The Amino Acid Sequence of Porcine Parathyroid Hormone†

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ABSTRACT: The complete amino acid sequence of porcine parathyroid hormone (PPTH) has been determined using both the automated and manual methods of the sequential Edman degradation. Overlapping peptides were generated by tryptic digestion of the maleylated hormone, and by cleavage at glutamic acid residues using staphylococcal protease. Porcine parathyroid hormone is similar in sequence to bovine parathyroid hormone, differing in only 7 of the 84 sequence positions. The amino terminal sequence of PPTH is also highly homologous with the amino terminal sequence of human parathyroid hormone. The results permit certain deductions about structure–activity relations among the porcine, bovine, and human hormones.

Studies of parathyroid hormone from several species have established a common hormonal role in the prevention of hypocalcemia and the maintenance of normal calcium homeostasis by acting directly on bone to increase the rate of calcium resorption, and by acting both directly and indirectly in the kidney and gastrointestinal tract to elevate blood calcium levels (Potts and Deftos, 1973; Parsons and Potts, 1972). The isolation and characterization studies of parathyroid hormone from three species have suggested that the similarity in biological action of bovine, porcine, and human parathyroid hormones may reflect similarities in their primary structures, and there has thus been considerable interest in the detailed study of the comparative amino acid sequences of these hormones.

The chemistry of bovine parathyroid hormone (BPTH)[1] has been extensively studied, and the complete amino acid sequence of the major isohormone of BPTH has been determined (Brewer and Ronan, 1970; Niall *et al.*, 1970). It has been further established that a fragment containing the amino terminal 29 residues of BPTH is sufficient for expression of the biological activity of the hormone (Keutmann *et al.*, 1972). Additional studies using synthetic peptides have further defined the precise requirements of sequence and chain length necessary for full expression of the *in vivo* and *in vitro* biological activity of the hormone (Tregear *et al.*, 1973).

Porcine parathyroid hormone has been isolated in pure form by Woodhead *et al.* (1971), and a preliminary report of its amino terminal sequence has appeared (O'Riordan *et al.*, 1971). Recently, human parathyroid hormone has been isolated (Keutmann *et al.*, 1974), and two conflicting studies of its amino terminal sequence have been published (Brewer *et al.*, 1972; Jacobs *et al.*, 1973; Niall *et al.*, 1974).

In this paper we present the methods and results that have allowed the elucidation of the complete amino acid sequence of porcine parathyroid hormone, and discuss conclusions of interest with regard to comparative sequence and structure–activity considerations in the parathyroid hormones.

Materials and Methods

Isolation. The PPTH used in these studies was isolated from porcine parathyroid glands by phenol extraction, trichloroacetic acid precipitation, gel filtration on Sephadex G-100, and ion exchange chromatography on carboxymethylcellulose, as described (Woodhead *et al.*, 1971). The purified hormone was homogeneous by the criteria of disc gel electrophoresis, thin-layer chromatography (tlc), and PhNCS end group determination.

Amino Acid Analysis. Samples for amino acid analysis were hydrolyzed *in vacuo* in 1.0 ml of 5.7 N HCl, at 110°, for 24 hr with addition of 2-mercaptoethanol (1:2000) to the HCl prior to hydrolysis to protect methionine residues (Keutmann and Potts, 1969). Analyses were performed on a Beckman Model 121 Amino Acid Analyzer.

Edman Degradations. Automated Edman degradations were performed in a Beckman Model 890 sequencer. The single coupling, double cleavage program of Edman and Begg (1967) was used with slight modifications to adapt to our instrument. Sequencer reagents and solvents were obtained from Beckman Instruments (Spinco Div., Palo Alto, Calif.); 1,4-butanedithiol (1:20,000) was added to the 1-chlorobutane to protect the labile phenylthiohydantoins of serine and threonine.

