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Conformational preferences of the sequential fragments of the hinge region of the human IgA1 immunoglobulin molecule

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The mean solution conformation of tetrapeptide fragments spanning the hinge region of human IgA1 was investigated by CD and ^{13}C-NMR methods. Distinct conformational differences for the partial sequences of IgA1 were found. In a series of tetrapeptides having the Thr-Pro-Pro-Thr sequence, the Pro-Pro fragment was ordered to the structure of a type II polyproline helix, but with unordered forms prevailing in the equilibria. In the case of the Pro-Pro-Thr-Pro sequence, a distinct preference for the β-turn conformation was found. Acetylation of this tetrapeptide shifts the equilibrium towards unordered forms containing some elements of the type II polyproline helix. The peptide Thr-Pro-Ser-Pro exists predominantly in the β-turn conformation whereas Pro-Ser-Pro-Ser-NH_2 has, for the most part an unordered conformation.

1. Introduction

The hinge region of the human IgA1 immunoglobulin molecule contains the sequence [1,2]:

... Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-
225
Pro-Ser-Thr-Pro-Pro-Thr...
235

This sequence is sensitive to the action of proteolytic enzymes. Several human bacterial pathogens produce extracellular proteases which are specific for human IgA1. Recently [3], the amino acid sequence of the first IgA1 protease, isolated from the Gram-negative diplococcus *Neisseria gonorrhoeae*, was determined.

In order to investigate the fragments of the IgA1 peptide chain as IgA1 proteinase inhibitors, one of us (J.B.) synthesized the following series of tetrapeptides

Thr-Pro-Pro-Thr	(I)
Thr-Pro-Pro-Thr-NH_2	(II)
Ac-Thr-Pro-Pro-Thr	(III)
Ac-Thr-Pro-Pro-Thr-NH_2	(IV)
Pro-Pro-Thr-Pro	(V)
Pro-Pro-Thr-Pro-NH_2	(VI)
Ac-Pro-Pro-Thr-Pro	(VII)
Ac-Pro-Pro-Thr-Pro-NH_2	(VIII)
Thr-Pro-Ser-Pro	(IX)
Pro-Ser-Pro-Ser-NH_2	(X)

Ultimately, we shall compare the inhibitory properties of these peptides with their conformational preferences. The mean solution conformation of peptides I–X was investigated by circular dichroism (CD) and ^{13}C-NMR measurements. These were performed in aqueous solution, at three different pH values (i.e., in acidic, neutral and basic media). In the analysis of the data, results obtained during the investigation of tetra-

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peptides of the general formula $(Ala)_3Pro$ and $(Ala)_3Thr$ [4–6] were used. As important conformational characteristics the chemical shifts of the C^{β} atoms of specific residues (recalculated onto the hydantoin scale [7], were employed. During this investigation, clear evidence of conformational differences between peptides I–IV and peptides V, VI and IX was obtained. Peptides belonging to the first group are probably unordered (with the structure of the poly-Pro II type helix dominating in the fragment Pro-Pro) while those in the other prefer the β-turn structure. Acetylation of the sequence Pro-Pro-Thr-Pro leads to an unordered conformation.

2. Materials and methods

Details of the syntheses of tetrapeptides I–X will be given in a separate paper by one of us (J.B.). All compounds were purified using HPLC. Homogeneity was verified by TLC in three different solvent systems.

CD measurements were carried out using a Jobin-Yvon mark III dichrograph at room temperature with light paths of 1, 2 and 10 mm. For each compound spectra were recorded at three pH values: acidic (pH 2.2–2.6), neutral (pH 6.0–6.7) and alkaline (pH 10.3–11.1). The concentration of solutions varied from 0.0379 to 0.1085 g/dm^3. Results of CD measurements are expressed as the molar ellipticity values.

^{13}C-NMR spectra were recorded in ^2H_2O on a Tesla 567 spectrometer at an operating frequency of 25.142 MHz with dioxane as an internal standard. The chemical shift of dioxane carbon atoms was assumed to be 67.4 ppm on the TMS scale. For each compound, measurements were carried out at three pH values: acidic (pH 2.0–2.4), neutral (pH 6.5–7.5) and alkaline (pH 10.0–10.8). The concentration of solutions varied from 12.0 to 36.0 g/dm^3. The pH of solutions was adjusted with either 4 N HCl or NaOH and measured with a Mera-Elwro N-517 pH-meter.

