A Reinvestigation of the Amino-Terminal Sequence of Human Parathyroid Hormone†

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ABSTRACT: The sequence of the amino-terminal portion of human parathyroid hormone, particularly the identity of residues 22, 28, and 30 (the subject of discrepancies in recent published reports), has been reexamined by two basic methods of structural analysis. A fresh lot of human parathyroid hormone isolated from pooled adenoma tissue was analyzed by Edman degradation with identification of critical residues by thin-layer chromatography and gas–liquid chromatography. In the second approach, ^{14}C or tritiated amino acids were incorporated during biosynthesis of the human hormone in slices of parathyroid glands in vitro; the appropriate amino acid residues were then determined as the ^{14}C or tritiated phenylthiohydantoin derivatives of the amino acid after Edman degradation, or by peptide isolation after appropriate cleavage with endopeptidases, or both. The results confirm our previous findings that residue 22 is glutamic acid, residue 28 is leucine, and residue 30 is aspartic acid.

Investigation of the structure of human parathyroid hormone has been limited by the scarcity of available starting material for hormone isolation. Whereas the bovine and porcine hormone can be extracted from gland tissue from slaughterhouse sources, the human hormone must be obtained from adenoma tissue removed at surgery. Recently, however, two laboratories reported partial sequence analysis of the human hormone from pooled parathyroid adenomas.

Brewer et al. (1972) reported the sequence of the amino-terminal 34 residues of the 84-residue molecule, whereas studies in our own laboratory dealt with the sequence of residues 1–37 (Niall et al., 1974). The proposed structures of Brewer et al. and Niall et al. differ from each other in three sequence positions.

The structure proposed by us (Niall et al., 1974) contains glutamic acid at position 22, leucine at position 28, and aspartic acid at position 30 (Figure 1). These residues are identical with the corresponding positions in the sequences originally reported for the bovine (Brewer and Ronan, 1970; Niall et al., 1970) and porcine (Sauer et al., 1974) hormones. In the sequence proposed by Brewer et al. (1972) positions 22, 28, and 30 contain glutamine, lysine, and leucine, respectively. This represents a charge difference of plus three in a sequence of nine residues, a rather profound difference when compared with the sequence of Niall et al. (1974). In a more recent report, Brewer et al. (1974) also presented evidence that residue 22 of the bovine and porcine hormones is glutamine.

Since the residues in question are located within the biologically active region of the molecule (Potts et al., 1971), resolution of these discrepancies is of considerable importance, if structure–function and immunologic investigations of the human hormone are to be properly conducted and interpreted.

Accordingly, we have reexamined the sequence positions 22, 28, and 30 of the human hormone, as well as position 22 of the bovine hormone, using two independent approaches: in vitro biosynthetic labeling of the hormone with radioactive amino acids, and a repeated sequence analysis by standard techniques with a new preparation of hormone purified from pooled human adenomas. These studies, described in detail in this report, confirm the sequences as found originally in our laboratory (Niall et al., 1970, 1974) for the amino-terminal region of the human, as well as the bovine, hormone.

Materials and Methods

Biosynthetic Labeling. Human parathyroid adenoma tissue was obtained directly at surgery, the calf parathyroid glands were obtained at a local slaughterhouse. Tissue slices were prepared for incubation as described by Habener et al. (1973). Incubations of 250 mg of tissue/flask were carried out for 2 hr at 27° in 2 ml of Earle's balanced salt solution containing 1.0 m*M* calcium chloride, 0.4 m*M* magnesium chloride, and 5% fetal bovine serum (Grand Island Biologicals). The appropriate labeled amino acids, purchased from New England Nuclear (Wilmington, Mass.), were added as follows: [^{14}C]glutamine, 10 μCi/ml; [^{14}C]glutamic acid, 20 μCi/ml; [^{3}H]leucine, 50 μCi/ml; [^{3}H]glutamic acid, 50 μCi/ml; [^{3}H]tryptophan, 25 μCi/ml. The remaining, unlabeled amino acids were added individually to the medium to provide a concentration of 5×10^{-4} *M*.