Manual Edman degradations were performed by a modification of the three-stage method of Edman (Edman, 1960; R. T. Sauer and H. D. Niall, in preparation). Samples (5–200 nM) were lyophilized in 5-ml centrifuge tubes prior to degradation. Coupling was carried out using 5 μl of PhNCS and 100 μl of 0.4 M dimethylallylamine buffer in propanol–water (60:40, v/v) adjusted to pH 9.7 with trifluoroacetic acid. The coupling reaction was carried out under nitrogen, for 20 min, at 55°. Following coupling, a single 0.2-ml benzene extraction was performed, and the organic phase discarded. The aqueous phase, containing the PTC–peptide, was then dried with nitrogen at room temperature, and the reaction tube placed under vacuum (less than 50 μ) for 10 min to remove trace amounts of water.

Cleavage was performed using 50 μl of F_3CCOOH, and reaction was carried out under nitrogen, for 5 min, at 55°.

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[1] Abbreviations used are: Tos-PheCH_2Cl, L-1-tosylamido-2-phenylethyl chloromethyl ketone; PPTH, porcine parathyroid hormone; BPTH, bovine parathyroid hormone; HPTH, human parathyroid hormone; PTC, phenylthiocarbamyl; PhNCS, phenyl isothiocyanate.

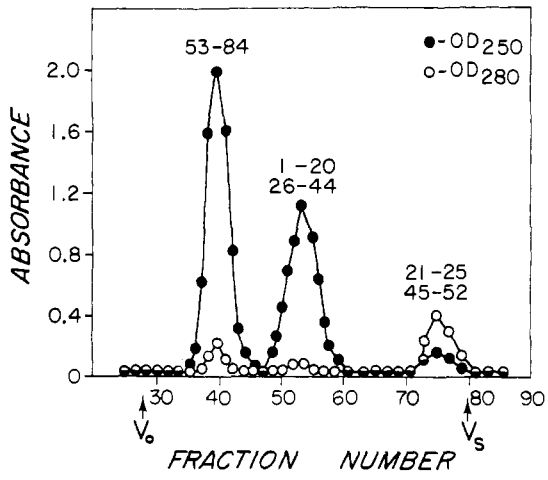

FIGURE 1: Sephadex G-50 column chromatography of a tryptic digest of maleylated porcine parathyroid hormone (Methods). Numbers above the peaks represent the residue assignments of the peptides found in each peak (Results). The strong absorbance at 250 nm in peaks 1 and 2 results from the absorption by the maleyl groups in these peptides.

Following cleavage, the F_3CCOOH was evaporated quickly with nitrogen, and 0.2 ml of 1-chlorobutane was added to the reaction tube to extract the thiazolinone amino acid derivatives. The 1-chlorobutane was then aspirated, transferred to another tube, and evaporated with nitrogen. Conversion of the thiazolinone derivatives to the phenylthiohydantoins was performed in 0.2 ml of 1 N HCl, for 10 min, at 80° (Ilse and Edman, 1963). An ethyl acetate extraction (2 × 0.8 ml) was then performed to extract the phenylthiohydantoins except for those of Arg, His, and $CysSO_3H$.

Prior to the next cycle of degradation, the residual peptide in the reaction tube was placed under vacuum for 10 min to remove traces of F_3CCOOH. A single cycle of degradation routinely took from 45 to 55 min.

Identification of Amino Acid Phenylthiohydantoins. The ethyl acetate soluble phenylthiohydantoins were identified and quantitated by gas–liquid chromatography (Pisano and Bronzert, 1969) using a Beckman Model 45 gas chromatograph. The phenylthiohydantoins of alanine, dehydroserine, glycine, valine, and proline were identified by chromatography at 180° on a 10% DC-560 support. The phenylthiohydantoins of leucine and isoleucine coeluted in this support, but were resolved on 1.5% AN-600 resin at 135°. The phenylthiohydantoins of aspartic acid, methionine, glutamic acid, and phenylalanine were identified as their trimethylsilyl derivatives by chromatography on the 10% DC-560 support at 220°. The phenylthiohydantoins of asparagine, glutamine, lysine, tyrosine, and tryptophan were identified on 2% OV-25 support at 250°.

