

Human Parathyroid Hormone: Amino-Acid Sequence of the Amino-Terminal Residues 1-34

(automated Edman degradation/mass spectrometry/calcium metabolism/metabolic bone disease)

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Communicated by Robert W. Berliner, October 3, 1972

ABSTRACT Human parathyroid hormone has been isolated in highly purified form from human parathyroid adenomas. The primary sequence of the amino-terminal 34 residues of the human hormone was obtained by automated degradation with a Beckman Sequencer. The phenylthiohydantoin amino acids were identified by gas chromatography and mass spectrometry. The first 34 residues of human parathyroid hormone differ from the bovine hormone by six residues, and from the porcine hormone by five residues. The amino-terminal residue is serine, similar to the porcine parathyroid hormone; bovine parathyroid hormone contains an amino-terminal alanine. Human parathyroid hormone contains two methionine residues, similar to the bovine species, whereas porcine parathyroid hormone contains a single methionine residue. Amino-acid residues in the first 34 that are unique to the human sequence include an asparagine at position 16, glutamine at position 22, lysine at position 28, and a leucine at position 30.

During the last few years, a significant core of information has been obtained by several laboratories on the chemistry, biosynthesis, and secretion of parathyroid hormone (PTH). These studies have indicated that this hormone is initially synthesized as a prohormone, proparathyroid hormone (1-4). Proparathyroid hormone contains about 106 amino acids, and has an apparent molecular weight of 12,500 (4). The prohormone is rapidly converted into the storage or glandular form of the hormone, which consists of 84 amino acids and has a molecular weight of 9500. The complete amino-acid sequences of the 84 amino-acid parathyroid hormone from bovine (5, 6) and porcine (7) species have been reported. After appropriate physiological stimuli, the 9500 molecular weight form of the parathyroid hormone is secreted into the circulation (8). Shortly after entering the peripheral circulation, the glandular form of the hormone is cleaved into smaller fragments. Gel filtration of human hyperparathyroid serum by several investigators has revealed a major immunoreactive fragment(s), with a molecular weight of 5000-8000, and several minor components (8-10). Immunochemical heterogeneity of the circulating human parathyroid hormone, presumably due to the different molecular forms of PTH, was initially reported by Berson and Yalow (11), and has been confirmed by others (12, 13). The specific site(s) of cleavage in the 84-amino-acid polypeptide chain of the parathyroid hormone in the general circulation is unknown. A biologi-

cally active peptide fragment of bovine PTH, prepared by dilute acid cleavage, has been reported (14, 15), a result indicating that the intact 84-amino-acid polypeptide is not needed for biological activity. This peptide has been identified as the amino-terminal peptide of the hormone, and is composed of the initial 30 residues of the sequence (15). Synthetic peptides of the first 34 residues of the bovine hormone (16) and the initial 30 residues of the porcine hormone (17) have been prepared and are biologically active, thereby confirming the localization of the biologically active region of the parathyroid hormone to the amino-terminal third of the 84-amino-acid polypeptide chain.

The purpose of this communication is to report the amino-terminal sequence of the first 34 residues of human parathyroid hormone, and to compare the amino-terminal sequence of the human hormone to that of the bovine and porcine species.

MATERIALS AND METHODS

The human parathyroid hormone used in these studies was isolated from parathyroid adenomas obtained from patients undergoing surgery for hyperparathyroidism. Dried, defatted parathyroid tissue was initially extracted with 8 M urea in 0.2 N hydrochloric acid, and fractionated with ether, acetic acid, sodium chloride, and trichloroacetic acid (TCA powder) (18). The TCA powder was further purified by gel filtration, followed by ion-exchange chromatography on CM-sephadex with an ammonium acetate gradient. Isolation of the hormone was monitored by radioimmunoassay and disc-gel electrophoresis. The procedures used in the isolation and characterization of the hormone will be described in detail in a separate report.