After incubation, the tissue was pulverized, extracted with 8 *M* urea–0.2 *N* HCl, and precipitated with trichloroacetic acid as described by Kemper et al. (1972).

Purification of Labeled Parathyroid Hormone. The pooled trichloroacetic acid preparations, anticipated to contain about 10% hormone in the bovine (Kemper et al., 1972) and 1–5% in the human preparations (J. F. Habener, personal communication) were further purified by gel filtration followed by ion exchange chromatography.

Gel filtration was carried out using Bio-Gel P-100 (Bio-Rad) equilibrated with 0.1 *M* ammonium acetate (pH 5.0) with bovine parathyroid hormone as carrier. The columns had also been presaturated with bovine hormone to improve

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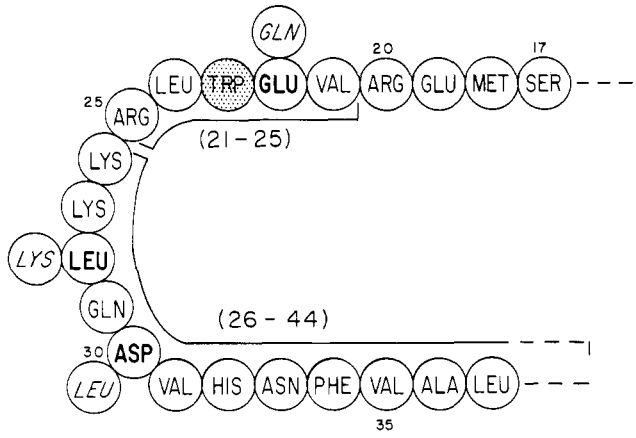

FIGURE 1: Portion of amino-terminal sequence of human parathyroid hormone, showing location of sequence differences between two proposed structures. The backbone sequence is that proposed by Niall et al. (1974) in which residues 22, 28, and 30 are glutamic acid, leucine, and aspartic acid, respectively (boldface type). In the sequence proposed by Brewer et al. (1972), these positions are glutamine, lysine, and leucine, as shown by the circles alongside (italicized type). Brackets enclose the tryptic peptides used in our reevaluation of these sequence positions. Tryptophan (position 23; stippled) provided a marker for isolation of peptide 21-25, used in reevaluation of position 22.

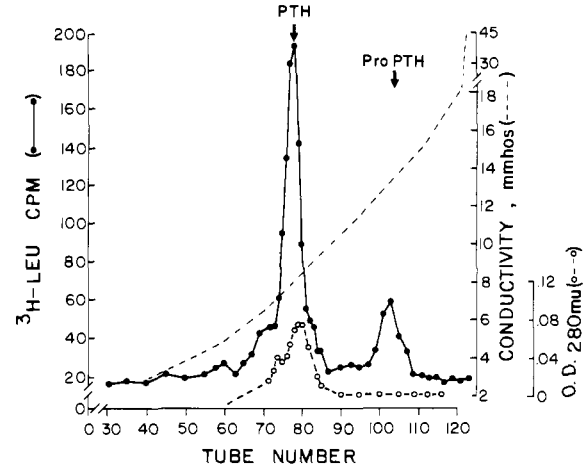

FIGURE 2: Elution profile from carboxymethylcellulose chromatography of [^{3}H]leucine-labeled human parathyroid hormone. Plot of counts in aliquots from successive tubes shows elution of native human hormone and proparathyroid hormone; peak of optical density represents elution of carrier bovine hormone.

recovery, as described by Keutmann et al. (1974). Parathyroid hormone eluting at a K_d of 0.35 was detected as a peak of optical density at 280 nm (from the carrier hormone) and of radioactivity as monitored by scintillation counting.

Ion exchange chromatography was performed on 0.9 × 6 cm CM-cellulose[1] columns, presaturated with bovine hormone. Elution was accomplished with a linear gradient of ammonium acetate buffer (Keutmann et al., 1974).