All identifications were based upon a fourfold or greater rise in yield of the particular residue above the background level of amino acid phenylthiohydantoins at that stage of the degradation. Asynchrony (overlap) in the degradations did not at any point exceed 25% of the yield of the in-step component.

All glc columns were 2 mm ID × 4 ft U-shaped columns and contained from 3 to 3.5 ft of packing. Nitrogen was used as the carrier; gas and flow rates were 55–60 cm^3/min for the DC-560 and OV-25 columns, and 25 cc/min for the AN-600 column. Inlet temperature was 260° and detector temperature was 320°. Detection was by hydrogen flame ionization.

ε-Maleyllysine phenylthiohydantoin was synthesized essentially according to Edman (1970). α-PTC-ε-maleyllysine was prepared by reaction of DL-lysine with an equimolar amount of PhNCS for 2 hr at 25°, in 50% pyridine–water (v/v). The pH was continuously monitored and maintained at pH 6.5. Following completion of the reaction the buffer was evaporated, and the ε-aminolysine phenylthiohydantoin was formed, by incubation in 1 N HCl, 60°, for 1 hr. ε-Maleyllysine phenylthiohydantoin was formed by reaction with a 1.5 molar excess of solid maleic anhydride, 25°, 30 min in 0.4 M dimethylallylamine in propanol–water (60:40, v/v), pH 9.5. This derivative was recrystallized from ethanol–water and ran as a single spot (R_F 0.11) in the H_1 tlc system of Edman (1970) using 250-μ silica gel plates (Analtech, Newark, Del.). Degradation of peptides with maleyllysine in known positions yielded a phenylthiohydantoin that ran identically with the synthesized product. The recovery of ε-maleyllysine phenylthiohydantoin from degradations of maleylated peptides was generally low, as judged by comparative uv intensity on tlc. This was presumably due to partial demaleylation and regeneration of ε-aminolysine phenylthiohydantoin during the acidic conversion stage of the Edman degradation.

Maleylation and Tryptic Digestion of PPTH. PPTH (10 mg) was treated with a 50-fold excess (over total amino groups) of solid maleic anhydride. The reaction was performed in 0.2 M $Na_2B_4O_7$ buffer at 25° for 30 min. The pH was main-

TABLE I: Automated Amino Terminal Degradation of Porcine Parathyroid Hormone.

Cycle	Residue	Yield[a]	Cycle	Residue	Yield[a]
1	Ser	396.1	26	Lys	65.2
2	Val	420.4	27	Lys	69.9
3	Ser	231.6	28	Leu	115.0
4	Glu	365.1	29	Gln	56.4
5	Ile	343.5	30	Asp	65.1
6	Gln	321.9	31	Val	83.3
7	Leu	338.7	32	His	*b*
8	Met	318.0	33	Asn	48.4
9	His	*b*	34	Phe	61.5
10	Asn	286.2	35	Val	66.5
11	Leu	275.2	36	Ala	61.5
12	Gly	215.3	37	Leu	59.7
13	Lys	159.0	38	Gly	44.0
14	His	*b*	39	Ala	52.1
15	Leu	187.5	40	Ser	18.9
16	Ser	63.8	41	Ile	44.7
17	Ser	103.4	42	Val	49.0
18	Leu	149.3	43	His	*b*
19	Glu	120.6	44	Arg	*c*
20	Arg	*c*	45	Asp	38.1
21	Val	159.7	46	Gly	16.5
22	Glu	116.4	47	Gly	28.7
23	Trp	14.6	48		
24	Leu	127.1	49	Gln	9.9
25	Arg	*c*	50		

[a] The yields (nmoles) for the major amino acid phenylthiohydantoins identified at each cycle were determined by gas–liquid chromatography, and were not corrected for either overlap or background. [b] The phenylthiohydantoin derivative of histidine was identified by the Pauly reaction. [c] The phenylthiohydantoin derivative of arginine was identified by the phenanthrenequinone reaction.

