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

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The mean solution conformation of tetrapeptide fragments of the hinge region of human IgA1 molecule was investigated by CD and ^{13}C-NMR methods. Distinct conformational differences for the partial sequences were found. Tetrapeptides with the Thr-Pro-Ser-Pro sequence were found to show a clear preference for the β-turn conformation. Conformational equilibria of these peptides are only slightly affected by acetylation or pH changes. In the case of Pro-Thr-Pro-Ser tetrapeptides conformational equilibria are dominated by unordered forms.

1. Introduction

The hinge region of the human IgA1 immunoglobulin molecule contains the sequence (1, 2):

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...Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-
                    225
Pro-Ser-Thr-Pro-Pro-Thr...
                  235
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which is sensitive to the action of extracellular proteases produced by several human bacterial pathogens. The sequence of the first IgA1 protease, isolated from the Gram-negative diplococcus *Neisseria gonorrhoeae* was determined by Pohlner et al. [3].

Continuing our studies [4] on the mean solution conformation of sequential fragments of the hinge region of human IgA1, we investigated the six following tetrapeptides:

Thr-Pro-Ser-Pro-NH_2	(I)
Ac-Thr-Pro-Ser-Pro	(II)
Ac-Thr-Pro-Ser-Pro-NH_2	(III)
Pro-Thr-Pro-Ser-NH_2	(IV)
Ac-Pro-Thr-Pro-Ser	(V)
Ac-Pro-Thr-Pro-Ser-NH_2	(VI)

The mean solution conformation of each compound was determined by CD and ^{13}C-NMR measurements. The data obtained during our investigation of model tetrapeptides $(Ala)_3Pro$ and $(Ala)_3Thr$ [5–7] were used in the interpretation of ^{13}C-NMR spectra. Chemical shifts of the C^{β} carbon atoms of specific residues (recalculated onto the hydantoin scale [8]) were employed to define the main conformational characteristics. Clear evidence of conformational differences between peptides I–III and IV–VI was obtained. Peptides I–III show a preference for a β-turn

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structure, while IV–VI are most probably in an unordered state.

2. Materials and methods

Details of the syntheses of investigated peptides will be published separately. Each compound was purified by HPLC. Purity and homogeneity of the products were proved 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, 5 and 10 mm. For each compound CD spectra were recorded at acidic (pH ~ 2), neutral (pH ~ 7) and alkaline (pH ~ 10) pH values. The concentration of solutions varied from 0.0358 to 0.1090 g/dm^3. Results of the CD measurements are expressed as the molar ellipticity (in degree cm^2 dmol^{-1}).

^{13}C-NMR spectra were recorded in ^2H_2O on a Tesla 567 spectrometer with an operating frequency of 25.142 MHz. Dioxane was used 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 acidic (pH 2.1–2.6), neutral (pH 6.7–7.4) and alkaline (pH 10.1–10.9) pH values. The concentration of solutions varied from 13.0 to 38.0 g/dm^3. The pH of solutions was adjusted with 4 N NaOH or 4 N ^{2}HCl and measured with a Mera-Elwro N-517 pH-meter. Absolute values of θ angles (angle in the moiety C^{α}-C^{β}-C′-O) were calculated from the equation [5]:

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

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

3. Results and discussion

The results of CD measurements are given in table 1. Selected curves which illustrate the observed regularities are shown in figs. 1 and 2. The CD spectra of peptides I–III (fig. 1) have the same shape. They exhibit a single minimum at approx. 210 nm, resembling spectra of the β-turn conformation [9,10]. pH changes do not influence the position of the minimum of the CD spectra of peptides I–III (which is the same as the free tetrapeptide Thr-Pro-Ser-Pro [4]). There are slight changes in molar ellipticity with an increase in pH in the spectra of peptides I and III. For peptide II, the influence of pH on ellipticities is significant (-0.68×10^4 degree cm^2 dmol^{-1} in acidic and -2.35×10^4 degree cm^2 dmol^{-1} in alkaline medium). The same tendency was observed for the free tetrapeptide Thr-Pro-Ser-Pro [4]. These facts suggest that ellipticity changes are connected with the ionization of the carboxyl group.

