CONFORMATIONAL STUDIES OF THE SYNTHETIC FRAGMENT 1–34 OF HUMAN PARATHYROID HORMONE BY NMR TECHNIQUES

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1. Introduction

Extensive investigations have been devoted to studies of the structure–function relations in polypeptide hormones. Particular interest focused on the identification of the structural features by which the hormone would be recognized at the receptor site of the target organ, or in immunological reactions. Features of both the amino acid sequence and the three-dimensional molecular conformation can be of relevance in this context [1,2]. Progress in this area of research, therefore depends to a considerable extent on the availability of detailed structural data on polypeptide hormones. The present paper describes an investigation of the molecular conformation of a biologically active synthetic fragment of the human parathyroid hormone in aqueous solution.

Parathyroid hormone is a major factor in calcium homeostatis. Its prime target organs are bone in which it induces resorption, and kidney in which it enhances reabsorption of calcium [3]. The molecule consists of one polypeptide chain with 84 amino acid residues [4]. The amino acid sequence of the NH_2-terminal 34 residues of human parathyroid hormone has been determined [5,6]. Synthesis of the structure proposed by Brewer et al. [5] yielded a biologically active peptide PTH (1–34) [7]. The ^{1}H n.m.r. studies described in this paper showed that PTH (1–34) in aqueous solution is predominantly in a flexible extended form, but that a short segment of the polypeptide chain forms a rigid spatial structure. From comparative studies of a series of peptide fragments, this non-random spatial structure was localized in the peptide fragment PTH (20–24), i.e. –Arg–Val–Gln–Trp–Leu–.

The amino acid sequences of human parathyroid hormone proposed by two different groups [5,6] differ in several positions. One of the differences concerns the amino acid residue 22, which is either Gln [5] or Glu [6]. This paper includes also a study of the influence of this amino acid substitution on the peptide conformation.

2. Materials and methods

The synthesis of PTH (1–34) and several partial sequences thereof was described previously [7]. Additional partial sequences of PTH (1–34) which were used in the present study were synthesized according to very similar procedures.

^{1}H n.m.r. spectra were recorded on a Varian HR 220 spectrometer and a Bruker HXS 270 spectrometer. Chemical shifts are given in parts per million (ppm) relative to internal sodium-2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Additional experimental details are given in the figure captions.

3. Results and discussion

Fig.1A shows the ^{1}H n.m.r. spectrum of PTH (1–34) in aqueous solution at neutral pH and ambient temperature. Comparison with a hypothetical spectrum computed as the sum of the resonances of the constituent amino acid residues (table II.7 of [8]) indicated that this spectrum corresponds very nearly to that

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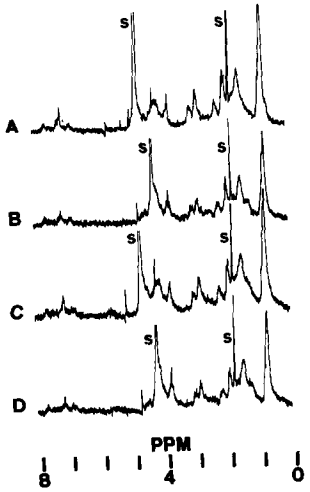

Fig.1. ^{1}H n.m.r. spectra at 220 MHz of a 0.01 M solution of the synthetic fragment 1–34 of human parathyroid hormone under different conditions of solvent medium and temperature. (A) Solution in ^2H_2O, p^{2}H = 6.8, T = 20°C; (B) Same solution as A, T = 75°C; (C) Solution in 6 M urea in ^2H_2O, p^{2}H = 6.8, T = 20°C; (D) Same solution as C, T = 75°C. (S = solvent).