TABLE II: Amino Acid Analysis of 24-Hr Hydrolysates of PPTH and of Pooled Fractions from G-50 Sephadex Chromatography of a Tryptic Digest of Maleylated PPTH (see Figure 1).[a]

	Maleylated Tryptic Pools						Native PPTH 1–84	
	Pool 1 53–84		Pool 2 1–20, 26–44		Pool 3 21–25, 45–52			
Aspartic acid	4.03	4	2.92	3	1.04	1	8.05	8
Threonine	0.00	0	0.00	0	0.00	0	0.00	0
Serine	2.07	2	4.28	5[b]	0.98	1	7.15	8[b]
Glutamic acid	4.78	5	3.98	4	1.96	2	11.25	11
Proline	0.94	1	0.04	0	0.97	1	1.95	2
Glycine	1.15	1	1.98	2	1.89	2	4.90	5
Alanine	3.72	4	1.87	2	0.00	0	6.05	6
Valine	3.70	4	3.87	4	1.00	1	8.50	9[c]
Methionine	0.00	0	0.78	1	0.00	0	0.80	1
Isoleucine	0.95	1	1.44	2[c]	0.00	0	2.50	3[c]
Leucine	3.13	3	5.72	6	1.07	1	10.00	10
Tyrosine	0.00	0	0.00	0	0.00	0	0.00	0
Phenylalanine	0.00	0	1.07	1	0.00	0	1.00	1
Tryptophan	0.00	0	0.00	0	0.00	1[d]	0.00	1[d]
Lysine	5.95	6	2.91	3	0.00	0	8.95	9
Histidine	1.02	1	4.20	4	0.00	0	4.85	5
Arginine	0.00	0	2.09	2	2.83	3	5.20	5
		32		39		13		84

[a] Values expressed as moles of amino acid per mole of PPTH, using best fit of all stable residues for normalization. [b] Serine value assumed after correction for losses during hydrolysis. [c] Values of valine and isoleucine corrected for incomplete hydrolysis after 24 hr. [d] One mole of tryptophan assumed on basis of spectrum.

tained at 9.0 by addition of 1 N NaOH. Following completion of the maleylation, the maleylated PPTH was separated from unreacted reagents and salt by gel filtration on a 2.0 × 45 cm Bio-Gel P-2 (100–200 mesh) column, equilibrated with 0.2 M NH_4HCO_3 (pH 8.2). The column effluent was monitored by OD_{250} and OD_{280} and the peak eluting at the void volume was pooled and lyophilized.

The maleylated PPTH was digested with TosPheCH_2Cl trypsin (Worthington Biochemicals) for 2 hr, at 37°, in 0.2 M trimethylamine–acetate buffer (pH 8.2), using an enzyme–substrate ratio of 1:100. The digest was then directly applied to a 1.2 × 140 cm G-50 (superfine) column equilibrated with 0.2 M NH_4HCO_3 (pH 8.2); 2.2-ml fractions were collected and monitored by OD_{250} and OD_{280}. The elution profile is shown in Figure 1. The three major peaks were pooled and lyophilized.

Peptides were demaleylated by incubation in 1 N formic acid, at 80°, for 45 min.

Digestion of PPTH with Staphylococcal Protease. Staphylococcal protease was kindly provided by Dr. G. Drapeau, Department of Microbiology, University of Montreal, Montreal, Canada. PPTH (4.0 mg) was treated with staphylococcal protease for 24 hr, at 37°, in 0.05 M NH_4OAc buffer (pH 4.0), using an enzyme–substrate ratio of 1:25 (mol/mol). The digest was then directly applied to a 1.2 × 140 cm G-50 (superfine) column equilibrated with 20% acetic acid. 2.0-ml fractions were collected and monitored by OD_{280}; 25-μl aliquots were taken from each tube and treated with fluorescamine (Udenfriend *et al.*, 1972) using 1.5 ml of 0.1 M $Na_2B_4O_7$ buffer (pH 8.6) and 0.5 ml of fluorescamine–acetone (5 mg/100 ml). Fluorescence was read in an American Instrument Company Fluoro-Microphotometer. Four peaks were detected (Figure 2), pooled, diluted ×4 with distilled water, and lyophilized.