Amino-acid analyses were performed on a Beckman-Spinco automatic amino-acid analyzer, model 120B or 121 adapted for high sensitivity or with a Durrum model 500 analyzer. Analytical disc-gel electrophoresis was performed in 8 M urea at pH 4.4 (19). Immunoassays were performed by the procedure of Arnaud *et al.* (20).

Automated Edman degradations were performed with the Beckman Sequencer, model 890B, in 1 M Quadrol buffer. The phenylthiohydantoin (PTH) amino acids were identified by regeneration to the constituent amino acid by hydrolysis with hydroiodic acid for 20 hr at 130° (21), gas-liquid

Abbreviation: PTH, parathyroid hormone

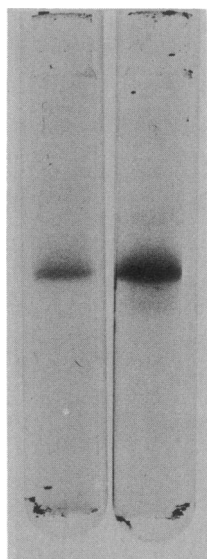


FIG. 1. Disc-gel electrophoresis of purified human (*left*) and bovine (*right*) parathyroid hormones.

chromatography (22, 23), and mass spectrometry (24–26). Chemical ionization mass spectrometry was performed on a Finnigan mass spectrometer equipped with a PDP-8/e Digital computer, and a Complot Plotter. Isobutane was used as the carrier gas, and the source was maintained at 200°. The samples were applied by a direct insertion probe, and the probe was heated from 30° to 250° over a 90-sec period. Electron impact mass spectrometry was performed on an LKB mass spectrometer, model 9000, with a direct insertion probe and an electron energy of 70 eV.

RESULTS

The purified human parathyroid hormone migrated as a single component on disc-gel electrophoresis, with a mobility identical to that of the bovine parathyroid hormone (Fig. 1). Amino-terminal analysis of the purified peptide by the Edman technique revealed serine.

350 nanomoles of the purified hormone were degraded on the Beckman Sequencer by use of a single cleavage of hepto-

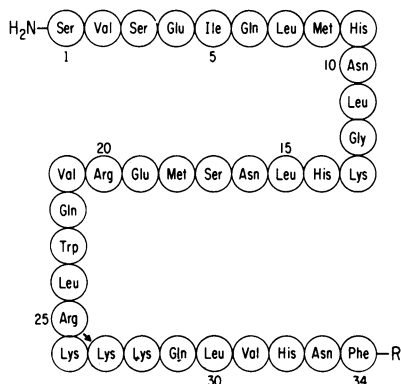


FIG. 2. Amino-acid sequence of the amino-terminal 34 residues of human parathyroid hormone.

fluorobutyric acid at each degradation. The results of the degradation of the first 34 residues of the human parathyroid hormone are shown in Fig. 2. The chemical ionization mass spectra of the phenylthiohydantoin (PTH) amino acids obtained at each of the 34 steps in the sequence are shown in Fig. 3. A “quasimolecular” (QM^+) or major fragmentary ion is observed in each spectrum (25). At step 12 in the sequence, a quasimolecular ion for glycine (m/e 192) and leucine (m/e 249) are observed (Fig. 3). Quantitation by the gas chromatography method of glycine (0.28 μ M) and leucine (0.09 μ M) permits definitive identification of glycine as the twelfth amino acid in the sequence, with the leucine resulting from overlap from step 11 (Fig. 2). The ion at m/e 292 and 293 in the mass spectra of step 20 are contaminant ions often observed in variable amounts in the aqueous layer of the Edman reaction. Leucine/isoleucine and lysine/glutamine yield identical masses of m/e 249 and m/e 264, respectively, on chemical ionization mass spectrometry. Lysine, however, can be distinguished from glutamine by the fragmentary ion at m/e 306, as illustrated in the spectra of residues 26, 27, and 28. Lysine/glutamine and leucine/isoleucine were also readily differentiated by gas chromatography on the CFC blend (23) and by electron impact mass spectrometry (24, 25).