Absolute values of the angles, θ (angle in the moiety C^{β}–C^{α}–C′–O) were calculated from the equation (7):

$$|\theta| = 49.7 + 19.4\Delta_h$$

The coefficients, Δ_h, were evaluated assuming the following C^{β} chemical shifts for the corresponding hydantoins: C^{β}_{Pro}, 26.65; C^{β}_{Thr}, 65.13; C^{β}_{Ser}, 60.19.

3. Results and discussion

The results of the CD measurements are summarized in table 1. As an illustration of these data, selected CD curves are also shown (figs. 1–3). The CD spectra of tetrapeptides I–IV are quite similar, which signifies that blocking of the terminal amine and carboxylate groups has only a secondary influence on the mean conformation of the peptides. In the case of the free tetrapeptide I, there appear in the CD spectrum (fig. 1) two distinct Cotton effects in acidic solution: a small positive one at 220 nm and a strong negative one at 200 nm. This spectrum closely resembles those of collagen and the poly-Pro II helix [8]. It is, however, also quite similar to that spectrum of polylysine in unordered conformation (ref. 9 and references cited therein). Increasing the pH causes the disappearance of the positive CD band. At the same time, the negative extremum increases distinctly and in the region of 220 nm a new negative shoulder is formed. The small positive Cotton effect was not observed in the case of peptides II–IV. Their CD spectra are similar to those of peptide I in neutral and basic solutions. Increasing pH influences the CD spectra of compounds II–IV differently: whereas the negative band at 200–203 nm is most intense in the case of peptide II in acidic solution, for peptide III the effect increases with increasing pH. The increase in the negative band with increasing pH was also noted for the fully protected peptide IV, which suggests that the changes observed in the CD spectra are not directly connected with ionization of the terminal amino and carboxylate groups. It appears that they are connected, rather, with the influence of pH on the phenomenon of peptide aggregation, which may indirectly influence the conformational equilibria in solution. It is difficult to decide on the basis of CD measurements, whether the mean solution conformation of these peptides is dominated by unordered forms or if the same ordering seen with a poly-Pro II type helix occurs.

Table 1

CD spectral data of tetrapeptides I–X

Wavelengths expressed in nm; molar ellipticities in degree $cm^2\ dmol^{-1}$ ($\times 10^{-4}$).

Tetrapeptide		λ (nm)/θ (°)										
		pH 2				pH 7				pH 10		
		Minimum	Shoulder	Maximum	Zero	Minimum	Shoulder	Maximum	Zero	Minimum	Shoulder	Zero
(I)	Thr-Pro-Pro-Thr	200/−4.12	215.5/0	222/0.41	234/0	200/−4.93	222/0	225/0.06	232/0	200/−6.58	232/−0.52	248/0
(II)	Thr-Pro-Pro-Thr-NH_2	200/−6.91	241/−0.53		255/0	200/−4.80	224/−0.05		225/0	200/−5.28	230/−0.44	240/0
(III)	Ac-Thr-Pro-Pro-Thr	200/−5.08	232/−0.24		244/0	202/−5.66	230/−0.21		238/0	203/−6.71	228/−0.52	244/0
(IV)	Ac-Thr-Pro-Pro-Thr-NH_2	202/−5.91	232/−0.40		245/0	202/−6.07	231/−0.50		346/0	203/−6.89		250/0
(V)	Pro-Pro-Thr-Pro	209/−2.69			244/0	212/−2.97			245/0	213/−3.08		244/0
(VI)	Pro-Pro-Thr-Pro-NH_2	210/−2.37			250/0	210/−2.36			247/0	211/−2.24		255/0
(VII)	Ac-Pro-Pro-Thr-Pro	203/−5.04	228/−0.58		244/0	204/−4.67			240/0	205/−4.78		245/0
(VIII)	Ac-Pro-Pro-Thr-Pro-NH_2	201/−4.81			242/0	203/−4.81	222/−1.47		244/0	202/−5.41		246/0
(IX)	Thr-Pro-Ser-Pro	209/−1.78			247/0	213/−2.01			246/0	214/−2.73		248/0
(X)	Pro-Ser-Pro-Ser-NH_2	198/−3.04	234/−0.41	239/−0.10	248/0	197/−3.02	213/−0.56		241/0	198/−3.04	220/−1.00	244/0