Isolation of Native Human Hormone from Pooled Frozen Adenomas. A new preparation of native human parathyroid hormone was isolated from pooled adenomas (350 g), using the methods employed in our previous extractions (Keutmann et al., 1974). The acetone-dried gland tissue was extracted with phenol, subjected to salt and ether precipitation, and then precipitated with trichloroacetic acid. The hormone was further purified from the CCl_3COOH precipitate by gel filtration on Bio-Gel P-100, followed by CM-cellulose chromatography. Purity of the hormone was assessed by automated Edman degradation, as previously described (Keutmann et al., 1974) and by polyacrylamide gel electrophoresis in 8 *M* urea (pH 4.4) (Habener and Potts, 1975).

ε-Aminolysine Blockade. Parathyroid hormone was treated with a 40-fold molar excess of maleic anhydride for 20 min at pH 9.0 (Klotz, 1967), and separated from the reagents by gel filtration.

Tryptic Peptide Preparation. Tryptic digestion was carried out in 0.2 *M* trimethylamine-acetate buffer (pH 8.2), using TPCK treated (Worthington) at an enzyme/substrate ratio of 1:125 (*M:M*).

Tryptic peptides from maleoylated hormone (including peptide 26-44; see Results) were fractionated by gel filtration on an 0.9 × 80 cm column of Sephadex G-50 and eluted with 0.2 *M* ammonium bicarbonate buffer (pH 8.5). Elution was monitored by optical density at 250 nm and, in the case of labeled preparations, by scintillation counting.

Digests of native hormone (untreated with maleic anhydride), used in preparation of peptide 21-25, were fractionated on a 1.2 × 140 cm column of Sephadex G-25, using 0.14 *M* ammonium acetate, pH 7.4, as eluent.

Purity of the separated peptides was evaluated by thin-layer chromatography, paper electrophoresis, amino acid analysis, and Edman degradation, as described further in the Results section.

Edman Degradation. Manual Edman degradation was carried out using the three-stage procedure (Edman, 1960; Niall and Potts, 1970). Phenylthiohydantoins were identified by gas-liquid chromatography (GLC) (Pisano and Bronzert, 1969) and thin-layer chromatography (Edman and Begg, 1967). Radioactive phenylthiohydantoins were identified by scintillation counting after scraping from the thin-layer plate.

Results

Purification of Native and Internally Labeled Human Parathyroid Hormone. The purification of the human parathyroid hormone used in these present studies was accomplished and assessed as previously described (Keutmann et al., 1974). The carboxymethylcellulose elution profile of a typical preparation—in this instance labeled with [^{3}H]leucine in vitro—is shown in Figure 2. Native human parathyroid hormone moved as a single band, mobility 0.38, on disc gel electrophoresis. In the radioactive preparations the native, labeled human parathyroid hormone consistently eluted from CM-cellulose at a slightly lower conductivity than did the carrier bovine hormone (Figure 2). A second peak of radioactivity eluted at a higher conductivity comparable to that found previously for elution of proparathyroid hormone by Cohn et al. (1972) and Chu et al. (1973) in work with similar labeled preparations. Mobility of this peptide by gel electrophoresis was 0.43. The radioactive tryptic peptides, and distribution of labeled residues within the peptides, obtained from this fraction were identical by gel filtration and electrophoresis to those obtained from the central fraction, indicating that this second peak was not an additional form of native hormone differing internally at the three sequence positions in question.

For the studies of glutamine and leucine incorporation sufficient hormone to provide 10,000-15,000 cpm per study was prepared. Incorporation of glutamic acid was found to

[1] Abbreviations used are: CM-cellulose, carboxymethylcellulose; TPCK, tosylamino-2-phenylethyl chloromethyl ketone.

