A NOVEL APPROACH FOR STUDIES OF THE MOLECULAR CONFORMATIONS IN FLEXIBLE POLYPEPTIDES

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1. Introduction

This paper outlines a novel experiment in which the occurrence of energetically favored conformations of proline-containing peptide molecules would be manifested in the equilibrium between the *cis* and *trans* forms of the X–Pro peptide bonds. The proposed method is based on ^{13}C NMR observations in simple linear oligopeptides which indicate that the ratio of *cis:trans* proline in 'random coil' polypeptide chains is characteristic for the amino acid sequence in the immediate environment of the prolyl residue, and that this equilibrium can be sensitive to the formation of energetically favored flexible molecular conformations which could otherwise in most cases not be detected by the common experimental techniques.

The proposed method should be of particular interest for investigations of medium size linear polypeptides, where the folding of the molecule is very little restricted by the covalent structure [1]. It might thus be used for studies of a variety of polypeptide hormones which have been found by other methods [2–4] to exist in flexible 'random coil' solution conformations. More detailed insight into the molecular species formed by this class of compounds is of much interest because it appears that different molecular conformations in solution, in complexes with carrier proteins, and at the receptor site might be intimately related with the biological roles of these polypeptide hormones [5], and there is little hope that relevant structural information on these molecules will come from single crystal X-ray studies [5]. Other applications of the proposed technique might include models for investigations of the minimal requirements of chain length, amino acid composition etc. for the existence of preferred flexible conformations in linear oligopeptides, or studies of solvent effects on the relative stabilities of different peptide conformations.

2. Principle of the proposed method

For the purpose of the present discussion we shall assume that the polypeptide chains consist of planar standard peptide groups [1] which are linked by single bonds. Except for the N-substituted peptide groups, where the *cis* and *trans* forms are included into the considerations, the peptide bonds are taken to be in the *trans* conformation. For a given primary structure, different backbone conformations can then be generated by variations of the torsion angles ϕ_i and ψ_i about the single bonds linking the peptide groups, and ω_i about X–Pro, and possibly other N-substituted peptide bonds [1]. This description, which does not allow for non-planar peptide bonds [6,7], should be adequate for flexible linear polypeptide chains. Additional torsion angles χ_i^j yield an analogous description for the orientations of the amino acid side chains [1].

For a variety of synthetic and biological linear polypeptides, studies with various experimental techniques, e.g. high resolution ^1H NMR [2,4] and ORD [3], have indicated that extended 'random coil' forms are the predominant species in solution. Because these non-globular extended forms can in general not be sizeably stabilized by mutual interactions among the different amino acid side chains, the energy contours for a peptide of this type will be characterized by wide regions encompassing a multitude of conforma-

tions of very similar energies, which differ in the torsion angles ϕ_i, ψ_i, and χ_i^j. Because of the low barriers for rotational motions about single bonds, there will be rapid interconversions among these species. Hence on the time scale of the common suitable experimental techniques, and with the available spectral resolution, e.g. in ^{1}H NMR and ORD, an average 'random coil' spectrum will often be observed at ambient temperature [2–4]. On the other hand, there is a barrier of $\Delta G^{\neq} \approx 20$ kcal Mol^{-1} for interchange between conformations containing the *cis* and *trans* forms of the X–Pro peptide bonds [8]. These interconversions will therefore be slow on the time scale of most of the relevant experimental techniques, so that the two species are observed separately if a sufficient spectral resolution can be obtained. The slow rate of the *cis–trans* isomerization of X–Pro is an essential factor for the proposed application of the *cis–trans* equilibrium as a probe for the detection of energetically favored flexible conformations.