These combined results provided a single unique sequence for the first 34 residues of human parathyroid hormone (Fig. 2).

DISCUSSION

The amino-acid sequence of the first 34 residues of human parathyroid hormone is of major importance, since previous studies of the bovine and porcine species have indicated that this is the biologically active region of the native hormone. The first 34 residues of human PTH differ from bovine PTH by six residues, and porcine PTH by five residues (Fig. 4). The amino-terminal 15 residues of human and porcine PTH are identical; however, bovine PTH differs from human and porcine PTH in position 1 and 7, where alanine substitutes for serine and leucine replaces phenylalanine (Fig. 4). In the remaining 16–34 region, human PTH differs from porcine PTH by five residues, and from bovine PTH by four residues (Fig. 4). Human PTH contains two methionine residues—similar to the bovine species—whereas porcine PTH contains a single methionine at position 8 (Fig. 4). The human sequence is unusual in that it contains four consecutive basic residues (arginine residue 25, and lysine residues 26–28). Amino-acid residues in the first 34 that are unique to the human sequence include an asparagine at position 16, glutamine at position 22, lysine at position 28, and a leucine at position 30.

One of the major problems in the clinical assessment of patients with disorders of mineral metabolism has been the difficulties encountered with the radioimmunoassay of human parathyroid hormone. There have been two basic problems with the immunoassay of PTH. The first problem, as discussed above, has been the presence in the peripheral circulation of peptide fragments of the 84 amino-acid polypeptide chain (8–10). Antisera from various laboratories undoubtedly have immunological determinants for different regions of the intact molecule, thus leading to variable and sometimes inconsistent results when applied to the measurement of PTH circulating in human blood (27). In addition, the differentiation by immunoassay of biologically active