In our earlier study [4] we observed that acetylation of the N-terminal amino group influenced the mean solution conformation of the tetrapeptide Pro-Pro-Thr-Pro. Acetylation destabilized the β-turn structure and shifted the conformational equilibrium toward unordered forms. The phenomenon was confirmed by CD and ^{13}C-NMR data [4]. In the case of the Thr-Pro-Ser-Pro tetrapeptide, investigated here, acetylation of the terminal amino group does not produce a similar destabilizing effect. This suggests that the acetyl derivatives (peptides II and III) remain largely in the β-turn conformation. This is probably due to the amino acid sequence of the peptide, which is highly correlated with β-turn formation, the preference of a Pro residue at position $i + 1$ for a type I β-turn conformation being well-known. The β-

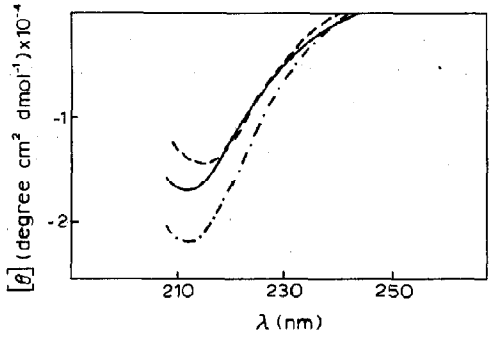

Fig. 1. CD spectra of tetrapeptides I–III in water at pH 7. (———) Thr-Pro-Ser-Pro-NH_2; (- - - - -) Ac-Thr-Pro-Ser-Pro; (·–·–·) Ac-Thr-Pro-Ser-Pro-NH_2.

Table 1

Results of CD measurements for tetrapeptides I–III

Tetrapeptide	pH	λ (nm)/[θ] (×10^{-4}) (degree cm^2 dmol^{-1})		
		Minimum	Shoulder	Zero
(I) Thr-Pro-Ser-Pro-NH_2	2	213/−1.85	–	245/0
	7	212/−1.83	232/−0.41	244/0
	10	211/−2.70	–	247/0
(II) Ac-Thr-Pro-Ser-Pro	2	199/−1.36 [a]	210/−0.70	240/0
	7	214/−1.48	–	243/0
	10	214/−2.51	–	247/0
(III) Ac-Thr-Pro-Ser-Pro-NH_2	2	211/−2.22	–	247/0
	7	212/−2.29	–	247/0
	10	213/−2.52	–	247/0
(IV) Pro-Thr-Pro-Ser-NH_2	2	200/−2.09 [a]	224/−0.72	248/0
	7	200/−4.39 [a]	224/−1.48	240/0
	10	204/−1.90 [a]	222/−0.86	248/0
(V) Ac-Pro-Thr-Pro-Ser	2	201/−2.24	230/−0.40	241/0
	7	201/−3.18	230/−0.48	241/0
	10	205/−2.86 [a]	230/−0.54	241/0
(VI) Ac-Pro-Thr-Pro-Ser-NH_2	2	203/−2.73	226/−0.67	241/0
	7	203/−3.27	230/−0.64	243/0
	10	204/−2.27	230/−0.64	243/0

[a] Position of the minimum not attained.

turn is also a preferred conformation of tetrapeptides with Pro in position 4 and Thr in position 1 [5,6]. Aubry and Maraud [12] showed that serine in position $i+2$ of the chain also induces β-turn conformation. Thus, for peptides I–III, all four amino acid residues stimulate β-turn formation.

The CD spectra of tetrapeptides IV–VI are very different from those of I–III. The CD-curve minima appear at approx. 200 nm, and there is a distinct shoulder in the range 224–230 nm. The spectra of IV–VI are strongly influenced by pH, but the origin of this observation is rather complex. Even the CD curve of the protected peptide VI shows significant dependence on pH (-2.73×10^4 degree cm^2 dmol^{-1} in acidic, and -3.27×10^4 degree cm^2 dmol^{-1} in neutral medium). This sug-