As was shown previously [7–11] and will be evidenced with some new examples in this paper, ΔG° for the equilibrium between the *cis* and *trans* forms of X–Pro is of the order –2.0 to +2.0 kcal Mol^{-1} in a variety of linear oligopeptides, so that the two forms are simultaneously present at ambient temperature. On the basis of the experiments described below, we propose that ΔG° for the *cis–trans* equilibrium of a single prolyl residue in a polypeptide chain can be decomposed into two terms $\Delta G^\circ_{str.}$ and $\Delta G^\circ_{conf.}$. The structural term $\Delta G^\circ_{str.}$ is typical for a sequence –CO–X(1)–Pro(2)–X(3)–, where X(1) and X(3) are two of the common amino acids.* Deviations from the proline cis:trans ratio determined by $\Delta G^\circ_{str.}$ for a given fragment of this type in a polypeptide chain would only arise if the free energy differences between the ensembles of rapidly interconverting conformations containing *cis* and *trans* proline, respectively, were different from $\Delta G^\circ_{str.}$. In other words, $\Delta G^\circ_{conf.}$ would only be different from O, if the populations of the rotational states described by the torsion angles ϕ_i, ψ_i, and χ_i^j were different in the

* It is quite likely that the relevant fragment is actually –CO–X(1)–P(2)–NH–. Additional experiments are in progress to investigate in more detail the role of the amino acid X(3).

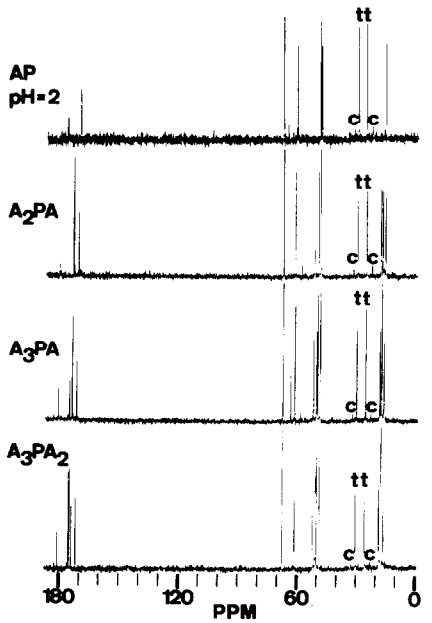

Fig. 1. Proton noise-decoupled ^{13}C NMR spectra at 25.16 MHz of solutions in D_2O of four peptides containing alanyl (A) and prolyl (P) residues. T = 25°. The letters c and t identify the resonances of the β- and γ-ring carbons of *cis* and *trans* proline, respectively [9–12].

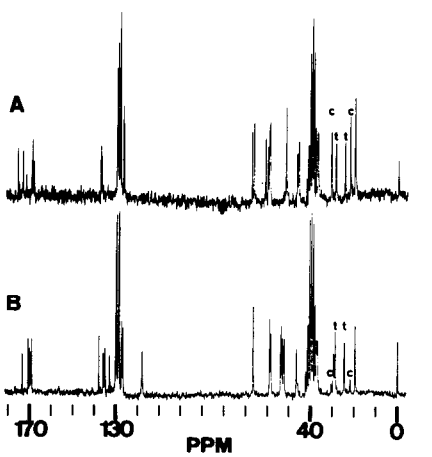

Fig. 2. Proton noise-decoupled [^{13}C]NMR spectra of solutions in DMSO-d_6 of two linear proline-containing peptides. T = 40°.
A) H–L–Thr–L–Phe–L–Pro–OH
B) H–L–Phe–L–His–L–Thr–L–Phe–L–Pro–OH
The letters c and t identify the resonances of the β- and γ-ring carbons of *cis* and *trans* proline, respectively [9–12]. The solvent resonance is at 39.8 ppm.

molecular species which contain *cis* and *trans* proline, respectively. The occurrence of energetically non-equivalent manifolds of flexible polypeptide conformations would thus in many cases be manifested in the *cis–trans* equilibrium of X–Pro, even if $\Delta G^{o}_{conf.}$ were as small as several tenth of one kcal Mol^{-1}.

