Structure-Activity Relation of NH₂-terminal Human Parathyroid Hormone Fragments*

(Received for publication, April 15, 1997, and in revised form, September 24, 1997)

Ute Charlotte Marx‡§, Knut Adermann§, Peter Bayer†, Markus Meyer§, Wolf-Georg Forssmann§, and Paul Rösch‡

From the §Lehrstuhl für Biopolymere, Universität Bayreuth, D-95440 Bayreuth and the ¶Niedersächsisches Institut für Peptid-Forschung, Fodor-Lyven-Straße 31, D-30625 Hannover, Federal Republic of Germany

Human parathyroid hormone (hPTH) is involved in the regulation of the calcium level in blood. This hormone function is located in the NH₂-terminal 34 amino acids of the 84-amino acid peptide hormone and is transduced via the adenylate cyclase and the phosphatidylinositol signaling pathways. It is well known that truncation of the two NH₂-terminal amino acids of the hormone leads to complete loss of in vivo normocalcemic function. To correlate loss of calcium level regulatory activity after stepwise NH₂-terminal truncation and solution structure, we studied the conformations of fragments hPTH-(2–37), hPTH-(3–37), and hPTH-(4–37) in comparison to hPTH-(1–37) in aqueous buffer solution under near physiological conditions by circular dichroism spectroscopy, two-dimensional nuclear magnetic resonance spectroscopy, and restrained molecular dynamics calculations. All peptides show helical structures and hydrophobic interactions between Leu-15 and Trp-23 that lead to a defined loop region from His-14 to Ser-17. A COOH-terminal helix from Met-18 to at least Leu-28 was found for all peptides. The helical structure in the NH₂-terminal part of the peptides was lost in parallel with the NH₂-terminal truncation and can be correlated with the loss of calcium regulatory activity.

All known extracellular biological activity of human parathyroid hormone (hPTH) is located in the NH₂-terminus of this 84-amino acid peptide hormone (1). hPTH-(1–37) is the naturally occurring bioactive hormone extractable from human blood (2, 3), and hPTH-(1–34) is known to maintain normocalcemia in blood via adenylate cyclase activation. To increase calcium flow into blood, the hormone acts directly on bone and kidney and indirectly on the intestine (1). In addition to the cyclic adenosine monophosphate (cAMP) pathway, involvement of the phosphatidylinositol hydrolysis signaling pathway is postulated for these functions (4). The receptor binding region mediating the calcium regulatory activity is located within sequence His-14 to Phe-34 (5, 6). The complete NH₂-terminal part of hPTH-(1–34) is required for stimulation of the cAMP-dependent pathway (4), and the minimum sequence affecting bone and kidney comprises amino acids 2–27 (1, 7). Adenylate cyclase activity is lost on deletion of the first NH₂-terminal amino acid, whereas receptor binding capacity is not influenced, indicating that the activation region for cAMP production and the receptor binding region are located in two distinct domains (4, 8). Adenylate cyclase activity measured in vitro does, however, not reflect the sequence-activity relationship indicated by various in vivo assays (4). hPTH-(2–34) is nearly inactive in an in vitro bioassay of cAMP stimulation, but in vivo the calcium level in blood is regulated with identical efficiency by hPTH-(2–34) and hPTH-(1–34) (Ref. 4 and references therein). This indicates that hPTH utilizes other second messengers in addition to cAMP for signal transduction and possibly additional receptors in vivo (9). Furthermore, hPTH is stimulating cell proliferation in skeletal derived cell cultures (10, 11) as well as DNA synthesis in chondrocytes (12). Different sequence regions of the peptide are responsible for these functions; for stimulation of DNA synthesis, amino acids Asp-30 to Phe-34 are postulated as an indispensable region, but flanking residues seem to be required in addition for this function (12).

hPTH stimulates an increase of bone formation and axial bone mass after periodic administration of the hormone (13). Thus, hPTH is useful in the treatment of patients with hypoparathyroidism and, moreover, in the treatment of osteoporotic patients. Therefore, it would be highly desirable to construct a stable mimic of this peptide hormone. Thus, recent studies focused on the determination of the three-dimensional structure of NH₂-terminal peptides in solution by nuclear magnetic resonance (NMR) spectroscopy. In particular, hPTH-(1–34) is an intensely studied hormone fragment as it contains all functional domains (14–17). From most experiments it was concluded that hPTH-(1–34) does not form secondary structure elements in the absence of TFE (14, 16, 18), but helix formation in TFE-free solution is nevertheless observed for hPTH-(1–34), residues 4–13 and 21–29 (19), and for hPTH-(1–37), residues 5–10 and 17–28 (20). In TFE-containing solution hPTH-(1–34) displays helical regions from Ser-3 to Gly-12 and from Ser-17 to Lys-26 (16, 18), but no tertiary interactions for hPTH-(1–34) are found under these conditions. It is commonly known that TFE stabilizes secondary structures, in particular helices (21–26), but bears the risk of weakening hydrophobically stabilized tertiary structure domains (24), an effect also observed for hPTH-(1–34).2

2 U. C. Marx, K. Adermann, W.-G. Forssmann and P. Rösch, unpublished data.
Since hPTH is of considerable medical importance, drugs mimicking this structure could be useful as therapeutics. In a first step in this direction, we determine here the structures of the NH₂-terminally truncated fragments hPTH-(2–37), hPTH-(3–37), and hPTH-(4–37) in comparison with the biologically active fragment hPTH-(1–37) (20) under near physiological conditions to elucidate a possible correlation between the loss of calcium regulatory activity after stepwise truncation of NH₂-terminal amino acids and structural features of the peptides.