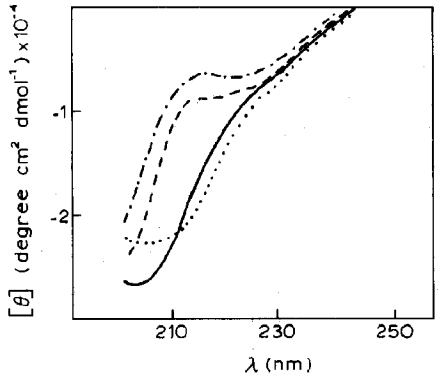

Fig. 2. CD spectra of tetrapeptides IV and VI in acidic and alkaline media. (·–·–·) Pro-Thr-Pro-Ser-NH_2, pH 2; (- - - - - -) Pro-Thr-Pro-Ser-NH_2, pH_{10}; (———) Ac-Pro-Thr-Pro-Ser-NH_2, pH 2; (· · · · · ·) Ac-Pro-Thr-Pro-Ser-NH_2, pH 10.

Table 2

Amounts of *cis*-Pro forms in tetrapeptides I–VI calculated from the ratio of intensities of C^β signals (in %)

pH	Tetrapeptide					
	I	II	III	IV	V	VI
2	15	15	15	15	20	25
7	10	10	10	20	15	15
10	15	15	20	25	20	15

Table 3

Chemical shifts of the carbon atoms of tetrapeptides I–III (*cis*-Pro signals are not included)

Carbon atom	Thr-	Pro-	Ser-	Pro-NH_2	Ac-	Thr-	Pro-	Ser-	Pro	Ac-	Thr-	Pro-	Ser-	Pro-NH_2	pH
C^{α}-Pro		61.35		61.35			59.93		60.45			61.35		61.35	2
C^{β}-Pro		30.28		30.28			29.68		29.68			30.29		30.28	
C^{γ}-Pro		25.42		25.42			25.42		25.42			25.50		25.30	
C^{δ}-Pro		48.87		49.32			48.58		48.58			48.87		49.32	
C^{α}-Thr	57.91					57.95					57.91				
C^{β}-Thr	66.95					68.07					68.89				
C^{γ}-Thr	19.59					19.67					19.52				
C^{α}-Ser			54.48					54.55					54.40		
C^{β}-Ser			61.38					61.27					61.35		
Ac					22.66					22.20					
C^{α}-Pro		61.35		61.35			59.85		60.60			61.35		61.35	7
C^{β}-Pro		30.35		30.15			29.68		29.68			30.35		30.15	
C^{γ}-Pro		25.50		25.32			25.35		25.35			25.65		25.50	
C^{δ}-Pro		48.87		49.32			48.57		48.57			48.87		49.32	
C^{α}-Thr	57.91					57.02					57.91				
C^{β}-Thr	67.03					68.00					68.89				
C^{γ}-Thr	19.67					19.60					19.52				
C^{α}-Ser			54.48					54.63					54.48		
C^{β}-Ser			61.35					61.20					61.35		
Ac					22.58					22.30					
C^{α}-Pro		61.42		61.42			59.93		63.07			61.35		61.35	10
C^{β}-Pro		30.35		30.15			30.28		30.28			30.28		30.28	
C^{γ}-Pro		25.35		25.35			25.35		25.35			25.34		25.34	
C^{δ}-Pro		48.87		49.27			48.65		48.65			48.87		49.10	
C^{α}-Thr	58.23					57.73					58.44				
C^{β}-Thr	70.05					68.82					[a]				
C^{γ}-Thr	19.30					19.67					19.44				
C^{α}-Ser			54.25					54.55					54.25		
C^{β}-Ser			61.20					61.57					61.20		
Ac					22.66					22.32					

[a] Resonance not identified.

gests that the influence of pH is not directly connected with the ionization of terminal amino or carboxyl groups.

The strong minima, present in the CD spectra of tetrapeptides IV–VI at approx. 200 nm, demonstrate the domination of unordered forms in the conformational equilibria. Their populations, as can be seen from comparison of ellipticities at different pH values, are highest at neutral pH.

The presence of the shoulder at 222–230 nm in CD spectra of peptides IV–VI does not have a ready explanation. It appears in the region of the β-turn n-π^* transition. Definite amounts of β-turn conformers may thus be present in the conformational equilibria. This conclusion, however, seems doubtful, because Pro-Thr-Pro-Ser is highly disadvantageous for β-turn formation [6].