Only ^{13}C NMR will be considered for the experimental measurements of the *cis–trans* equilibrium of X–Pro [9–12]. In smaller peptides, the ^{13}C NMR of cis and trans proline can be identified in the natural abundance ^{13}C NMR spectra (figs. 1 and 2) [9–12]. The application of the method to larger polypeptides with more than approximately 10 to 15 amino acid residues will probably require the introduction of ^{13}C-enriched proline into the molecules. This should not pose any major problems, since most of the polypeptide hormones and model peptides which are of interest in this context have already been synthesized by chemical methods.

3. Material and methods

The peptides mentioned in fig. 1 were purchased from Bachem AG, Liestal, Switzerland. The other peptides were obtained from Drs. W. Rittel and M. Brugger, Ciba-Geigy AG, Basel. The ^{13}C NMR spectra were recorded on a Varian XL-100 spectrometer using the Fourier Transform method.

4. Experimental results

This section reports ^{13}C NMR measurements of the equilibrium between *cis* and *trans* proline in a series of linear peptides with the generalized sequence $H–A_m–P–A_n–OH$, where A and P are L-alanine and L-proline, respectively. In addition, an example is presented where there is evidence for the occurrence of energetically preferred flexible conformations, i.e. $\Delta G^{o}_{conf.} \neq 0$.

The peptides consisting exclusively of alanine and proline were chosen to reduce to a few parameters the variations of the peptide structures which might affect the *cis–trans* equilibrium of proline, i.e. chain lengths m and n, and the electric charges on the end groups. In the dipeptide H–A–P–OH, the *cis* proline concentrations varies sizeably during a pH-titration [7]. In $^{\oplus}H_2–A–P–OH$, there is approximately 10% (fig. 1), in $^{\oplus}H_2–A–P–O^{\ominus}$ 40%, and in $H–A–P–O^{\ominus}$ 55% of cis proline. A comparatively small pH-dependence is observed for H–A–P–A–OH. In $H–A_2–P–A–OH$ (fig. 1), $H–A_2–P–A_2–OH$, $H–A_3–P–A–OH$ (fig. 1), and $H–A_3–P–A_2–OH$ (fig. 1), the equilibrium between *cis* and *trans* proline is essentially unaffected by the charges on the end groups. In aqueous solutions between pH 1.0 and 12.0 these molecules contain approximately 10% of *cis* proline [7]. We conclude that no energetically preferred flexible conformations are present in aqueous solutions of these molecules, i.e. that the rotational states described by the torsion angles ϕ_i, ψ_i, and χ^j_i are very nearly equally populated in the molecules which contain *cis* and *trans* proline, respectively.

The data on the peptides $H–A_m–P–A_n–OH$ indicate that the fragment –CO–A–P–A– could be used as a probe for the occurrence of preferred flexible conformations.* Since $\Delta G^{o}_{str.}$ for the equilibrium between the *cis* and *trans* forms of –X–Pro– appears to be quite sensitive to the nature of the amino acid X [7], this quantity will have to be determined experimentally for different fragments –CO–X(1)–P(2)–X.(3)– which might be applied as conformational probes.* In view of future experiments with the polypeptide hormone calcitonin M [3,4], we are very interested in the fragment –CO–L-Phe–L-Pro–X– [7]. Fig. 2 shows the ^{13}C NMR spectra of two partial sequences of calcitonin M [13] which contain the peptide group L–Phe–L–Pro. It is seen that the equilibrium between cis and trans proline is markedly affected by variation of the covalent structure outside the immediate environment of the prolyl residue. In solution in DMSO-d_6, H–L-Thr–L-Phe–L-Pro–OH contains approximately 60%, H–L-Phe–L-His–L-Thr–L-Phe–L-Pro–OH approximately 15% of *cis* proline. We conclude from fig. 2, and from other studies of L-Phe–L-Pro–containing peptides [7], that we have here an example where the cis–trans equilibrium of proline is affected by the overall molecular conformations, i.e. $\Delta G^{o}_{conf.} \neq 0$.

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