### MATERIALS AND METHODS

#### Peptide Synthesis—Synthesis of hPTH fragments was carried out using a PerSeptive 9050 automated peptide synthesizer on preloaded Fmoc-t-Leu-PEG-PS or Fmoc-t-Leu-TentaGel PS resin. HPLC was performed with a 4-fold excess of Fmoc amino acids in N,N-dimethylformamide were performed in the presence of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate/N,N-diisopropylpropyleneimine/1-hydroxybenzotriazole for 30 min. The following protective groups were used: Ser-(tert-butyloxycarbonyl), Glu-(O-t-butyloxycarbonyl), Gln-(tritylaminomethyl), His-(tritylaminomethyl), Asn-(tritylaminomethyl), Lys-(tert-butyloxycarbonyl), Arg-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl), and Tyr-(tert-butyloxycarbonyl). Fmoc groups were cleaved in 10 min with 20% piperidine in N,N-dimethylformamide. The peptides were deprotected and cleaved from the resin with trifluoroacetic acid/ethanediol/water, 94:3:3, for 120 min. After filtration and precipitation of the crude peptide by addition of cold acetone, the peptide was lyophilized from 10% acetic acid and purified by preparative reversed phase-high performance liquid chromatography (Biofocus 3000, Bio-Rad, München, Germany). TLC was performed with silica gel plates (Immobilon, Millipore, Germany). Gas phase sequencing (473A Protein Sequencer, Applied Biosystems/Perkin-Elmer, Weiterstadt, Germany), and amino acid analysis (Aminoquant 1090L, Hewlett Packard, Waldbronn) showed correct mass, amino acid sequence, and composition.

#### Biological Activity—In vitro biological activity of the synthetic hPTH-(1–37), hPTH-(2–37), hPTH-(3–37), and hPTH-(4–37) fragments was tested by observation of the stimulation of the CAM assay growth in osteogenic cells (rat osteosarcoma cells) compared with synthetic hPTH-(1–34) fragment. ROS 17/2.8 cells were grown in 25 cm² plastic flasks at 37 °C in a humidified atmosphere of air/CO₂ in Ham’s F12/Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum, 50 mg of streptomycin/ml, and 50 units of penicillin/ml. The medium was changed on alternate days. The cells reached confluence within 3–4 days and were plated into 24-well dishes for experiments. Assays were performed on confluent cultures 1–2 days after change in medium.

#### NMR Spectroscopy—Two-dimensional NMR spectra were obtained on a commercial Bruker AMX600 spectrometer at 298 K with standard methods (31, 32). For hPTH-(4–37) an additional set of spectra was measured at 285 K to resolve frequency degeneracy. The measurements were carried out in 50 mM phosphate buffer with 270 mM sodium chloride. Peptide concentrations were 1.8 mM, pH 6.0 (hPTH-(2–37)), 2.1 mM, pH 6.0 (hPTH-(3–37)), and 1.9 mM, pH 5.8 (hPTH-(4–37)).

### Table I

Energy contributions to the structures and deviations from standard geometry NOE and X-PLOR statistics

| hPTH(1–37) | hPTH(2–37) | hPTH(3–37) | hPTH(4–37) |
|------------|------------|------------|------------|
| 183°       | 171°       | 210°       | 159°       |
| i – j = 1  | 106        | 101        | 126        | 100        |
| i – j = 2  | 6          | 6          | 10         | 5          |
| i – j = 3  | 43         | 43         | 53         | 38         |
| i – j = 4  | 2          | 4          | 0          | 1          |
| i – j = 5  | 5          | 6          | 5          | 5          |
| i – j > 6  |            |            |            |            |

Average energies (kJ/mol)

- E<sub> noe</sub>  
  - hPTH(1–37) -1579.36  
  - hPTH(2–37) 268.77  
  - hPTH(3–37) 535.08  
  - hPTH(4–37) 109.99  

- E<sub> dW</sub>  
  - hPTH(1–37) -522.05  
  - hPTH(2–37) 300.77  
  - hPTH(3–37) 715.08  
  - hPTH(4–37) 148.24  

- E<sub> imp</sub>  
  - hPTH(1–37) -1083.16  
  - hPTH(2–37) 268.54  
  - hPTH(3–37) 584.73  
  - hPTH(4–37) 101.91  

- E<sub> CVOW</sub>  
  - hPTH(1–37) -2123.47  
  - hPTH(2–37) 2120.37  
  - hPTH(3–37) 772.71  
  - hPTH(4–37) 559.95  

### Notes

- a Total NOE number.
- b RMSD among backbone structures (nm).

Data from the following 600 MHz spectra were employed for the sequence-specific assignment of spin systems and the evaluation of the NOEY distance constraints for the different PTH fragments: double quantum filtered COSY spectra, DqT-COSY spectra, and nD/qD for the double quantum filtered COSY spectra. Base-line and phase correction of the 6th order was used. Data were evaluated on X-Window work stations with the NDee program package (Software Symbiose GmbH, Bayreuth, Germany).