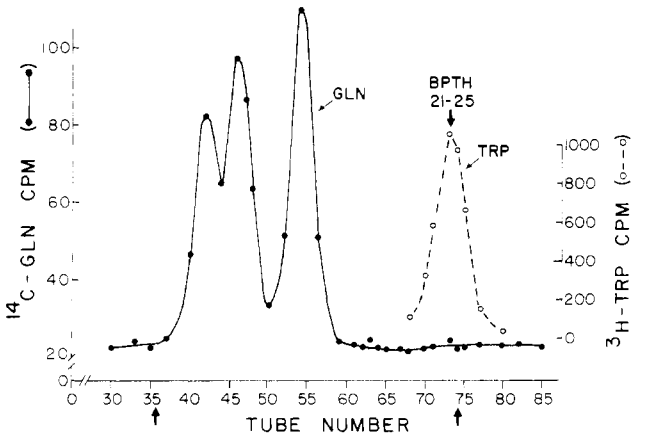

FIGURE 3: Sephadex G-25 gel filtration of tryptic digest of bovine parathyroid hormone internally labeled with [^{14}C]glutamine and [^{3}H]tryptophan. Elution of peptide 21-25, indicated by counts from tryptophan marker, showed no incorporation of glutamine at position 22. Other tryptic peptides, containing glutamine, eluted earlier from the column. Arrows beneath ordinate (tubes 35 and 75) mark the exclusion volume and salt volume, respectively, as determined by albumin and acetone.

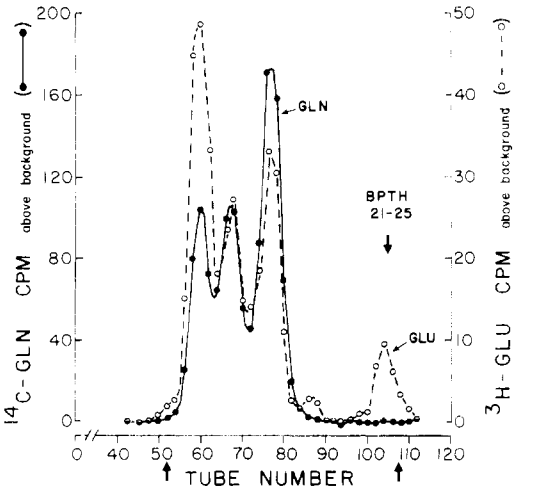

FIGURE 4: Sephadex G-25 gel filtration of tryptic digest of [^{14}C]glutamine-[^{3}H]glutamic acid-labeled bovine parathyroid hormone. A peak of glutamic acid counts, but no glutamine, eluted with peptide 21-25. Arrows mark (see Figure 3) exclusion and salt volumes.

be considerably less efficient; hence, these preparations contained 3000-5000 cpm of this isotope.[2]

Examination of Sequence Position 22. Bovine parathyroid hormone, purified as described after internal labeling with [^{14}C]glutamine and [^{3}H]tryptophan, was digested with trypsin. The chromatographic elution profile of this digest on Sephadex G-25 is shown in Figure 3. The tryptic peptide 21-25, containing position 22 and marked by the ^{3}H counts from the tryptophan residue at position 23, eluted late in the profile (due to adsorption to the polydextran column bed by the aromatic tryptophan residue).

This peptide was shown to be homogeneous by several criteria. It moved as a single spot on thin-layer chromatography (solvent system butanol-pyridine-water-acetic acid, 15:10:12:3; Eastman cellulose plates) with an R_f of 0.74 by paper electrophoresis in pyridine-acetate buffer, mobility (calculated relative to lysine) was 0.12 at pH 6.5 and 0.20 at pH 3.6. Compositional analysis after acid hydrolysis showed theoretical recoveries of amino acids, that is, equimolar amounts of arginine, glutamic acid, leucine, and valine, with tryptophan identified by Ehrlich's reagent. A single sequence was found on Edman degradation, coinciding with that found in our earlier analysis of the bovine hormone (Niall et al., 1970).

The plot of radioactivity showed that no glutamine counts eluted with this peptide (Figure 3). In another study, [^{14}C]glutamic acid was used instead of glutamine, again in the presence of the [^{3}H]tryptophan marker. The tryptic digest of this preparation was eluted from Sephadex G-25, and in the resulting profile a peak of counts from glutamic acid was found coinciding with elution of peptide 21-25.

These results were confirmed by a third experiment in which [^{14}C]glutamine and [^{3}H]glutamic acid were both introduced into the same incubation. The Sephadex G-25 elution profile of this preparation after tryptic digestion is shown in Figure 4. Again, no glutamine counts were found in the region of the peptide 21-25, whereas glutamic acid was incorporated as shown by the peak of ^{3}H from this peptide.