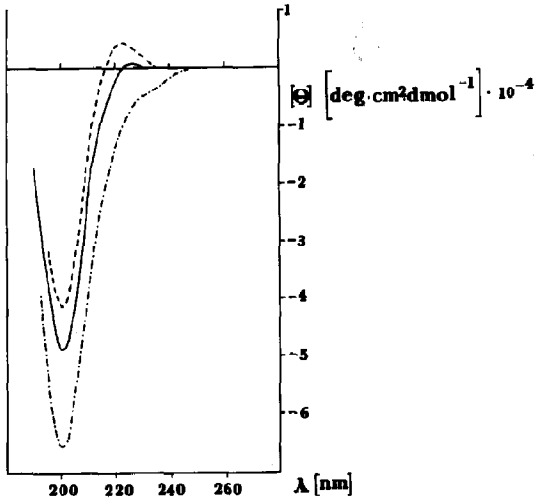

Fig. 1. CD spectra of H-Thr-Pro-Pro-Thr-OH in water at three pH values. (———) pH 6.4, (- - - - -) pH 2.3, (·-·-·) pH 11.3.

The last conformation may be taken into account for peptide I in acidic medium: increasing pH probably shifts the conformational equilibrium more towards the unordered forms. Some evidence of the appearance of ordering in the fragments Pro^2-Pro^3 of these peptides results, however, from ^{13}C-NMR data (see below).

A different situation occurs in the case of tetrapeptides V and VI (see fig. 2). In their CD spectra, only one negative effect located at 210 nm is seen. Increasing pH does not influence the CD spectrum of VI. In the case of V, it evokes a shift of the negative extremum to 213 nm, accompanied by an increase in intensity. The spectra resemble those given by a β-turn conformation [9,10]. This is probably due to the presence of Pro and Thr residues in positions 2 and 3 of the peptide chain, respectively. The preference of the Pro residue located in position $(i+1)$ for a type I β-turn conformation is very well known. From our investigation, it follows [6] that the Thr residue stimulates β-turn formation by occupying position $(i+2)$ in the definite sequence. It was also found [4,5] that Pro in position $(i+3)$ of the tetrapeptide sequence favors a β-turn. Thus, in peptides V and VI, we have the proper location of three amino acid residues which consonantly stimulate β-turn formation.

It is very interesting that acetylation of the N-terminal group in V and VI destabilizes the β-turns. This is demonstrated in the case of peptides VII and VIII by a strong increase in the negative extremum, which is additionally shifted to 205 nm. Comparison of the CD data obtained for peptides I–IV with those of VII and VIII suggests that the mean conformations may be similar in both cases. Increasing the pH does not influence the CD spectra of VII and VIII to a great extent. Thus, acetylation of the terminal residue strongly reduces the tendency of the Pro-Pro-Thr-Pro sequence to create a β-turn structure.

The CD spectra of tetrapeptide IX (fig. 3) also show the presence of a distinct amount of β-turn structure in conformational equilibrium. An increase in pH is accompanied here by a bathochromic shift of the maximum position (to 215 nm) and an increase in absolute ellipticity. A different situation is found in the case of peptide X (fig. 3). In the CD spectra of this compound, two negative Cotton effects, at 220 and 194–197 nm, are visible. It seems that the unordered forms dominate the equilibrium. Indeed, the amino acid sequence of this peptide does not stimulate forma-

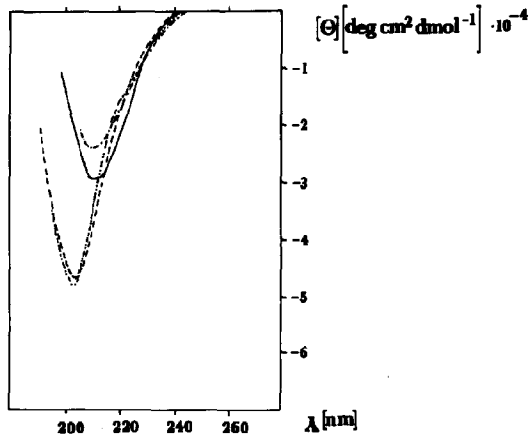

Fig. 2. CD spectra of compounds V–VIII in water at neutral pH. (———) H-Pro-Pro-Thr-Pro-OH, (- - - - -) Ac-Pro-Pro-Thr-Pro-OH, (·-·-·) H-Pro-Pro-Thr-Pro-NH_2, (·-·-·-) Ac-Pro-Pro-Thr-Pro-NH_2.