Results

Amino Terminal Sequence (Residues 1–47). PPTH (5.7 mg) was degraded using the automated sequenator method. Continuous identification of the major phenylthiohydantoin, above background and overlap levels, was possible through cycle 47, thus establishing the sequence of this region. The yields of the major amino acid phenylthiohydantoin identified at each cycle of this degradation are presented in Table I.

Maleylated Tryptic Peptides. Sephadex G-50 fractionation of the tryptic digest of maleylated PPTH resulted in the resolution of three peaks (Figure 1). Aliquots of each pool were taken for amino acid analysis and sequence determination.

Pool I contained 32 amino acids by composition (Table II) and maleyllysine was detected as the amino terminal residue.

Pool II gave a single end group, also maleyllysine, and contained 39 residues by composition. Following demaleylation of the pool, however, two end groups, lysine and serine, were observed, indicating the presence of two peptides. The compositional data (Table II), taken with the observed end groups, and the knowledge of the amino terminal sequence of PPTH, clearly indicated that pool II was a mixture of two peptides representing residues 1–20 and 26–44 of the native hormone.

Pool III was also found to be heterogeneous by sequence analysis, containing two peptides, with a total composition of 13 amino acids. (Table II).

Sequence Residues 45–52. Manual Edman degradation of the pool III peptide mixture revealed two amino acid phenylthiohydantoins at each of the first five cycles of degradation, but a single residue at cycles 6, 7, and 8 (Table III). The presence of the single tryptophan residue of PPTH at cycle three of this degradation indicated that one of the sequences represented residues 21–25 of native PPTH (MT 3b in Table III), and thus the results of the mixture analysis could be

TABLE III: Manual Edman Mixture Analysis of Peak 3 from G-50 Chromatography of Tryptic Digest of Maleylated Porcine Parathyroid Hormone (Figure 1).

	Cycle							
	1	2	3	4	5	6	7	8
MT 3a	Asp -	Gly -	Gly -	Ser -	Gln -	Arg -	Pro -	Arg - COOH
Yield[a]	104.3	91.6	94.3	51.2	48.3	*b*	47.3	50.1[c]
MT 3b	Val -	Glu -	Trp -	Leu -	Arg			
Yield[a]	115.9	90.4	80.6	41.3	*b*			

[a] The yields of the major amino acid phenylthiohydantoins identified at each cycle were determined by glc, except where noted, and are not corrected for background or overlap. [b] The phenylthiohydantoin derivative of arginine was identified by the phenanthrenequinone reaction. [c] This yield of arginine was determined by amino acid analysis (without hydrolysis) following seven cycles of Edman degradation.

easily interpreted to give the unknown sequence (MT 3a in Table III). This sequence overlapped the amino terminal sequence of PPTH at residues 45–47, and therefore was assigned as residues 45–52 of PPTH.

Sequence Residues 53–75. The composition of the 32 amino acid peptide recovered in pool I (Figure 1), when taken with the sequence of the amino terminal 52 residues of PPTH, accounted for all the remaining residues of the 84 amino acids of PPTH. The amino terminal residue, lysine, of this peptide (designated MT 1) was thus assigned as residue 53 of PPTH.

MT-1 (0.15 μM) was sequenced by the manual Edman procedure. Clear residue assignments were made for the first 23 cycles of this degradation. The yields of the amino acid phenylthiohydantoins identified at each cycle of this degradation are presented in Table IV. This sequence was assigned as residues 53–75 of the porcine hormone.

Cleavage of PPTH with Staphylococcal Protease. PPTH was cleaved with staphylococcal protease under conditions leading to specific cleavage at glutamyl residues (Houmard and Drapeau, 1972). Following cleavage, four peaks were fractionated by gel filtration on Sephadex G-50 (Figure 2). Peaks 1 and 4 were homogeneous by end group determination, while peaks 2 and 3 contained nonstoichiometric mixtures of 3–4 peptides each.