Data from the following 600 MHz spectra were employed for the sequence-specific assignment of spin systems and the evaluation of the NOEY distance constraints for the different PTH fragments: double quantum filtered COSY spectra, DqT-COSY spectra, and nD/qD for the double quantum filtered COSY spectra. Base-line and phase correction of the 6th order was used. Data were evaluated on X-Window work stations with the NDee program package (Software Symbiose GmbH, Bayreuth, Germany).
nealing refinement and subsequent energy minimization (protocol distance geometry simulated annealing (33)). For the refinement the dielectric constant was changed to $\varepsilon = 4$. Structure parameters were extracted from the standard paralleldg.pro and topparallelg.pro files (34). For each fragment 30 structures were calculated. Ten structures for every fragment were selected on the criteria of smallest number of NOE violations over 0.05 nm and lowest overall energy.

**Structure Analysis**—The final structures were analyzed with respect to stable idealized elements of regular secondary structure using the DSSP (definition of secondary structure of proteins) program package (35). To elucidate the stability of the structures, we calculated the local root mean square deviations with a five-amino acid window (36). For visualization of structure data the SYBYL 6.0 (TRIPOS Association), the RASMOL V 2.6 (37), and the MOLSCRIPT program packages (38) were used.

**RESULTS AND DISCUSSION**

**Activity Tests**—The biological activities of hPTH-(1–37), hPTH-(2–37), and hPTH-(3–37) are virtually identical in the in vivo activity test of calcium homeostasis in blood using Parsons’ Chicken Assay (30). Pure solvent without PTH served as control. The variation of the calcium level in blood is used as indicator for PTH activity. After subtraction of the control value (10.78 mg/dl Ca$^{2+}$), data are expressed as percentage of the hPTH-(1–34) value (14.60 mg/dl Ca$^{2+}$) and are plotted as the mean ± S.D. from eight separate experiments. In the in vitro activity test of hPTH-(1–37), hPTH-(2–37), hPTH-(3–37), and hPTH-(4–37) in comparison to synthetic hPTH-(1–34). The cAMP generation in rat osteosarcoma cells after stimulation with the different hPTH fragments is used as indicator for PTH activity. Data are expressed in picomoles/well as a response to the different hPTH fragments and the control, respectively, and are plotted as the mean ± S.D. from three separate preparations, each assayed in duplicate.

![Activity tests](http://www.jbc.org/)

**CD Spectroscopy**—To compare the overall content of helical structure of the different peptides, far UV CD spectroscopy was used (Fig. 2) with peptide concentrations ranging from 270 to 310 $\mu$M. The overall shape of the spectra of the different peptides indicates the presence of both $\alpha$-helical and random coil structural elements (40, 41). With the stepwise truncation of the NH$_2$-terminal amino acids the ellipticity at 222 nm changes to less negative values. The evaluation of the helix content of the different peptides by standard methods (42) shows the following approximate fractional helix contents: hPTH-(1–37), 29%; hPTH-(2–37), 24%; hPTH-(3–37), 23%; and hPTH-(4–37), 22%.

After truncation of the first two amino acids resulting in tol hydrolysis, and release of intracellular calcium by activating G protein-linked receptors in bone and kidney (4). A single receptor was shown to stimulate intracellular accumulation of both cAMP and inositol triphosphates (39). PTH has the concentration-dependent ability to stimulate two separate signal pathways (9), and different sequential regions of the hormone may be responsible for initiation of the adenylate cyclase and the phospholipase C activating pathway. The existence of these multiple pathways is possibly reflected by the fact that hPTH-(2–37) is virtually inactive in the adenylate cyclase assay but can induce substantial hypercalcemia in the in vivo model (Fig. 1, a and b).
hPTH-(3–37), the wavelength corresponding to zero ellipticity and the minimum between 200 and 210 nm are shifted to lower wavelength. For hPTH-(1–37) and hPTH-(2–37) the ellipticity vanishes at 197 nm, and for hPTH-(3–37) and hPTH-(4–37) the ellipticity vanishes at 194 nm. The CD spectrum shows a minimum at 205 nm for hPTH-(1–37) and hPTH-(2–37), whereas the minimum for hPTH-(3–37) and hPTH-(4–37) is at 203 nm, indicating a structural transition between hPTH-(2–37) and hPTH-(3–37). These changes in the shape of the spectra may be interpreted as relative increase of random coil structure upon truncation of the first two amino acids (40, 41, 43).

Analysis of C-α Proton Chemical Shifts—To allow an initial mutual comparison of the truncated fragments and hPTH-(1–37), we used the chemical shift data available from our experiments to perform a secondary structure estimation based on chemical shift index strategy (44, 45). The procedure depends on a direct correlation between the chemical shifts of C-α proton resonances of consecutive amino acids and the local secondary structure: an upfield shift of the C-α proton resonances relative to the corresponding “random coil” values indicates local α-helical structure (negative value in Fig. 3), and a downfield shift of C-α proton resonances compared with the corresponding random coil values indicates a local β-sheet structure (positive value in Fig. 3). Only deviations from the random coil values by more than 0.1 ppm are taken into account for secondary structure estimation. For hPTH-(1–37) and hPTH-(2–37), the chemical shifts of C-α proton resonances suggest two helical regions extending from Ser-17 to at least Leu-28 (20). For hPTH-(3–37) and Gln-6 to His-9 of hPTH-(4–37) are shifted downfield by 0.05 to 0.15 ppm relative to the corresponding random coil values of Wishart et al. (45).

Structures of hPTH Fragments

FIG. 2. Far UV CD spectra of hPTH-(1–37) (—), hPTH-(2–37) (— —), hPTH-(3–37) (— — —), and hPTH-(4–37) (— — —). Sample conditions are as follows: 50 mM phosphate buffer, pH 6.0, 270 mM NaCl, peptide concentration 270–310 μM, 0.1-mm cell.