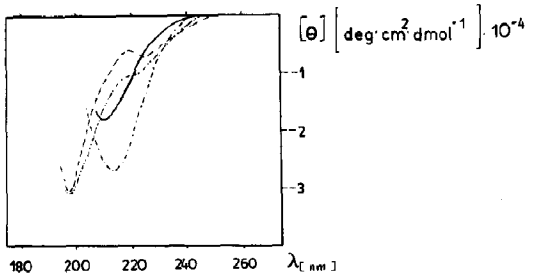

Fig. 3. CD spectra of compounds IX–X in water at acidic and alkaline pH. (———) H-Thr-Pro-Ser-Pro-OH, pH 2.3; (·–·–·) H-Thr-Pro-Ser-Pro-OH, pH 11.1; (- - - - -) H-Pro-Ser-Pro-Ser-NH_2, pH 2.3; (···–···–) H-Pro-Ser-Pro-Ser-NH_2, pH 11.1.

tion of the β-turn structure (according to our observations [5], Pro in positions 1 and 3 of a tetrapeptide destabilizes the β-turn).

In the ^{13}C-NMR spectra of peptides I–X, apart from resonances of *trans*-Pro forms (i.e., a *trans* configuration of the X–Pro amide bond) resonances of *cis* forms are also visible. Because of difficulties in the assignment of particular *cis* resonances to specific residues, they were not analyzed in detail. Approximative amounts of the *cis* forms were determined from the ratio of intensities of the *trans*- and *cis*-Pro resonances (table 2). The amount of *cis* form is often used as a measure of the conformational lability of a peptide, i.e., an increasing amount of *cis* form may be considered to be a demonstration of increasing conformational lability for a particular peptide. In the cases investigated here, the amount of the *cis* forms reaches 10–20%. It is worth noting that the least amount of *cis* form was observed (table 2) in the case of peptide IX, for which, on the grounds of the CD data, stabilization of the β-turn structure was postulated. The corresponding value for peptide X (for which an unordered conformation was postulated) is about 20%. The amount of *cis* form also increases, when compared with peptides V and VI, for the acetylated peptides VII and VIII. This is in good agreement with the conclusion that acetylation of Pro-Pro-Thr-Pro results in disordering of the peptide chain. A low content of *cis* form also characterizes peptide I; acetylation of this peptide gives rise to an increase in amount of *cis* isomers in the equilibrium. Thus, it may be concluded that peptides I and IX are characterized by having the greatest extent of conformational uniformity. This conclusion corresponds well with that from analysis of the CD data.

In tables 3–5 the results of the ^{13}C-NMR measurements are summarized. In the assignment of resonances, data obtained from model tetrapeptides with the formulae $(Ala)_3Pro$ and $(Ala)_3Thr$ were used. An additional control of the correctness of the assignments was the comparison of resonance positions for the entire series of peptides.

As can be seen from table 3, both proline residues in peptides I–IV differ in C^{β} chemical shifts; the difference reaches a value of about 1.0 ppm, e.g., in the case of peptide I, it is equal to 1.2 ppm at pH 2.0 and 0.97 ppm at pH 10.0. This difference is connected with the steric effect exerted by the Pro^3 residue on the antecedent Pro, since in the case of peptide IX (with sequence Thr-Pro-Ser-Pro) no such differentiation of Pro residues is observed. Such effects of Pro are well known. According to our interpretation [4], they consist of the closing of the dihedral angle θ of the moiety C^{β}–C^{α}–C′–O.

The coefficients, Δ_h, calculated for the Pro^2 and Pro^3 residues of peptides I–IV [7], range from

Table 2

Approximate percentages of *cis*-Pro forms in conformational equilibrium (accuracy ±5%), as estimated from the ratio of the intensities of resonances

pH	Tetrapeptide									
	I	II	III	IV	V	VI	VII	VIII	IX	X
2	10 [a]	<10 [b]	20 [c]	25 [a]	10 [a]	15 [b]	20 [a]	20 [a]	<10 [a]	15 [a]
7	15 [a]	10 [a]	20 [a]	15 [a]	15 [a]	15 [a]	30 [a]	15 [a]	10 [a]	15 [a]
10	10 [b]	20 [a]	15 [a]	10 [a]	20 [b]	10 [b]	20 [a]	20 [a]	10 [a]	20 [a]

[a] C^{β}; [b] C^{γ}; [c] C^{δ}.