Sequence Residues 70–84. The peptide isolated in peak 1 (Figure 2) was designated SP-1, and 0.035 μM was sequenced by the manual Edman method. Amino acid phenylthiohydantoins were identified at the first 14 cycles of degradation, and glutamine was identified at the 15th and carboxyl terminal residue by its detection by amino acid analysis, without hydrolysis, following the 14th cycle of degradation. The amino acid phenylthiohydantoins identified at each cycle of this degradation and their yields are listed in Table V. The

TABLE IV: Manual Edman Degradation of Peak 1 (MT-1) from G-50 Chromatography of Tryptic Digest of Maleylated Porcine Parathyroid Hormone (Figure 1).

Cycle	Residue	Yield[a] (nM)
1	Lys	*b*
2	Lys	*b*
3	Glu	97.2
4	Asp	65.7
5	Asn	105.6
6	Val	138.4
7	Leu	107.9
8	Val	106.3
9	Glu	48.9
10	Ser	28.0
11	His	*c*
12	Gln	31.4
13	Lys	*b*
14	Ser	8.7
15	Leu	26.3
16	Gly	17.4
17	Glu	6.2
18	Ala	15.5
19	Asp	9.1
20	Lys	*b*
21	Ala	6.3
22	Ala	6.8
23	Val	5.2
24		
25		

[a] The yields of the major amino acid phenylthiohydantoins identified at each cycle were determined by glc, and are not corrected for background or overlap. [b] Lysine was identified as ϵ-maleyllysine phenylthiohydantoin in the H_1 tlc system of Edman. [c] The phenylthiohydantoin derivative of histidine was identified by the Pauly reaction.

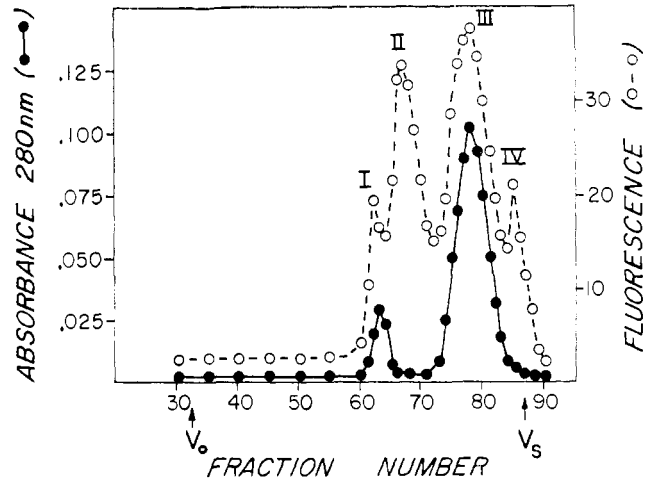

FIGURE 2: Sephadex G-50 column chromatography of native PPTH digested with staphylococcal protease under conditions specific for cleavage at glutamyl residues (Methods). Peak 1, which was used for sequence analysis of residues 70–84, was pooled on the ascending portion of the peak to prevent contamination with peak 2 components.

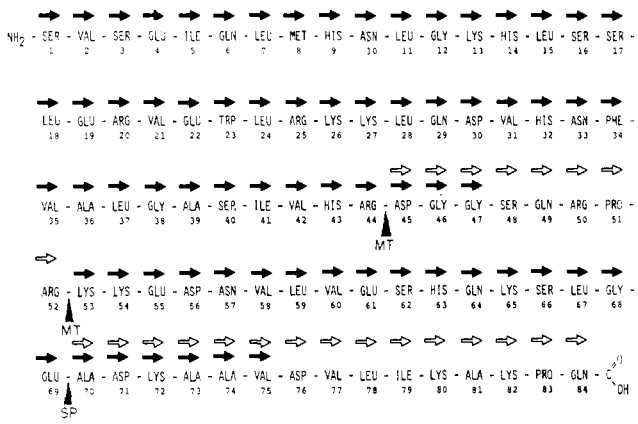

FIGURE 3: The amino acid sequence of porcine parathyroid hormone. The solid and open horizontal arrows are schematic representations of the Edman degradations used to determine the sequence (Results). Cleavages of native PPTH with staphylococcal protease (SP) and maleylated PPTH with trypsin (MT) which were important in the sequence determination are indicated by the solid vertical arrows.