Comparable incubations were carried out using human adenoma tissue. The labeled products, pooled from multiple adenomas and purified in the presence of bovine hormone carrier, were similarly subjected to tryptic digestion and G-25 gel filtration. The results are summarized in Figure 5. As with the bovine products, no glutamine incorporation into peptide 21-25 could be observed, despite good recovery of the tryptophan counts from residue 23. On the other hand, glutamic acid was found to elute with this peptide, again with no glutamine counts, after processing of hormone labeled with both amino acids. Comparable results, showing absence of glutamine and presence of glutamic acid in peptide 21-25, were found when the tryptic peptides were separated by electrophoresis instead of gel filtration.

It remained necessary to provide assurance that the labeled glutamine had not undergone deamidation during incubation before incorporation. In that event, radioactivity introduced as glutamine would instead be incorporated only at positions containing glutamic acid; if position 22 were in fact glutamine, no counts would then be found in the 21-25 peptide. Therefore, Edman degradations were carried out on each of the labeled preparations. Glutamine was found to be correctly incorporated into an expected sequence position. In three cases, good recovery of glutamine counts at position 6 (known to be glutamine) was obtained by amino-terminal degradation of an aliquot of labeled hormone taken before trypsin digestion. In the fourth preparation, from incubation with bovine tissue, incorporation of glutamine correctly at position 84 was shown by degradation of a tryptic peptide isolated from gel filtration. These findings indicate that labeled glutamine was in all studies being incorporated as glutamine. The absence of glutamine counts at position 22 along with presence of glutamic acid counts, in both human and bovine hormones, indicate that this residue is glutamic acid rather than glutamine.

Examination of Sequence Positions 28 and 30. The sequence positions 28 and 30 were investigated by means of Edman degradation of the tryptic peptide 26-44 obtained both from hormone internally labeled with [^{3}H]leucine and from a new preparation of human hormone purified from

[2] In separate experiments, [^{14}C]aspartic acid, 40 μCi/ml, was incorporated during incubations with human parathyroid tissue; however, the efficiency of incorporation of aspartic acid was very low into the entire molecule, precluding the examination by Edman degradation of the location of any aspartic acid residue.