Table 3

^{13}C chemical shifts for tetrapeptides I–IV in $^{2}H_2O$ at three pH values

pH:	Carbon atom	(I)				(II)					(III)					(IV)					
		Thr	Pro	Pro	Thr	Thr	Pro	Pro	Thr	NH_2	Ac	Thr	Pro	Pro	Thr	Ac	Thr	Pro	Pro	Thr	NH_2
2	α-Pro		60.00	61.20			60.08	61.20					59.78	61.20				59.78	61.20		
	β-Pro		28.78	29.98			28.93	30.05					29.08	30.12				29.00	30.05		
	γ-Pro		25.49	25.30			25.49	25.49					25.50	25.36				25.42	25.42		
	δ-Pro		48.58	49.25			48.58	49.25					48.65	49.25				48.65	49.17		
	α-Thr	57.91			59.41	57.91			59.48			58.06			59.03		57.99			59.48	
	β-Thr	66.88			68.15	66.88			67.85			68.09			68.20		67.85			67.85	
	γ-Thr	19.55			19.89	19.59			19.59			19.47			19.67		19.34			19.59	
	Ac										22.43					22.36					
7	α-Pro		61.42	61.42			60.00	61.13					61.42	61.20				60.00	61.27		
	β-Pro		29.01	30.05			29.01	30.05					29.08	30.05				29.08	30.05		
	γ-Pro		25.50	25.35			25.49	25.49					25.50	25.36				25.50	25.50		
	δ-Pro		48.65	49.32			48.58	49.25					48.73	49.25				48.73	49.25		
	α-Thr	57.91			60.08	59.91			59.56			58.06			59.78		57.99			59.56	
	β-Thr	66.88			68.81	66.95			67.85			67.85			68.89		67.92			67.92	
	γ-Thr	19.59			20.04	19.59			19.59			19.59			20.04		19.59			19.59	
	Ac										22.43					22.43					
10	α-Pro		61.35	61.20			59.63	61.20					61.35	61.20				59.78	61.27		
	β-Pro		29.08	30.05			28.93	30.05					29.08	30.05				29.08	30.05		
	γ-Pro		25.42	25.42			25.42	25.42					25.50	25.35				25.50	25.50		
	δ-Pro		48.65	49.10			49.65	49.02					48.65	49.25				48.65	49.25		
	α-Thr	58.51			59.71	58.57			59.48			58.06			59.78		57.99			59.56	
	β-Thr	70.16			68.97	70.16			67.85			67.91			68.89		67.92			67.92	
	γ-Thr	19.35			20.04	19.30			19.60			19.59			20.04		19.59			19.59	
	Ac										22.43					22.43					

2.16 to 2.43 for Pro^2 and 3.33–3.47 for Pro^3. This corresponds to a value of θ between 91 and 96.8° for Pro^2 and 114–117° for Pro^3. Correspondingly, the angles ψ of the Pro^3 residue are about −55° (the other possible value of about 175° is unlikely). This value is characteristic of a *cis'* configuration of the prolyl residue. Similar ψ values have been reported for model tetrapeptides from the $(Ala)_3Pro$ series [4,5], as well as in the case of tuftsin and its analogues [11].

From the θ values calculated for the Pro^2 residue, ψ values of −31.0 to −36.8 or 152 to 156.8° may be proposed. The second set of values corresponds to the conformation of prolyl residues being of the poly-Pro II type (i.e., the *trans'* configuration of Pro). Because of the distinct difference in Δ_h values observed for Pro^2 and Pro^3, it can be assumed that the conformation of these two residues must be markedly different, i.e., if it is assumed that the configuration of Pro^3 is *cis'*, Pro^2 appears to be in the *trans'* configuration. Thus, in the case of peptides I–IV, the central moiety (Pro^2-Pro^3-, dipeptide) favours a local conformation of the poly-Pro II type helix. This conclusion does not exclude the assumption that a distinct amount of unordered peptide conformations may be present in the conformational equilibria of peptides I–IV. Increasing pH appears to shift the equilibria towards the unordered forms.

Thus, summarizing the CD and ^{13}C-NMR data, we can conclude that in the case of peptides I–IV, the local ordering of the Pro^2-Pro^3 fragment of the peptide chain to a poly-Pro II structure appears probable. However, such forms remain in the equilibrium with unordered conformations of the peptides.