In the ^{13}C-NMR spectra of peptides I–VI, resonances of both *trans*-Pro forms (*trans* configuration of the X–Pro amide bond) and *cis* forms are present. They were not analyzed in detail because of the difficulties in unequivocal assignment of a particular *cis*-Pro signal to the definite Pro residue. Approximative amounts of the *cis* forms (table 2) were estimated from the ratio of intensities of C^{β}-*trans* of C^{β}-*cis* resonances of Pro residues in a peptide.

The amount of *cis* form is frequently used as a

Table 4

Chemical shifts of the carbon atoms of tetrapeptide IV–VI (*cis*-Pro signals are not included)

Carbon atom	Pro-	Thr-	Pro-	Ser-NH_2	Ac-	Pro-	Thr-	Pro-	Ser	Ac-	Pro-	Thr-	Pro-	Ser-NH_2	pH
C^{α}-Pro	60.38		61.67			60.68		61.57			60.83		61.65		2
C^{β}-Pro	30.58		30.13			30.72		30.20			30.80		30.20		
C^{γ}-Pro	24.45		25.50			25.12		25.50			25.12		25.57		
C^{δ}-Pro	47.38		49.25			49.04		49.36			49.25		49.62		
C^{α}-Thr		58.29					57.71					57.70			
C^{β}-Thr		67.77					68.00					67.92			
C^{γ}-Thr		19,52					19.60					19.52			
C^{α}-Ser				56.12					57.99					56.27	
C^{β}-Ser				61.87					63.29					61.95	
Ac					21.95					22.21					
C^{α}-Pro	60.45		61.57			60.75		61.72			60.75		61.57		7
C^{β}-Pro	30.65		30.13			30.72		30.20			30.72		30.20		
C^{γ}-Pro	24.52		25.57			25.12		25.52			25.12		25.50		
C^{δ}-Pro	47.57		49.25			49.25		49.55			49.25		49.55		
C^{α}-Thr		58.36					57.76					57.76			
C^{β}-Thr		67.84					67.85					67.84			
C^{γ}-Thr		19.59					19.74					19.44			
C^{α}-Ser				56.20					57.76					56.27	
C^{β}-Ser				61.94					62.92					61.95	
Ac					22.21					22.21					
C^{α}-Pro	60.66		61.65			60.75		61.32			60.75		61.58		10
C^{β}-Pro	31.10		30.13			30.72		30.20			30.72		30.20		
C^{γ}-Pro	24.82		25.50			25.12		25.42			25.12		25.50		
C^{δ}-Pro	47.38		49.25			49.25		49.62			49.25		49.55		
C^{α}-Thr		57.91					57.84					57.78			
C^{β}-Thr		68.00					68.00					67.85			
C^{γ}-Thr		19.59					19.44					19.52			
C^{α}-Ser				56.20					55.67					56.27	
C^{β}-Ser				61.87					61.95					61.95	
Ac					22.21					22.21					

measure of the conformational lability of a particular peptide (increasing amount of the *cis* form may be considered as a measure of conformational lability). It is worth noting that the amounts of *cis* forms are evidently smaller in the case of peptides I–III (10–15%) (for which stabilization of the β-turn was postulated), than for peptides IV–VI (which are most probably unordered). In peptides I–III as well as in V and VI the *cis*-Pro signals arise from two Pro residues of the peptide chain. In the case of peptide IV, however, only the Pro^3 residue can produce the *cis* resonances. We may thus conclude that the highest proportion of *cis*-Pro isomer (with respect to one Pro residue) occurs with peptide IV.

Tables 3 and 4 summarize the results of ^{13}C-NMR measurements. The data gathered during the investigation of model tetrapeptides having the general formulae $(Ala)_3Pro$ and $(Ala)_3Thr$ [5–7] were applied in the assignment of particular resonances. Additional confirmation of the assignments was gained by comparison of the observed resonance positions with those of tetrapeptides X-Thr-Pro-Pro-Thr-Y and X-Pro-Pro-Thr-Pro-Y (X = X, Ac; Y = OH, NH_2) which were investigated previously [4].