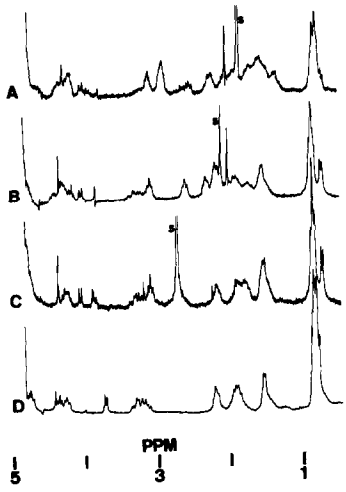

Fig.2. Spectral region from 0 to 5 ppm of the ^{1}H n.m.r. spectra at 220 MHz of four synthetic fragments of the human parathyroid hormone. Peptide concentration ca. 0.05 M, solvent ^2H_2O, T = 20°C. (A) PTH (18–34), p^{2}H = 5.0; (B) PTH (18–24), p^{2}H = 3.7; (C) PTH (20–24), p^{2}H = 6.3; (D) PTH (21–24), p^{2}H = 4.3. (S = solvent).

expected for the random coil form of PTH (1–34). This conclusion was further supported by the experiments of fig.1, B–D, which showed that the ^{1}H n.m.r. spectra of PTH (1–34) obtained under denaturing conditions were very similar to that of fig.1A. There is one small spectral difference between spectrum A and the spectra B–D in fig.1, however, which appeared from experience with other peptides and proteins [8] to be of potential interest, i.e. the shoulder on the high field side of the strong resonance of the methyl protons at approximately 0.9 ppm. This spectral difference showed that a small number of protons in PTH (1–34) experienced a noticeable conformation dependent high field chemical shift [8], indicating the presence of a local non-random spatial structure in the predominantly flexible extended polypeptide conformation.

Next we investigated the influence of the length of the polypeptide chain on the high field resonance. It was found that this spectral feature was present in PTH (18–34) (fig.2A) and in PTH (18–24) (fig.2B). The observation that the spectra of PTH (1–12) and PTH (25–34) did not contain this line gave confirmatory evidence that the spatial structure causing the high field resonance was located in PTH (18–24). Because of the comparatively small size of the peptide and the correspondingly small number of mutually overlapping methyl proton resonances at around 0.9 ppm, the high field resonance in PTH (18–24) appeared as a well resolved doublet of intensity corresponding to three protons (fig.2B). It could thus be assigned to a methyl group of an aliphatic amino acid side chain. In subsequent experiments, the high field methyl doublet resonance was also observed in PTH (19–24) and PTH (20–24) (fig.2C); it was not present in the shorter peptides PTH (21–24) (fig.2D), PTH (22–24) and PTH (23–24).

The amino acid sequence of PTH (20–24) is H-Arg–Val–Gln–Trp–Leu-OH [5]. The peptide thus contains four aliphatic methyl groups. From the relative resonance intensities, three of the methyls are observed in the 'random coil position' [8] at approximately 0.90 ppm and one methyl group is at 0.79 ppm. In analogy to PTH (1–34) (fig.1), PTH (20–24) could be denatured, e.g. by dissolving the peptide in dimethylsulfoxide. In the denatured peptide all four methyl resonances were at 0.90 ppm (fig.3). This experiment thus showed again that the high field shift of one methyl resonance was a consequence of the three-dimensional molecular conformation rather

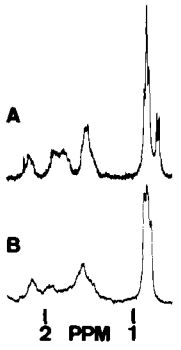

Fig.3. Spectral region from 0 to 3 ppm of the ^1H n.m.r. spectra at 220 MHz of 0.05 M solutions of the synthetic fragment 20–24 of the human parathyroid hormone in different solvents, $T = 20°C$. (A) solvent ^2H_2O, $p^2H = 6.3$; (B) solvent d_6-DMSO.

than the amino acid sequence.

Considering the primary structure [5], the high field methyl line could come either from Val-21 or from Leu-24. With spin decoupling experiments at 270 MHz and by comparison with the spectra of the shorter peptides PTH (n–24), n = 20, 21, 22 and 23, all the resonances in the hexapeptide PTH (19–24), H-Glu–Arg–Val–Gln–Trp–Leu-OH, were identified (fig.4). The high field shifted methyl resonance was thus found to come from Val-21.

In the amino acid sequence of human parathyroid hormone proposed by Potts' group [6] the amino acid residue 22 is Glu. The ^1H n.m.r. spectrum of H-Arg–Val–Glu–Trp–Leu-OH was found to be essentially identical with that of PTH (20–24) in fig.2C. Hence the amino acid substitution Gln-22 → Glu had no noticeable effect on the local conformational feature manifested in the high field methyl resonance.