FIG. 3. Difference values of the observed C-α proton chemical shifts relative to the random coil values of Wishart et al. (45). The threshold of ±0.1 ppm is indicated by dashed lines. a, hPTH-(1–37); b, hPTH-(2–37); c, hPTH-(3–37); d, hPTH-(4–37).

The threshold of ±0.1 ppm is indicated by dashed lines. a, hPTH-(1–37); b, hPTH-(2–37); c, hPTH-(3–37); d, hPTH-(4–37).

not allow their C-α proton resonance chemical shift values to be compared with those of the amino acids with more than one flanking residue. The C-α proton resonances of Ile-5 to Met-8 of hPTH-(3–37) and Gln-6 to His-9 of hPTH-(4–37) are shifted downfield by 0.05 to 0.15 ppm relative to the corresponding values of hPTH-(1–37), suggesting a loss of the NH2-terminal helix after deletion of the first two amino acids of hPTH-(1–37) (Fig. 3).

Analysis of Medium Range NOEs—The NOEs observed for the various hPTH fragments were determined from the 200-ms NOESY spectra at 298 K (Table I and Fig. 4). The $d_{\alpha\beta}$($i,i+3$) and $d_{\alpha\alpha}$($i,i+3$) NOESY cross-peaks fully corroborate the existence of two helical regions for hPTH-(1–37) and hPTH-(2–37). Indications for an NH2-terminal helix for hPTH-(3–37), however, are weak and are entirely missing for hPTH-(4–37), thus confirming the results from the chemical shift index procedure. In particular, helix typical ($i,i+3$) NOEs are clustered from Ile-5 to Leu-11 and Ser-17 to Phe-34 for hPTH-(1–37) and hPTH-(2–37), respectively. For hPTH-(1–37), two helical regions were found earlier, a short one from Ile-5 to Asn-10 and a longer one from Ser-17 through at least Leu-28 (20). For hPTH-(3–37) two weak helix typical NOEs are found in the NH2-terminal region, and for hPTH-(4–37) no helix typical NOE could be found in the NH2-terminal region, and frequency degenerations of possible ($i,i+3$) NOEs were not present. In contrast, clear evidence of the COOH-terminal helix in these
Structures of hPTH Fragments

**Figure 4**

*SVSEIQLHNLGKHLNMLRRVWRLAKQDVHNFVAL*

**a**

- $d_{\text{NN}}(i+1)$
- $d_{\alpha\alpha}(i+1)$
- $d_{\beta\beta}(i+1)$
- $d_{\alpha\beta}(i+1)$
- $d_{\alpha\alpha}(i+2)$
- $d_{\alpha\beta}(i+2)$
- $d_{\beta\beta}(i+2)$
- $d_{\alpha\alpha}(i+3)$
- $d_{\alpha\beta}(i+3)$
- $d_{\beta\beta}(i+3)$
- $d_{\alpha\alpha}(i+4)$
- $d_{\alpha\beta}(i+4)$
- $d_{\beta\beta}(i+4)$

*SEIQLHNLGKHLNMLRRVWRLAKQDVHNFVAL*

**c**

- $d_{\text{NN}}(i+1)$
- $d_{\alpha\alpha}(i+1)$
- $d_{\beta\beta}(i+1)$
- $d_{\alpha\beta}(i+1)$
- $d_{\alpha\alpha}(i+2)$
- $d_{\alpha\beta}(i+2)$
- $d_{\beta\beta}(i+2)$
- $d_{\alpha\alpha}(i+3)$
- $d_{\alpha\beta}(i+3)$
- $d_{\beta\beta}(i+3)$
- $d_{\alpha\alpha}(i+4)$
- $d_{\alpha\beta}(i+4)$
- $d_{\beta\beta}(i+4)$

*VSEIQLHNLGKHLNMLRRVWRLAKQDVHNFVAL*

**b**

- $d_{\text{NN}}(i+1)$
- $d_{\alpha\alpha}(i+1)$
- $d_{\beta\beta}(i+1)$
- $d_{\alpha\beta}(i+1)$
- $d_{\alpha\alpha}(i+2)$
- $d_{\alpha\beta}(i+2)$
- $d_{\beta\beta}(i+2)$
- $d_{\alpha\alpha}(i+3)$
- $d_{\alpha\beta}(i+3)$
- $d_{\beta\beta}(i+3)$
- $d_{\alpha\alpha}(i+4)$
- $d_{\alpha\beta}(i+4)$
- $d_{\beta\beta}(i+4)$

*EIQLHNLGKHLNMLRRVWRLAKQDVHNFVAL*

**d**

- $d_{\text{NN}}(i+1)$
- $d_{\alpha\alpha}(i+1)$
- $d_{\beta\beta}(i+1)$
- $d_{\alpha\beta}(i+1)$
- $d_{\alpha\alpha}(i+2)$
- $d_{\alpha\beta}(i+2)$
- $d_{\beta\beta}(i+2)$
- $d_{\alpha\alpha}(i+3)$
- $d_{\alpha\beta}(i+3)$
- $d_{\beta\beta}(i+3)$
- $d_{\alpha\alpha}(i+4)$
- $d_{\alpha\beta}(i+4)$
- $d_{\beta\beta}(i+4)$
two fragments is found from Ser-17 to Phe-34 and His-32, respectively. To investigate whether the missing \( (i,i+3) \) NOEs in the NH\(_2\)-terminal region of hPTH-(4–37) can be accounted for by the lower concentration of this peptide (1.9 mM), two-dimensional NMR spectra of a sample of hPTH-(1–37) with 1.8 mM concentration were measured with the same buffer, temperature, and spectrometer conditions. From the 200-ms NOESY spectrum of this sample, two \( d_{\alpha\alpha}(i,i+1) \) and five \( d_{\alpha\alpha}(i,i+3) \) NOEs in the NH\(_2\)-terminal region of hPTH-(1–37) could be assigned. Thus, the lower concentration cannot account for the missing \( (i,i+3) \) NOEs in the NH\(_2\)-terminal region of hPTH-(4–37). The loss of the NH\(_2\)-terminal helix on removal of the first two amino acids was fully confirmed by the NOESY cross-peak patterns (Fig. 4).