TABLE V: Manual Edman Degradation of Peak 1 (SP-1) from G-50 Chromatography of Staphylococcal Protease Digest of Porcine Parathyroid Hormone (Figure 2).

Cycle	Residue	Yield[a] (nM)
1	Ala	35.0
2	Asp	20.6
3	Lys	*b*
4	Ala	20.9
5	Ala	19.4
6	Val	15.3
7	Asp	10.9
8	Val	12.2
9	Leu	9.1
10	Ile	7.1
11	Lys	*b*
12	Ala	6.4
13	Lys	*b*
14	Pro	4.2
15	Gln	5.6[c]

[a] The yields of the major amino acid phenylthiohydantoins identified at each cycle were determined by glc, and are not corrected for background or overlap. [b] Lysine was identified as ϵ-maleyllysine phenylthiohydantoin in the H_1 tlc system of Edman. [c] This yield of glutamine was determined by amino acid analysis (without hydrolysis), following 14 cycles of Edman degradation.

sequence of SP-1 overlaps the PPTH sequence from residues 70–75 (Figure 3), and thus comprises residues 70–84 of porcine parathyroid hormone, completing the sequence determination.

The complete sequence of PPTH is shown in Figure 3 together with a schematic representation of the four degradations and the specific cleavage sites used to elucidate the sequence.

Discussion

The completion of the amino acid sequence of porcine parathyroid hormone permits a comparison of this sequence with that determined for bovine parathyroid hormone and with the two sequences proposed for the amino terminal region of human parathyroid hormone (Figure 4).

PPTH and BPTH are identical at 77 of the 84 sequence positions. However, three of the seven point mutations which do occur are found within the amino terminal 34 residues, a region known to include the biologically active fragment of parathyroid hormone. Since both the bovine and porcine hormones are active in multiple bioassay systems employing several mammalian and avian species (for a recent review, see Parsons and Potts (1972)), it is apparent that some variation in the "active fragment" is allowed without compromise of hormonal function.

One of the differences between the porcine and bovine molecules involves the replacement of Met[18] in BPTH with Leu[18] in PPTH. This finding is of interest since it has been shown that mild oxidation of the two methionines in BPTH leads to a complete loss of biological activity (Tashjian *et al.*, 1964; Rasmussen and Craig, 1962). Woodhead *et al.* (1972) have likewise observed that oxidation results in inactivation of PPTH. This seems to imply that the single methionine residue at position 8 of PPTH is essential for activity. However, the functional acceptability of the Leu[18]-Met[18] shift between PPTH and BPTH may indicate that the loss of activity following oxidation of Met[8] in PPTH results from an adverse effect of the methionine sulfoxide residue, rather than an absolute necessity for the methionyl residue. Substitution of either leucine or norleucine at position 8 in synthetic PPTH should prove a valuable test of this hypothesis.

Of the four sequence differences between BPTH and PPTH that occur exterior to the "active fragment," three of the mutations are located within the six-residue sequence 42–47,

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Porcine	Ser	-	Val	-	Ser	-	Glu	-	Ile	-	Gln	-	Leu	-	Met	-	His	-	Asn	-	Leu
Bovine	Ala	.	.	.	.	.	.	.	.	.	Phe	.	.	.	.	.	.	.	.	.	.
Human[a]	Ser	.	.	.	.	.	.	.	.	.	Leu	.	.	.	.	.	.	.	.	.	.
Human[b]	Ser	.	.	.	.	.	.	.	.	.	Leu	.	.	.	.	.	.	.	.	.	.