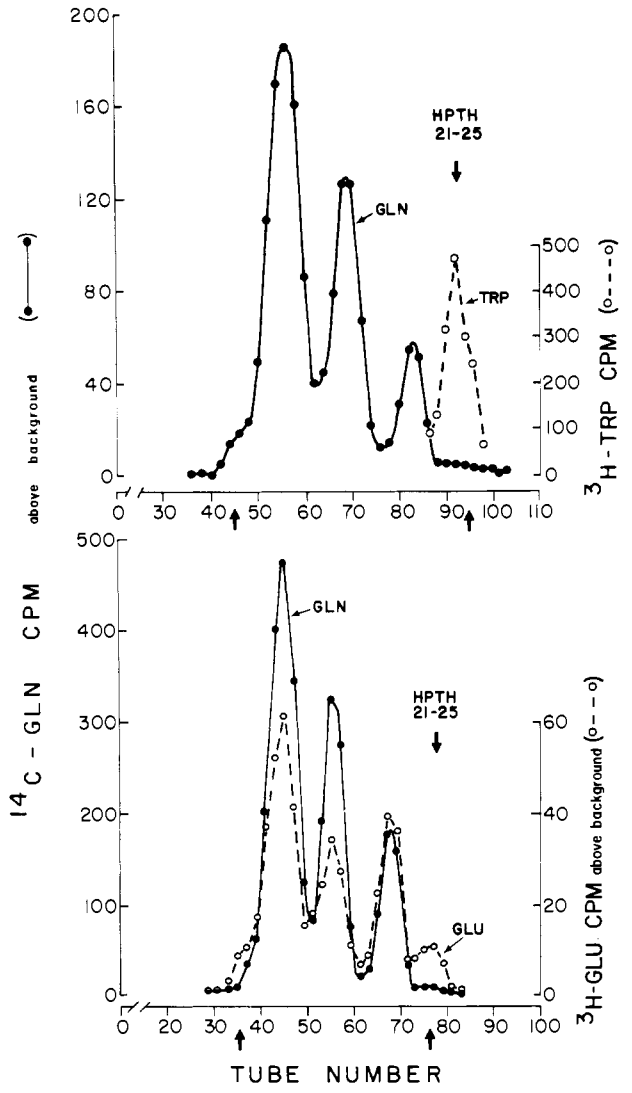

FIGURE 5: Elution patterns from gel filtration of separate human parathyroid hormone preparations, internally labeled with [^{14}C]glutamine and [^{3}H]tryptophan (top) and [^{14}C]glutamine and [^{3}H]glutamic acid (bottom). As with the respective bovine preparations (Figures 3 and 4), no glutamine counts eluted with peptide 21–25, located by tryptophan marker (top), whereas glutamic acid counts were found in this peptide (bottom). The volumes collected per tube differed in the two runs (note change in numbers on ordinate) but peptide 21–25 eluted in identical positions relative to the salt volume marker (arrow at 93, top, and 76, bottom).

pooled parathyroid adenomas.

[^{3}H]Leucine-labeled human hormone, purified in the presence of carrier bovine hormone (Figure 2), was subjected to blockade of the ϵ-amino group of lysine residues and digested with trypsin. The peptides were separated by gel filtration on Sephadex G-50. Elution of the labeled peptides from the human hormone coincided with those from the carrier bovine hormone; peptide 26–44 eluted at a K_d of 0.5.

Degradation of this peptide was carried out for six cycles. The ^{3}H counts obtained at each cycle were assessed both directly by quantitation of the organic phase, and specifically as PTH-leucine after thin-layer chromatography. The leucine counts recovered at the successive cycles are plotted in Figure 6, along with the GLC profiles of phenylthiohydantoins from the bovine hormone carrier. A marked rise in leucine counts from the human hormone was obtained at step 3 (residue 28), coinciding with the leucine from the bovine hormone as identified by GLC. No rise in leucine

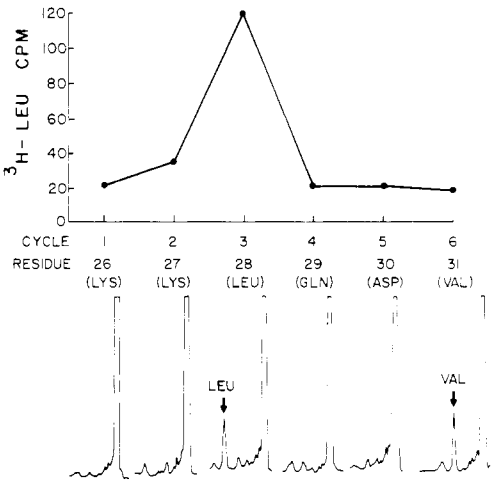

FIGURE 6: Edman degradation of tryptic peptide 26–44 of [^{3}H]leucine-labeled human parathyroid hormone. Counts of leucine recovered at each cycle are shown at bottom; gas–liquid chromatographic patterns of phenylthiohydantoins from the carrier bovine peptide are at top. Rise in the leucine counts at step 3 indicated presence of leucine at position 28 of the human hormone, coinciding with leucine at the same position in the carrier.

counts was obtained at step 5 (position 30), although peptide was still present in the degradation as shown by presence of valine from the carrier hormone, identified by GLC at step 6. Identical results were obtained from a repeat degradation of a second preparation of [^{3}H]leucine-labeled human hormone.

For the direct study of the unlabeled peptide 26–44 obtained from human hormone, 0.74 mg of purified hormonal peptide was subjected to maleic blockade of lysine residues and the tryptic peptides separated by Sephadex G-50 chromatography as before. The peptide (26–44) moved as a single spot, R_f 0.64, on thin-layer chromatography in the solvent system, butanol–pyridine–water–acetic acid (15:10:12:3) (maleoyl groups were removed from the peptide by treatment with formic acid before thin-layer chromatography).