Relative NOE Intensities—Relative intensities of sequential and medium range NOEs may be used to estimate the perfection and stability of helical structures, in addition to the upfield shift of the \( \alpha \)-proton resonances. For an ideal \( \alpha \)-helix the \( d_{\alpha\alpha}(i,i+1) \) and \( d_{\alpha\alpha}(i,i+3) \) distances should be nearly identical, whereas the \( d_{\alpha\alpha}(i,i+1) \) distances should be shorter, yielding higher intensity NOEs (47–49). For all PTH fragments employed in our experiments, most of the sequential \( d_{\alpha\alpha}(i,i+1) \) NOEs are of higher intensity than the corresponding \( d_{NN}(i,i+1) \) and \( d_{NN}(i,i+3) \) NOEs (Fig. 4), indicating that the helices are not ideal but are in an equilibrium with a more extended conformation, possibly a \( 3_{10} \)-helix. The helices of the PTH fragments are clearly more stable than nascent helices that do not show \( (i,i+3) \) NOEs (48, 50). Simultaneous observation of \( d_{\alpha\alpha}(i,i+2) \) and \( d_{\alpha\alpha}(i,i+4) \) NOEs may arise from a mixture of \( 3_{10} \)- and \( \alpha \)-helix type structures (50). This effect was observed for the COOH-terminal region of the different PTH fragments. Karle and Balaram (51, 52) suggest that six-residue sequences for the COOH-terminal region of the different PTH fragments we studied seem to represent an equilibrium between an \( \alpha \)-helix and \( 3_{10} \)-helical conformation, the NH\(_2\)-terminal helix having a higher tendency to a more extended \( 3_{10} \)-helical conformation. This phenomenon is also reflected by values of the upfield shift of the \( \alpha \)-proton resonances (Fig. 3).

Other NOEs—For each fragment, four to six long range \( (i-j) > 5 \) NOEs could be assigned (Table I). All fragments show several long range NOEs between Leu-15 and Trp-23. For hPTH-(1–37), five NOEs were found between Leu-15 and Trp-23 (20), four NOEs for hPTH-(2–37) and hPTH-(3–37), and five NOEs for hPTH-(4–37). Additionally, two NOEs between Leu-15 and Val-21 were observed in hPTH-(3–37). These NOEs indicate a spatial proximity between Leu-15 and Trp-23, probably due to hydrophobic interactions between these two residues. The observed NOEs are responsible for a clear restriction of the conformational space of the calculated structures and lead to a defined loop region around His-14 to Ser-17. Furthermore, due to the ring current field of the spatial neighboring aromatic ring system of Trp-23 the \( \delta \) proton resonances of Leu-15 are shifted upfield in comparison to the analogous resonances of other leucines for all four fragments.

Structure Calculation and Analysis—159–210 interresidual NOEs per fragment were collected from 200-ms NOESY spectra at 298 K and used in restrained MD calculations (Table I). For structure calculation of the NH\(_2\)-terminally truncated fragments, the combined distance geometry/simulated annealing protocol described earlier (20, 33) was used. For each fragment a family of 30 structures was calculated, and the 10 structures with lowest energy values and lowest number of NOE violations were selected from each group. To resolve frequency degenerations of proton resonances in the spectra of hPTH-(4–37) an additional set of spectra was obtained at 288 K. From this NOESY spectrum, 175 unambiguous interresidual NOEs could be assigned. Only NOEs were taken into account for structure calculation that were also observed, albeit ambiguously, in the NOESY spectrum at 298 K. For each of the four fragments the COOH-terminal helix extending from Met-18 to at least Leu-28 is found by DSSP analysis. For hPTH-(1–37) an NH\(_2\)-terminal helix from Gln-6 to His-9 exists. For hPTH-(2–37), five structures show an NH\(_2\)-terminal \( \alpha \)-helix around Leu-7; the others show turns or \( 3_{10} \)-helix in this region. None of the 10 calculated structures of hPTH-(3–37) displays an NH\(_2\)-terminal \( \alpha \)-helix, and only two structures exhibit a \( 3_{10} \)-helix from Glu-4 to Gln-6. No structure of hPTH-(4–37) shows an NH\(_2\)-terminal helix, and only in one case a turn is indicated by DSSP in this region.

The extension of the COOH-terminal helix of hPTH-(4–37) is virtually identical to that of the corresponding helix in the other fragments. The loss of the NH\(_2\)-terminal helix after truncation of the first two amino acids is corroborated by the structure calculations.

The two inactive fragments hPTH-(3–37) and hPTH-(4–37) show the same loop region around His-14 to Ser-17 and the following COOH-terminal helix from Met-18 to at least Leu-28 as the fragments hPTH-(1–37) (20) and hPTH-(2–37). This well defined region from His-14 to at least Leu-28 is part of the postulated receptor binding region from His-14 to Phe-34 (5, 6). Indeed, NH\(_2\)-terminally truncated biologically inactive hPTH fragments such as PTH-(3–34) have complete receptor binding ability (8, 53).