In the case of peptides V and VI (table 4), the C^{β}_{Pro} chemical shifts (and Δ_h coefficients) of the Pro^2 and Pro^4 residues are typical of *cis'* configurations. The corresponding signal for Pro^1 is shifted to higher fields. The origin of the shift, however, is complex. Besides the steric influence of the Pro^2 residue, the effect of protonation of the Pro^1 residue on its C^{β} chemical shift must be taken into acount. The CD spectra suggest that steric interaction of Pro^1 and Pro^2 residues is of secondary significance in determining the mean solution conformation of these two peptides. The tendency of the Pro-Thr-Pro sequence to form the β-turn structure seems to be more important here. Another situation arises when the Pro^1 residue is acetylated (peptides VII and VIII). Here, the conformation of the Pro^2 and Pro^4 residues remains *cis'*. The interaction of residues Pro^1 and Pro^2 probably becomes the most important factor influencing mean peptide conformation, shifting the conformational equilibrium towards the unordered state (see CD data) with perhaps some contribution from the poly-Pro II type conformation. The conformational differences between the residues Pro^1 and Pro^2 in peptides VII and VIII are not as marked as these observed for Pro^2 and Pro^3 in the series I–IV. This conclusion results from a comparison of the ^{13}C-NMR data. In the series I–IV, the difference in C^{β} chemical shifts of both prolines is about 1.0 ppm. This reaches values of 0.60–0.67 ppm for VII and 0.53–0.61 ppm for VIII. Values of ψ for residue Pro^2 in peptides V–VIII are within the range −54 to −58.6°. Such values are indeed near typical of the $(i+1)$ residue in a β-turn conformation.

Our data (table 4) suggest that the mean value of the conformational angle ψ of the Thr^3 residue in peptides V–VIII is similar to that of residues Pro^2 and Pro^4. This is demonstrated by the similarity of the Δ_h coefficients. They occur, in the case of Thr^3, in the range 2.73–2.94 ppm. Blocking the terminal amino and carboxylate group in the sequence Pro-Pro-Thr-Pro does not change the C^{β} chemical shift of the Thr^3 residue significantly.

The conformational similarity (with respect to the mean value of ψ) of Pro and Thr residues has also been observed by us for model tetrapeptides [6], as well as for some tuftsin analogues [11]. A different mean conformation, however, is possessed by Pro and Thr in the corresponding *N*-acetyl-*N'*-methylamides (I.Z. Siemion and B. Picur, unpublished results).

The $C^{\beta}_{Thr^3}$ chemical shift is very similar to that observed for Thr in tetrapeptides of the $(Ala)_3Thr$ series. Practically the same positions are observed for the C^{β} signal of Thr^1 in the spectrum of peptide IX and in the spectrum of the tetrapeptide Thr-Ala-Ala-Ala. This suggests that proline does not exert any significant steric effect on the antecedent Thr residue.