Edman degradation was carried out for six cycles on 25 nmol of peptide. A single phenylthiohydantoin derivative was identified at each step. Lysine, identified as the maleylated derivative, was found by thin-layer chromatography at cycles 1 and 2 (positions 26 and 27). At cycle 3 (position 28), only leucine was found by both thin-layer chromatography and gas–liquid chromatography. Yield of this residue when quantitated using the Beckman SP-400 column at 180° was 11.4 nmol. Glutamine was identified by thin-layer chromatography at cycle 4. Aspartic acid, 8.1 nmol, was identified at cycle 5 (position 30) using the same chromatographic column at 210°. No evidence of leucine was found when the product from this cycle was chromatographed at 180°. The finding of solely aspartic acid at this position was confirmed by thin-layer chromatography. At cycle 6 (position 30), valine was identified by both gas–liquid chromatography (yield 3.9 nmoles) and thin-layer chromatography.

The results of this degradation indicated that the sequence commencing with residue 26 of the human hormone is Lys-Lys-Leu-Gln-Asp-Val .... Combined with the additional evidence provided by the degradation of the internally labeled preparations, these data reaffirm the previous findings of Niall et al. (1974) that the residue at position 28 is leucine and position 30 is aspartic acid.

Discussion

By application of refinements in methodology designed to enhance sensitivity, both Brewer et al. (1972) and our group (Niall et al., 1974) were able to carry out sequence analysis of the active amino-terminal region of human parathyroid hormone using only a few milligrams of purified peptide.

The structures proposed by the two groups were identical for the first 21 residues, but three differences between the proposed structures were found through the next nine residues (Figure 1).

There is urgent need for resolution of these discrepancies, which represent a net charge difference of plus three and occur within that region of the molecule known to be required for biological activity. Synthetic peptides have been prepared according to both proposed sequences (Andreatta et al., 1973; Tregear et al., 1974); however, their widespread use for the preparation and characterization of antisera for clinical radioimmunoassay applications has been restrained by lack of consensus regarding the structure.

The structure of bovine and porcine parathyroid hormone has presented an additional problem. After Brewer et al. (1974) found glutamine at position 22 in the human hormone, they reexamined this position in the bovine and porcine hormones. Based on their results they postulated that, in previous analyses by both laboratories, the glutamine had undergone deamidation during hormone extraction and purification or during sequence analysis, resulting in the erroneous identification of the amino acid at position 22 as glutamic acid rather than the residue actually present, glutamine. In a more recent study, they reported that the glutamine at position 22 is particularly liable to loss of the side chain amide (Brewer et al., 1975).

In our previous work by Edman degradation of both intact hormone and the tryptic peptide 21–25 with bovine and porcine, as well as human parathyroid hormone, we have consistently found only glutamic acid at position 22 without trace of glutamine. Other amidated dicarboxylic acid residues were readily detected, including glutamine at position 29 of the intact hormone. Hence, we had and still have no basis for identification of position 22 as anything but glutamic acid. Clearly, therefore, a means for investigation of the nature of this residue, other than simply more repeated Edman degradations, seemed indicated.

The technique of internal labeling with radioactive amino acids appeared to us to be particularly appropriate for this purpose. Tager et al. (1973) have employed this highly sensitive method in the analysis of rat insulin connecting peptides. In the parathyroid field, the method has been applied effectively to the structural analysis of human proparathyroid hormone (Cohn et al., 1974; Jacobs et al., 1974). In this approach, the labeled residue can be detected with extreme sensitivity during sequence analysis, and the correct residue will be identified even if extensive alteration of the residue should take place during purification and chemical treatment. Specifically with regard to [^{14}C]glutamine, with the radioactive counts located within the internal carbons of the residue, these counts will be found at the correct sequence position, regardless of any loss of the side chain amide subsequent to incorporation. The logic of the approach is thus solely dependent upon the well-known specificity of amino acid incorporation. If position 22 is glutamine, radioactivity will be found at position 22 after incubation with radioactive glutamine; if this position is glutamic acid, no radioactivity will be found in this peptide in preparations incubated with [^{14}C]glutamine; counts will be found only when radioactive glutamic acid is used for incorporation.