Local RMSD Values—To elucidate the stability of the structures in the helical regions and the defined loop, we calculated the local root mean square deviations (RMSD) using a five-amino acid window (36) (Fig. 5). The upper trace represents the local RMSD values for all heavy atoms, and the lower trace represents the values for the peptide backbone. The regions with defined structure show substantially reduced local RMSD values compared with the flexible regions at the termini and around Gly-12. For hPTH-(1–37) and hPTH-(2–37) two regions with local backbone RMSD values lower than 0.07 nm were found from Gln-6 to His-9 and Asn-16 to Lys-26 for hPTH-(1–37) and from Leu-7 to His-9 and Asn-16 to Asp-30 for hPTH-(2–37), respectively. Comparatively high RMSD values for the amino acids Leu-11 to Lys-13 for fragments hPTH-(1–37) and hPTH-(2–37) indicate a flexible hinge region between the NH\(_2\)-terminal helix and the loop region followed by the COOH-terminal helix. For hPTH-(3–37) and hPTH-(4–37) a decrease of the RMSD values is found in the region of the COOH-terminal helix from Ser-17 to Gln-29. Compared with the fragments hPTH-(1–37) and hPTH-(2–37) the NH\(_2\)-terminal region is structurally less well defined for the fragments hPTH-(3–37) and hPTH-(4–37) (Fig. 5).

A best fit superposition of the peptide backbone atoms of the 10 final structures selected from the MD calculation of hPTH-(2–37) shows two well defined regions linked by a hinge region around Gly-12: a loop region from His-14 to Ser-17 followed by the COOH-terminal helix up to at least Leu-28 (Fig. 6, a and b) and a short NH\(_2\)-terminal helix around Leu-7 (Fig. 6c). The loop region and the following COOH-terminal helix is very similar to the region from His-14 to Leu-28 of hPTH-(1–37) (20). The same is true for hPTH-(3–37) and hPTH-(4–37) (Fig. 6d), indicating that the truncation of the first amino acids only
influences the NH2-terminal structure, whereas the loop region and the COOH-terminal helix remain unimpaired. This is also confirmed by similar RMSD values for the region His-14 to Leu-28 of the four peptides (Table I). For most of the calculated structures the COOH-terminal helix ends at Leu-28, but the \((i,i+3)\) NOE pattern may be interpreted to indicate that the helical region extends to Phe-34 or His-32, respectively, for all four PTH fragments. The upfield shifted Cα proton resonances also extend to Gln-29 or His-32.

**Helix Content**—From NMR and structure calculation data the following helix contents for the different peptides were estimated. The secondary structure analysis using the DSSP program (35) result in a helix content of 43\% for hPTH-(1–37), 44\% for hPTH-(2–37), 37\% for hPTH-(3–37), and 32\% for hPTH-(4–37). For this calculation only amino acids that reside in a helical conformation in more than 50\% of the calculated structures were taken into account. Under the assumption that residues which contribute to medium and strong \((i,i+3)\) NOEs are part of helical structures (Fig. 4), the helix content is 59\% for hPTH-(1–37), 55\% for hPTH-(2–37), 43\% for hPTH-(3–37), and 38\% for hPTH-(4–37). The helix content according to the chemical shift indexing procedure is 46 and 50\% for hPTH-(1–37) and hPTH-(2–37), respectively, 31\% for hPTH-(3–37), and 32\% for hPTH-(4–37) (Fig. 3). From the NMR results a clear decrease in the helix content is derived between hPTH-(2–37) and hPTH-(3–37). Assuming that the length of helical regions is reflected correctly by the combined NMR results, there is a significant underestimation of the helix content from CD spectra (22–29\%), which is also reported for other peptides (54, 55).

One explanation for the apparent lower helix content estimated from CD spectra is that the helical sequences are in helical conformation in 50–70\% on time average in the case of PTH. Other explanations are the absolute length of the helices and the associated end group effects (56–60) as well as a possible contribution of the aromatic side chain of Trp-23 to the far UV CD signal (59, 61). Additionally, the shape and intensity of the CD signal depends on the geometry of a peptide helix. An ideal \(\alpha\)-helix has a stronger CD signal than a \(3_1\)helix (59) with a different shape (50, 58). These phenomena lead to a lower percentage of helicity estimated from the \(\theta_{222}\) value. Thus, changes in the short NH2-terminal helix could not be detected on the basis of the CD signal at 222 nm alone. The possibility of an equilibrium with a \(3_1\)helic is also reflected by the values of the upfield shifts of the Cα proton resonances (Fig. 3).

Progressive destabilization of a helix by successive removal of residues at the NH2-terminal end was observed earlier (62). Among others, backbone hydrogen bonding, loss of conformational entropy, interactions between side chains, electrostatic interactions between polar and charged groups at the termini with the helix macrodipole, and capping interactions at the helix termini influence helix stability (54, 63, 64). The unfavorable interaction of the positively charged NH2-terminal |NH3 group with the helix macrodipole is known as a destabilizing factor (63, 65, 66). This terminal charge gets closer to the region of the NH2-terminal helix by successive deletion of the NH2-terminal amino acids, which would provide an explanation for the destabilization of the NH2-terminal helix of hPTH.