The conformational feature of PTH (1–34) manifested in the high field shift of one methyl proton resonance of Val-21 thus localized to the peptide segment 20–24, –Arg–Val–Gln(Glu)–Trp–Leu– , the question arises as to the details of this spatial structure. Two additional n.m.r. parameters of Val-21 appear to be particularly relevant in this context. These are an upfield shift of the Val β-proton resonance of approx. 0.3 ppm as compared to the random coli position [8], and a value of approx. 8.0 Hz for the vinical spin–spin coupling constant $^3J_{\alpha\beta}$. Independently, these two

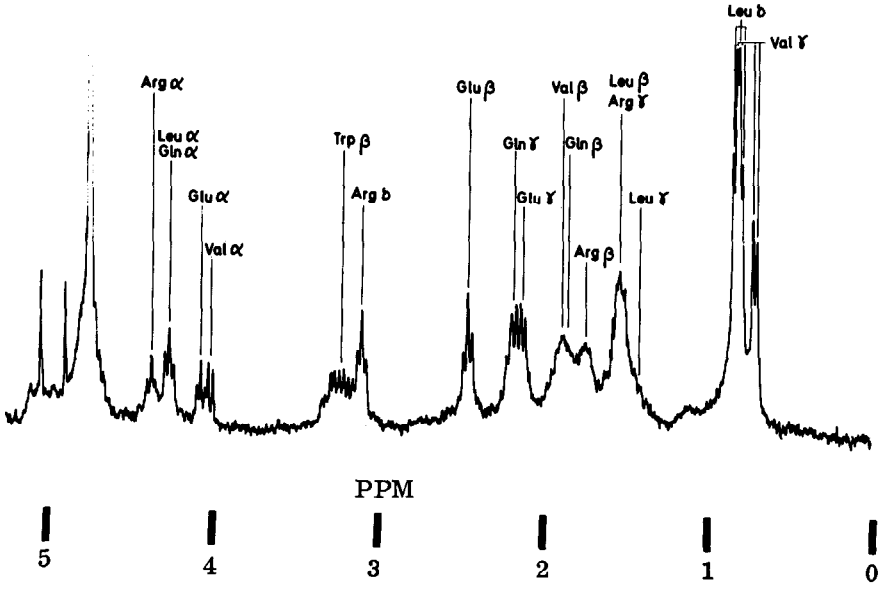

Fig.4. Spectral region from 0 to 5 ppm of the ^1H n.m.r. spectrum at 270 MHz of a 0.05 M solution in ^2H_2O of the synthetic fragment 19–24 of the human parathyroid hormone, $p^2H = 3.5$, $T = 24°C$. The resonance assignments obtained from comparison with smaller peptide fragments and from double resonance experiments are also indicated. All the resonances were observed with the exception of the C^α proton of Trp, which is probably covered by the HDO resonance at 4.8 ppm.

observations give evidence for a non-random population of the rotation states about the $C^{\alpha}-C^{\beta}$ bond of valine; $^{3}J_{\alpha\beta}$ = 8 Hz corresponds to a preferred population of the trans orientation of the valine side chain of approx. 0.5. Even though this information is not sufficient for a detailed description of the spatial structure, it makes readily comprehensible that the two methyl groups experience different conformation dependent chemical shifts. Considering the amino acid sequence of PTH (20–24), different exposure of the two methyls to the ring current field of Trp-23 [8] appears to be a likely cause for the upfield shifts of one methyl resonance and the β-proton resonance of Val-21. Alternatively, high field shifts could also arise from the electrostatic effects of nearby carbonyl groups [9].

In conclusion, the experiments described in this paper showed that PTH (1–34) is overall predominantly in an extended flexible conformation. It includes, however, a local non-random spatial structure involving exclusively residues of the peptide segment 20–24. This unique structural feature is maintained after the amino acid substitution Gln-22 → Glu. Therefore, in as far as they depend on the spatial arrangement of the segment 20–24, the functional properties of the human parathyroid hormone primary structures proposed by Brewer [5] and by Potts [6] should be very similar.

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