Table 4

^{13}C chemical shifts for tetrapeptides V–VIII in $^{2}H_2O$ at three pH values

pH	Carbon atom	(V)				(VI)					(VII)					(VIII)					
		Pro	Pro	Thr	Pro	Pro	Pro	Thr	Pro	NH_2	Ac	Pro	Pro	Thr	Pro	Ac	Pro	Pro	Thr	Pro	NH_2
2	α-Pro	60.00	61.27		61.95	59.93	61.28		61.28			59.11	61.05		61.45		59.11	61.27		61.05	
	β-Pro	29.16	30.20		30.05	29.16	30.20		30.35			29.45	30.05		29.75		29.45	29.98		30.35	
	γ-Pro	24.67	25.42		25.42	24.68	25.42		25.42			25.05	25.43		25.43		25.05	25.42		25.42	
	δ-Pro	47.53	48.95		48.58	47.58	49.18		48.58			48.43	49.55		48.95		48.43	49.47		49.17	
	α-Thr			57.91				57.84						57.76					57.66		
	β-Thr			67.92				67.85						68.07					67.92		
	γ-Thr			19.45				19.45						19.37					19.37		
	Ac										22.06					22.06					
7	α-Pro	60.00	61.35		63.07	59.93	61.27		61.27			59.11	61.13		62.92		59.11	61.27		61.13	
	β-Pro	29.16	30.20		30.20	29.23	30.20		30.42			29.62	30.20		28.83		29.53	30.05		30.42	
	γ-Pro	24.67	25.42		25.42	24.67	25.42		25.42			25.05	25.35		25.35		25.12	25.42		25.42	
	δ-Pro	47.45	48.87		48.58	47.55	49.25		48.58			48.25	49.55		48.90		48.50	49.55		49.17	
	α-Thr			57.76				57.91						57.46					57.69		
	β-Thr			67.85				67.85						67.92					68.00		
	γ-Thr			19.44				19.44						19.37					19.37		
	Ac										22.06					22.14					
10	α-Pro	59.78	61.27		63.07	59.86	61.27		61.27			59.11	61.13		62.99		59.09	61.27		61.27	
	β-Pro	29.38	30.20		30.20	29.30	30.20		30.42			29.53	30.20		29.98		29.52	30.13		30.42	
	γ-Pro	24.97	25.42		25.42	24.82	25.42		25.42			25.10	25.35		25.35		25.10	25.50		25.50	
	δ-Pro	47.38	48.87		48.50	47.57	49.25		48.58			48.43	49.55		48.80		48.50	49.54		49.14	
	α-Thr			57.69				57.91						57.46					57.50		
	β-Thr			67.85				67.93						67.92					67.92		
	γ-Thr			19.44				19.44						19.37					19.35		
	Ac										22.06					22.10					

Table 5

^{13}C chemical shifts for tetrapeptides IX–X in ^2H_2O at three pH values

pH	Carbon atom	(IX)				(X)				
		Thr	Pro	Ser	Pro	Pro	Ser	Pro	Ser	NH_2
2	α-Pro		59.50		64.27	60.45		61.20		
	β-Pro		30.20		29.90	30.57		30.13		
	γ-Pro		25.49		25.34	25.42		25.42		
	δ-Pro		48.57		49.32	47.38		48.95		
	α-Thr	57.91								
	β-Thr	66.85								
	γ-Thr	19.52								
	α-Ser			54.63			54.93		56.12	
	β-Ser			61.42			61.72		61.87	
7	α-Pro		59.71		62.99	60.54		61.20		
	β-Pro		30.20		30.20	30.57		30.13		
	γ-Pro		25.49		25.42	25.52		25.49		
	δ-Pro		48.05		49.32	47.46		48.95		
	α-Thr	57.91								
	β-Thr	66.95								
	γ-Thr	19.59								
	α-Ser			54.55			55.00		56.20	
	β-Ser			61.42			61.27		61.95	
10	α-Pro		61.20		63.07	60.68		61.50		
	β-Pro		30.28		30.28	31.25		30.13		
	γ-Pro		25.35		25.35	26.19		25.42		
	δ-Pro		48.73		49.10	47.23		48.95		
	α-Thr	58.51								
	β-Thr	70.02								
	γ-Thr	19.22								
	α-Ser			54.40			54.25		56.20	
	β-Ser			61.17			61.72		61.87	

In the case of peptide IX (table 5), both prolyl residues exist mostly in the *cis'* conformation as indicated by the Δ_h coefficients. The Δ_h coefficient for Ser^3 of this peptide amounts to 1.23 and 0.98 ppm for acidic and basic solution, respectively. This corresponds with absolute θ values of 68.7 and 73.6°, respectively. Correspondingly, the mean value of ψ is -8.7 and -13.6 for acidic and basic solution, respectively. Both are near to the typical values for the $i+2$ residue in a β-turn conformation. Thus, the mean solution conformation of this peptide may be dominated, as suggested by the CD measurements, by the β-turn structure.

In the last case, formation of β-turn structure is stimulated not only by the proper location of both prolines, but also by Ser^3 in the $i+2$ position of the potential turn. Stabilization of the β-turn in cases where Ser occupies the $i+2$ position has been indicated recently by Aubry and Marraud [12].

The Δ_h coefficients of Ser^2 and Ser^4 in peptide X (table 5) are greater than those in the previous case (1.53 ppm for Ser^2 and 1.68 ppm for Ser^4). This may result from increased disordering of peptide X in comparison to peptide IX. It is also remarkable that the position of the C^β resonances for the Ser^2 and Ser^4 residues of this peptide are practically identical. Thus, as observed for Thr, there is no noticeable steric effect on the antecedent serine residue exerted by proline.

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