	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Porcine	Gly	-	Leu	-	Arg	-	Lys	-	Leu	-	Gln	-	Asp	-	Val	-	His	-	Asn	-	Phe
Bovine	.	.	.	.	.	.	Leu	.	.	.	Asp	.	.	.	.	.	.	.	.	.	Ala
Human[a]	.	.	.	.	.	.	Leu	.	.	.	Asp	.	.	.	.	.	.	.	.	.	.
Human[b]	Gln	.	.	.	.	.	Lys	.	.	.	Leu	.	.	.	.	.	.	.	.	.	.

	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63
Porcine	Val	-	Ser	-	Leu	-	Gly	-	Glu	-	Ala	-	Asp	-	Lys	-	Ala	-	Ala	-	Val
Bovine	.	.	.	.	Ser	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84
Porcine	Asp	-	Val	-	Leu	-	Ile	-	Lys	-	Ala	-	Lys	-	Pro	-	Gln				
Bovine	.	.	.	.	.	.	.	.	.	.	Asp	.	.	.	.	.	.				

FIGURE 4: A comparison of the sequence of porcine parathyroid hormone with the published sequences of bovine and human parathyroid hormone. See text for a discussion of the observed differences: (a) human sequence as determined in our laboratory (Jacobs *et al.*, 1973; Niall *et al.*, 1974); (b) human sequence as determined by Brewer *et al.* (1972).

while in the carboxyl terminal 37 residues there is only a single change, at residue 74. Since the carboxyl terminal region of the hormone has not been implicated in hormonal function at the receptor level (Potts *et al.*, 1971), the preservation of such a high degree of structural identity in the COOH-terminal region is rather surprising.

In this regard it will be of extreme interest to investigate the carboxyl terminal sequence of human parathyroid hormone. If the same high degree of homology is observed in the carboxyl terminal sequence of HPTH, it will be tempting to speculate that this region plays some important role in hormone metabolism, possibly in the biosynthesis, storage, intracellular transport, or secretion of the hormone.

It will be of similar interest to compare the sequences comprising residues 42–47 of PPTH, BPTH, and HPTH to see if the high incidence of variation (50%) observed in this region between PPTH and BPTH also exists among the three hormones.

The active region of PPTH is highly homologous with the amino terminal sequence proposed by our group for HPTH (Niall *et al.*, 1974). These two sequences differ at only two of the first 37 positions. Brewer *et al.* (1972) have proposed a different structure for the amino terminal sequence of HPTH which results in differences from PPTH at a total of five positions within the amino terminal 30 residues.

Although agreement on the correct amino terminal sequence of HPTH has not yet been reached, a comparison of the active regions of PPTH, BPTH, and the two sequences reported for HPTH leads to several observations. First, although some variation is allowed, there is a general conservation of identity of residues. Secondly, the mutations which do occur are, for the most part, favorable. If the changes found in the active regions of the parathyroid hormones are compared with the frequency of observed mutations among homologous proteins (see Dayhoff, 1972), all the mutations, except for the Leu^{28}-Lys^{28} and Asp^{30}-Leu^{30} substitutions for HPTH proposed by Brewer *et al.* (1972), fall within the two most commonly observed classes of allowed mutations. This combination of structural identity and functionally favored mutations provides a logical molecular basis for the common biological effects of these three species observed in many assay systems.

On the other hand, although there are overall similarities in the biological properties of these molecules, interesting differences in potency are observed in some assay systems, particularly in the *in vitro* renayl adenylyl cyclase assay where BPTH is at least three times as active as either PPTH or HPTH. These differences in relative biological potency are of great interest since there are only a limited number of possibilities for alteration of activity through the observed structural changes, and thus a few sequence positions may provide the basis for revealing structure–activity relations. For example, HPTH and PPTH both differ from BPTH in having Ser^{1} and Leu^{7} rather than Ala^{1} and Phe^{7}. These positions would then seem to be logical first points of study in the synthesis of hormonal analogs for the purpose of studying hormone–receptor interactions in the kidney membrane.

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