 75%, and the half-life of circulating intact hormone is prolonged twofold. Further studies are necessary to determine whether hormone cleavage can be blocked entirely by administration of a sufficient dose of inhibitor for some appropriate time before and during injection of intact hormone. Quantities of bPTH-(28–48) presently available and ultimately needed for detailed studies of cleavage inhibition necessitate examination of related issues before selecting ideal conditions for blocking cleavage of native hormone. We now plan to undertake systematic studies to determine: (a) the half-life and distribution of the synthetic peptide in vivo; (b) the optimal delivery system for bPTH-(28–48), including the time course of onset and duration of effectiveness of bPTH-(28–48) on survival of and biological response to native hormone in vivo; and (c) the minimum and maximum effective ratio of bPTH-(28–48) to native hormone.

Along these lines, preliminary studies with ^{131}I-labeled bPTH-(28–48) demonstrate rapid disappearance of the synthetic peptide from the circulation. Much of this disappearance presumably reflects renal excretion and/or nonspecific destruction of the fragment, rather than delivery to cleavage sites. It should be emphasized that in these preliminary experiments a very large molar excess (several thousandfold) of cleavage-inhibiting peptide to intact native labeled hormone was used. However, this large dose of bPTH-(28–48), for the reasons outlined above, does not necessarily reflect a low-binding affinity of the unmodified sequence for the cleavage-mediating enzyme. If it is found that indeed very high molar ratios of bPTH-(28–48) to native hormone are required to block cleavage, further studies might include the design and synthesis of analogues of bPTH-(28–48) bearing structural modifications that prolong its survival in blood, or analogues such as those containing D-amino acids at positions 33 and 34 which might prolong survival at the site of enzymatic cleavage and thereby enhance inhibitory properties. Ultimately, the presently reported fragment, bPTH-(28–48), or analogues of this sequence should prove valuable in furthering our understanding of the metabolism and mode and control of action of parathyroid hormone, as well as the problem of heterogeneity of circulating parathyroid hormone (Canterbury and Reiss, 1972; Goldsmith et al., 1973; Segre et al., 1972; Silverman and Yalow, 1973).

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