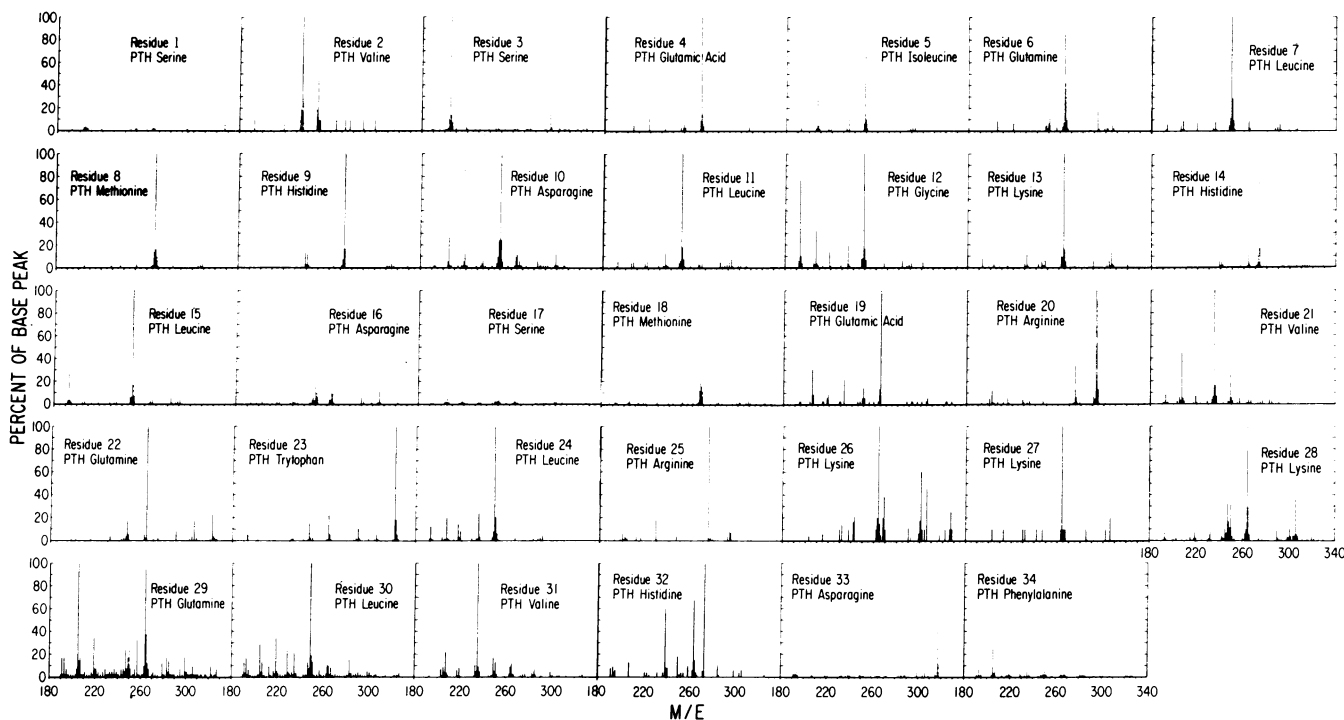


FIG. 3. Mass spectra of the phenylthiohydantoin (PTH) amino acids obtained during the automated Edman degradation of the amino-terminal 34 residues of the human parathyroid hormone.

amino-terminal fragments from inactive fragments has so far been impossible. The second difficulty has been the utilization of heterologous assays that use radioactively labeled bovine hormone as the tracer, and antibodies prepared against the bovine or porcine hormone (28-30, 20). The sensitivities of these assays are variable, and depend on the cross reactivity of the particular antiserum with the human hormone. As noted above, the human sequence in only the initial third of the molecule differs from the bovine by six residues and the porcine by five amino acids.

Habener *et al.* (31) have attempted to circumvent some of these problems with the immunoassay by the development of

amino- and carboxyl-specific antisera. These investigators have used an antibody prepared against the bovine hormone, and have absorbed their antiserum with either the synthetic 1-34 bovine fragment, or a 53-84 fragment prepared by chemical cleavage of the native bovine hormone. The amino-terminal specific antiserum was further characterized by displacement with synthetic bovine fragments, and the recognition site of this absorbed antiserum was shown to be directed toward residues 14-19 in the bovine sequence. Using this approach, they have concluded that the major fragment in the human circulation is carboxyl-terminal, and biologically inactive. They were, however, unable to identify the amino-