At pH 6.0, as was used in our experiments, the side chains of Glu-4 and His-9 are charged. These charges stabilize the helix macrodipole (64–66) and are of importance for stabilizing short helices in particular (66). The negative charge of the Glu-4 side chain that interacts favorably with the helix macrodipole may be screened by the closer proximity of the positively charged NH3\(_+\) group in the truncated fragments hPTH-(3–37), and even more so in hPTH-(4–37). The truncation of Ser-3 leads to a complete loss of the NH2-terminal helix. Ser-3 may possibly serve as an N-cap (54, 63, 64) as its side chain may form a hydrogen bond to the main chain of the NH2-terminal helix, thus stabilizing the NH2-terminal helix. Gly-12 is at the very COOH-terminal end of the NH2-terminal helix. Indeed, Gly has a propensity to function as a helix C-cap (64). Stabilizing effects within the COOH-terminal helix were discussed earlier (20).

Most structural investigations of PTH fragments so far were carried out on hPTH-(1–34) using NMR spectroscopy in TFE containing solution to stabilize the helical regions (14–16, 18, 67). Under these conditions no long range NOEs were found, and thus no tertiary interactions could be derived. Spatial proximity of Leu-15 and Trp-23, indicated by several long range NOEs and a hydrogen bond to the main chain of the NH2-terminal helix, thus stabilizing the NH2-terminal helix. Gly-12 is at the very COOH-terminal end of the NH2-terminal helix. Indeed, Gly has a propensity to function as a helix C-cap (64). Stabilizing effects within the COOH-terminal helix were discussed earlier (20).

**Conclusion**—After deletion of the NH2-terminal two or three amino acids, PTH’s biological activity is lost, but its receptor binding ability remains unimpaired (8, 53). Table II summa-
FIG. 6. a, best fit superposition of the backbone atoms of His-14 to Leu-28 of the 10 final structures selected from the MD calculation of hPTH(2–37). Only the backbone atoms of amino acids His-14 to Leu-28 are shown. b, same structures and superposition as in a, but all backbone atoms are shown to illustrate the inherent flexibility of the peptide. c, best fit superposition of the backbone atoms of Gln-6 to His-9 of the 10 final structures selected from the MD calculation of hPTH(2–37). Only the backbone atoms of amino acids Val-2 to Lys-13 are shown. d, best fit superposition of the backbone atoms of His-14 to Leu-28 of the 10 calculated and selected structures of hPTH(4–37). Only the backbone atoms of amino acids His-14 to Leu-28 are shown.

Table II

| Loop (fragments 14–17) | NH₂-terminal helix (amino acids 18–28) | Activity |
|------------------------|----------------------------------------|----------|
| hPTH(1–37)             | +                                      | +        |
| hPTH(2–37)             | +                                      | +        |
| hPTH(3–37)             | +                                      | +        |
| hPTH(4–37)             | +                                      | +        |

Structures of hPTH Fragments

Summary of the structural features and biological activity of the different hPTH fragments.

The NH₂-terminal helix, however, is present only in the in vivo bioactive fragments hPTH(1–37) and hPTH(2–37), but not in the inactive fragments hPTH(3–37) and hPTH(4–37) (Table II). This may indicate that the NH₂-terminal helix is correlated with the in vivo bioactivity of the PTH fragments concerning the calcium level in blood. Existence of the NH₂-terminal helix, however, cannot be connected to the ability to stimulate adenylate cyclase, as hPTH(2–37) is nearly inactive in the cAMP assay. This result may imply different structural requirements for triggering the different signal transduction pathways (4), and may thus indicate the occurrence of different PTH receptors as discussed in the literature (69). To decide whether or not the in vivo biological activity is determined on a structural level by the NH₂-terminal helix or depends on a direct functional role of the first two amino acids, structure calculations and activity tests of stabilized PTH fragments are currently under investigation.