However, since tissue preparations such as those used here do contain amino transferase enzymes that use glutamine as substrate—e.g., in production of amino sugars or other amides (Meister, 1962)—it was essential to rule out the possibility that deamidation of labeled glutamine might take place in the incubation medium prior to incorporation. This eventuality could result in a failure to detect radioactivity at position 22, even though this residue is in fact glutamine; radioactive glutamine converted to glutamic acid cannot be incorporated by the tRNA for glutamine. Our degradations, which located glutamine counts at other sequence positions known to contain glutamine, provided the necessary evidence that deamidation of glutamine prior to incorporation into the peptide chain had not taken place. It could thus be concluded that the absence of glutamine counts from peptide 21–25 means that the residue at position 22 is not glutamine. A further advantage of this technique, the use of double-isotope labeling, enabled us to demonstrate not only the absence of [^{14}C]glutamine but also the presence of [^{3}H]glutamic acid incorporation at position 22 in the same tissue preparation.

A situation somewhat different from position 22 was presented by reexamination of positions 28 and 30. In this case, stable residues were involved, and there was little likelihood of misidentification consequent to breakdown during purification. Hence, reevaluation by conventional Edman sequence analysis was appropriate and useful. The degradation of the tryptic peptide generated by cleavage between residues 25 and 26 enabled the two positions to be reached early in the degradation, thereby minimizing any potential problems of the Edman procedure (including "overlap" or nonspecific cleavages) that would obscure identification.

Clear-cut identification of leucine as the residue at position 28 and of aspartic acid at position 30 was made once again in our degradations of purified human hormone. Combined with the earlier studies of Niall et al. (1974), this constitutes a total of three separate verifications of the nature of the residues at these two positions. In addition, we chose to employ the biosynthetic approach with these residues as well. This approach was complementary to our repeated sequence analysis. The ready detection of leucine counts at position 28 strengthened our conclusion that residue 28 is leucine; the absence of leucine counts at position 30 confirmed our conviction that leucine is not the residue present at position 30.

We conclude from the evidence provided by the overall findings reported here that our previously proposed sequence for the amino-terminal portion of parathyroid hormone is correct. However, in contrast to our findings, Brewer et al. (1975) recently presented additional evidence, based on methods different from those reported here, in support of their own previously reported sequence (Figure 1).

It is difficult for us to account for the continuing disparity between the findings of the two laboratories, each applying rigorous chemical methodology; a single hormonal peptide obviously cannot have two different sequences depending upon the laboratory in which it is studied. There appear to be only two possible explanations.

Firstly, there may be two different hormonal forms, reminiscent of the earlier finding that in pooled bovine parathyroid tissue there are three isohormonal forms, differing at

least with regard to threonine and valine content (Keutmann et al., 1971). Both groups have used human hormone extracted and purified from large pools of adenomatous and hyperplastic parathyroid tissue. The material used during our recent biosynthetic studies represented, in each case, products pooled from incubations of 12–15 different parathyroid adenomas. We have not found, however, another distinct hormonal form nor is there any evidence for more than one hormonal peptide reported in the findings of Brewer et al. (1972, 1975). If there are indeed two forms of the human hormone, selection by each laboratory group entirely for one form without evidence for presence of the other form would appear unusual. We are, however, in the course of further biosynthetic studies continuing to look for evidence of isohormonal forms of human parathyroid hormone.

The second explanation for the discrepancy might be that some systematic error is involved in the methods, or conclusions, or both, reported from one or both of the two laboratories. Despite a careful consideration of the issue, we can find no factor within our own results that might have misled us with regard to the structure we propose, nor do we discern any flaw in the studies reported by Brewer et al. (1975).

It is the purpose of this communication to simply present and interpret the data we have found. It is possible that evaluation of biological and immunological properties of the respective synthetic peptides in comparison with the native human hormone may ultimately provide clues to the resolution of the present discrepancies concerning the structure. Nevertheless, such studies will not supplant the need for further direct chemical investigation of the structure.

Analysis of the sequence of the hormone by independent laboratories would be particularly helpful. Unfortunately, the supplies of human parathyroid tissue available for isolation and characterization of the hormone are extremely limiting. Perhaps the biosynthetic approach that we have detailed in the present report may make it possible for other groups to investigate the sequence of human parathyroid hormone even with availability of only a small number of human parathyroid glands.

Acknowledgments

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