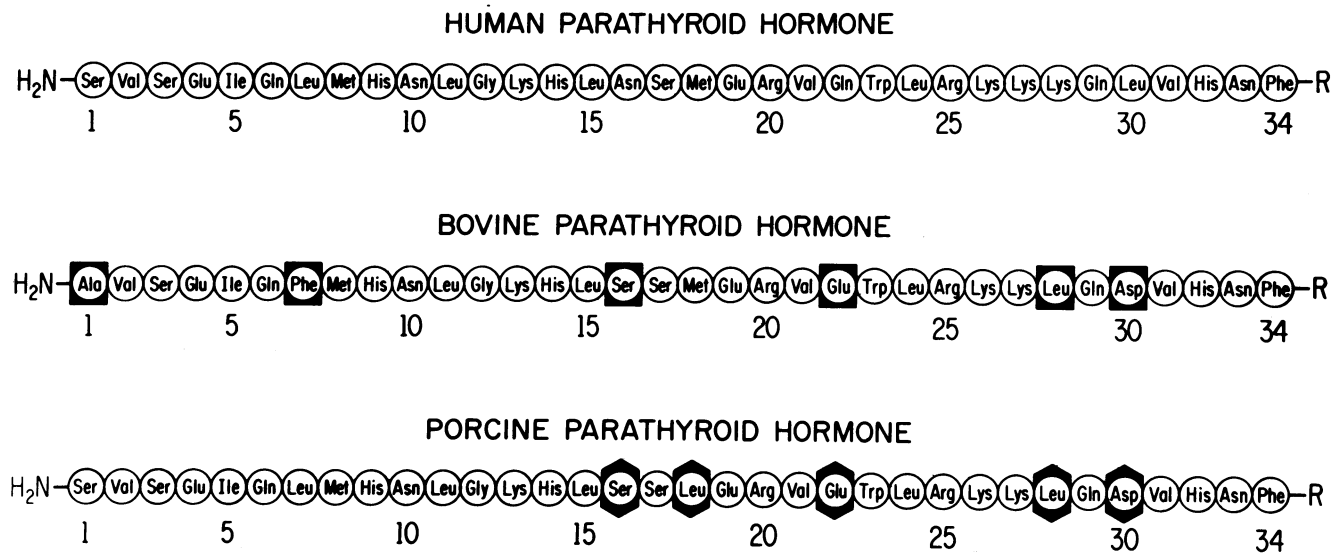


FIG. 4. Comparison of the amino-acid sequence of the amino-terminal 34 residues of human-, bovine-, and porcine-parathyroid hormones.

terminal fragment in the circulation of human subjects. This may be due either to rapid clearance of the amino-terminal fragment from the circulation, or to poor cross-reactivity of the amino-terminal specific bovine antiserum with the amino-terminal region of the human hormone. It is of interest that the human sequence differs in the 14-19 region from the bovine hormone by the substitution at residue 16 of an asparagine for a serine residue (Fig. 4). The significance of this substitution in the human hormone to the results obtained by Habener *et al.* with their amino-terminal specific bovine antiserum is unknown. Canterbury and Reiss have reported results on the nature of the circulating fragment of the parathyroid hormone that are in contrast to those reported by Habener *et al.* Using an antiserum prepared against bovine parathyroid hormone, these investigators have identified three different immunochemical forms of the parathyroid hormone in the peripheral circulation of hyperparathyroid patients (32). The molecular weights of these three components, as determined by gel filtration, were 9500 (presumably glandular PTH), 7000-7500, and 4500-5000. Recently, these investigators have directly assessed the biological activity of these three fragments in a renal adenylate cyclase system (33). Both the 9500 and the 4500-5000 fragment stimulated the adenylate cyclase system, whereas the 7000-7500 component was inactive. These results are consistent with the presence of an amino-terminal biologically active fragment of PTH of about one-half the size of the glandular hormone in human hyperparathyroid serum.

The determination of the amino-terminal sequence of the human parathyroid hormone will now permit the synthesis of peptides based on the human sequence for both clinical and investigative use. Synthetic fragments, as well as chemical analogues, will permit more definitive studies to be performed on the chemistry of the human hormone, including the specific residues and the minimum length of the polypeptide chain that is required for biological activity. In addition, these synthetic fragments will enable investigators to characterize the heterologous antisera currently in use in the immunoassay, and to develop specific antisera directed toward the amino-terminal region of the human hormone. Antisera based on the human sequence will enable more detailed studies to be performed on the nature of the circulating hormone in man, and its role in calcium homeostasis and metabolic bone disease.

An international cooperative effort has made the work reported in this manuscript possible. More than 150 individual laboratories, physicians, surgeons, and pathologists donated human parathyroid tissue for use in the extraction and purification of the human parathyroid hormone that was used in the determination of the amino-terminal sequence of the human hormone. Space does not permit a listing of their names here; however, they are represented by human PTH study groups from Australia, Canada, Europe (Belgium, Germany, Holland, and Switzerland), France, Japan, Mexico, Spain, Sweden, and the United States. A great deal of the credit for the results reported in this manuscript is due to the untiring efforts of these individuals. We also thank Drs. Henry Fales and Bill Milne for their assistance in the mass spectrometric analyses. The excellent technical assistance of Mr. Wayne Blanchard, Mrs. M. Juliariva, Mrs. Judy Larsen, Miss Ann Kelly, and Miss Janice Leoffler is gratefully acknowledged. This work was supported in part by grants from the U.S. Public Health Service (NIH-Am 12302) and from the Mayo Foundation.

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