REFERENCES

1. Potts, J. T., Jr., Kronenberg, H. M., and Rosenblatt, M. (1982) Adv. Prot. Chem. 35, 323–306
2. Forssmann, W. G., Schulz-Knappe, P., Meyer, M., Adermann, K., Forssmann, K., Hock, D., and Aoki, A. (1993) in Peptide Chemistry (Yanaihara, N., ed) pp. 553–557, ESCOM, Leiden
3. Hock, D., Magerlein, M., Heine, G., Ochlich, P. P., and Forssmann, W. G. (1997) FEBS Lett. 400, 221–225
4. Coleman, D. T., Fitzpatrick, A., and Bilezikian, J. P. (1994) in The Parathyroid (Bilezikian, J. P., Levine, M. A., and Marcus, R., eds) pp. 239–258, Raven Press, New York
5. Cunfield, M. P., McKeever, R. L., Goldmann, M. E., Dung, L. T., Fisher, J. E., Gay, C. T., DeHaven, P. A., Levy, J. J., Roubini, E., Nutt, R. F., Chorev, M., and Rosenblatt, M. (1990) Endocrinology 127, 83–87
6. Lopez-Hilker, S., Martin, K. J., Sugimoto, T., and Slatopolsky, E. (1992) J. Lab. Clin. Med. 119, 728–743
7. Tregear, G. W., van Rietbergen, J., Greene, E., Keutmann, H. T., Niall, H. D., Reit, B., Parsons, J. A., and Potts, J. T., Jr. (1973) Endocrinology 93, 1349–1353
8. Segre, G. V., Rosenblatt, M., Reiner, B. L., Mahaffey, J. E., and Potts, J. T., Jr. (1979) J. Biol. Chem. 254, 6980–6986
9. Jouihomme, H., Whitfield, J. F., Chakravarty, B., Durkin, J. P., Gagnon, L., Issacs, R. J., MacLean, S., Neugebauer, W., Willick, G., and Rixon, R. H. (1992) Endocrinology 130, 53–69
10. Sonnen, D., Bindermann, I., Schluter, K-D., Wingender, E., Mayer, H., and Kaye, A. M. (1999) Biochem. J. 327, 781–785
11. Sonnen, D., Schluter, K-D., Wingender, E., Mayer, H., and Kaye, A. M. (1999) Biochem. J. 327, 863–868
12. Schluter, K-D., Hellstern, H., Wingender, E., and Mayer, H. (1989) J. Biol. Chem. 264, 11087–11092
13. Reeve, J., Meunier, P. J., Parsons, J. A., Bernat, M., Bijvoie, O. L. M., Coupon, P., Edouard, C., Kienerman, L., Neer, R. M., Renier, J. C., Slovick, D., Vismans, F. J., and Potts, J. T. (1990) Br. Med. J. 280, 1340–1344
14. Klaus, W., Dieckmann, T., Wray, V., Schomburg, D., Wingender, E., and Mayer, H. (1991) Biochemistry 30, 6936–6942
15. Barden, J. A., and Cuthbertson, R. M. (1993) Eur. J. Biochem. 215, 315–321
16. Strickland, L. A., Bozzato, R. P., Kronis, K. A. (1999) Biochemistry 38, 3211–3217
34. Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S., Wagner, G., Braun, W., Havel, T. F., Schaumann, T., Go, N., and Wuthrich, K. (1994) Biochemistry 33, 1684–1693
35. Kabsch, W., and Sander, C. (1983) Biopolymers 21, 211–217
36. Dyson, H. J., Merutz, G., Waltho, J. P., Lerner, R. A., and Wright, P. E. (1992) J. Mol. Biol. 226, 795–817
37. Sonnichsen, F. D., van Eyk, J. E., Hodges, R. S., and Sykes, B. D. (1992) Biochemistry 31, 8790–8798
38. Abbott, E., and Sheppard, R. C. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford
39. Morrisett, J. D., David, J. S. K., Pownall, H. J., and Gotto, A. M. (1973) Biochemistry 12, 1290–1299
40. Wishart, D. S., Sykes, B. D., and Richards, F. M. (1992) Biochemistry 31, 1647–1651
41. Wright, P. E., Dyson, H. J., and Lerner, R. A. (1988) Biochemistry 27, 7167–7175
42. Karle, I. L. (1996) Biochemistry 35, 387–397
43. Wishart, D. S., Sykes, B. D., and Richards, F. M. (1991) J. Mol. Biol. 222, 311–353
44. Kuipers, K., Billiter, M., and Braun, W. (1984) J. Mol. Biol. 180, 715–740
45. Dyson, J. H., Rance, M., Houghten, R. A., Wright, P. E., and Lerner, R. A. (1988) J. Mol. Biol. 201, 201–217
46. Wright, P. E., Dyson, H. J., and Lerner, R. A. (1988) Biochemistry 27, 7167–7175
47. Kuipers, K., Billiter, M., and Braun, W. (1984) J. Mol. Biol. 180, 715–740
48. Dyson, J. H., Rance, M., Houghten, R. A., Wright, P. E., and Lerner, R. A. (1988) J. Mol. Biol. 201, 201–217
49. Wright, P. E., Dyson, H. J., and Lerner, R. A. (1988) Biochemistry 27, 7167–7175
50. Kuipers, K., Billiter, M., and Braun, W. (1984) J. Mol. Biol. 180, 715–740
51. Dyson, J. H., Rance, M., Houghten, R. A., Wright, P. E., and Lerner, R. A. (1988) J. Mol. Biol. 201, 201–217
52. Kuipers, K., Billiter, M., and Braun, W. (1984) J. Mol. Biol. 180, 715–740
53. Dyson, J. H., Rance, M., Houghten, R. A., Wright, P. E., and Lerner, R. A. (1988) J. Mol. Biol. 201, 201–217
54. Kuipers, K., Billiter, M., and Braun, W. (1984) J. Mol. Biol. 180, 715–740
55. Dyson, J. H., Rance, M., Houghten, R. A., Wright, P. E., and Lerner, R. A. (1988) J. Mol. Biol. 201, 201–217
56. Kuipers, K., Billiter, M., and Braun, W. (1984) J. Mol. Biol. 180, 715–740
57. Dyson, J. H., Rance, M., Houghten, R. A., Wright, P. E., and Lerner, R. A. (1988) J. Mol. Biol. 201, 201–217
58. Kuipers, K., Billiter, M., and Braun, W. (1984) J. Mol. Biol. 180, 715–740
59. Dyson, J. H., Rance, M., Houghten, R. A., Wright, P. E., and Lerner, R. A. (1988) J. Mol. Biol. 201, 201–217
60. Kuipers, K., Billiter, M., and Braun, W. (1984) J. Mol. Biol. 180, 715–740
Structure-Activity Relation of NH$_2$-terminal Human Parathyroid Hormone Fragments

Ute Charlotte Marx, Knut Adermann, Peter Bayer, Markus Meyer, Wolf-Georg Forssmann and Paul Rösch

J. Biol. Chem. 1998, 273:4308-4316.
doi: 10.1074/jbc.273.8.4308

Access the most updated version of this article at http://www.jbc.org/content/273/8/4308

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 58 references, 10 of which can be accessed free at http://www.jbc.org/content/273/8/4308.full.html#ref-list-1