Table 5 lists the values of the coefficient Δ_h calculated for the consecutive amino acid residues of peptides I–VI (Δ_h values for Thr^1 in peptide I, Pro^4 in peptide II, Pro^3 in peptide IV and Ser^4 in

Table 5

Δ_h coefficients of the specific residues of tetrapeptides I–VI.

Residue	pH	Tetrapeptide					
		I	II	III	IV	V	VI
1	2		2.94	3.76		4.07	4.35
	7		2.87	3.76		4.07	4.07
	10		3.69	[a]		4.07	4.07
2	2	3.63	3.03	3.64	2.64	2.87	2.79
	7	3.70	3.03	3.70	2.71	2.72	2.71
	10	3.70	3.83	3.63	2.87	2.87	2.72
3	2	1.19	1.08	1.16	3.48	3.55	3.55
	7	1.16	1.07	1.16	3.48	3.55	3.55
	10	1.01	1.38	1.01	3.48	3.55	3.55
4	2	3.63		3.63	1.68		1.76
	7	3.50		3.50	1.75		1.70
	10	3.50		3.63	1.68		1.66

[a] Resonance not identified.

peptide V are omitted because the hydantoin scale is valid only for amino acid residues located within the peptide chain). The Δ_h coefficients (table 5) calculated for Pro^2 and Pro^4 in peptides I–III are typical of the *cis'* conformation (Pro carbonyl group in a *cis* position to Pro α-hydrogen). Possible values of ψ angles obtained for Pro^2 from the hydantoin scale [8] are in the range -38.5 to $-61.5°$. Such values are typical of the $i+1$ residue in a type I β-turn conformation ($\psi - 30°$). The Δ_h coefficients for Ser^3 (table 5) in peptides I–III vary from 1.01 to 1.38. Possible ψ angles determined for this residue from the hydantoin scale are -9.3 to $-16.5°$, i.e., close to the typical values of the $i+2$ residue in a type I β-turn (ψ 0°). This result is in good agreement with those of CD measurements of peptides I–III. The close proximity of Δ_h coefficients of residues Pro^2, Ser^3 and Pro^4 in peptides I–III indicates the similarity of the mean solution conformation of these peptides. Thus, neither acetylation nor amidation influences the conformational equilibria to a great extent. The only difference was observed for the Pro^2 residue of peptide II. In acidic and neutral solution the C^β resonance of this residue is distinctly shifted towards higher fields, indicating a different mean solution conformation of Pro^2 of this peptide at the mentioned pH values.

Interestingly, Δ_h values of Thr^1 in peptides II and III differ in acidic and neutral media by about 0.8 ppm. The only difference in the chemical structure of II and III consists of the presence of the amide group (peptide III) instead of the carboxyl group (peptide II). This indicates that amidation of the C-terminal Pro residue to the acetylated tetrapeptide increases the average θ angle in the Thr^1-C^β-C^α-C'-O moiety. Thus, the chemical changes in position 4 of the peptide chain influence the conformation of the N-terminal amino acid residue. This is, however, possible only when there is an interaction between N-terminal and C-terminal residues in a peptide, i.e., in folded conformations (presumably of the β-turn type). These observations provide additional support for the conclusion that conformational equilibria in the series I–III are dominated by folded structures. It should also be noted that pH changes influence the Δ_h coefficients in peptides I–III only to a small extent. Therefore, we conclude, in agreement with the CD data, that ionization of terminal amino or carboxyl groups does not markedly affect the mean solution conformation of peptides I–III.

In the case of peptides IV–VI with the sequence Pro-Thr-Pro-Ser the Δ_h coefficients of consecutive residues differ very slightly. The mean conformation in this series differs, however, distinctly from that of series I–III. The influence of pH on Δ_h values in this series is very small (Δ_h coefficients change to a small extent with increase in pH) which points to the minor influence that pH has on mean peptide conformation. We must note, however, that the CD spectra of peptides IV–VI were distinctly influenced by pH. Thus, there is an inconsistency between CD and ^{13}C-NMR data.

The Δ_h coefficients for Pro^3 residues in the series IV–VI (table 5) vary from 3.48 to 3.55 (indicating a *cis'* conformation). The chemical shifts of Thr^2, however, are very close to those of the model tetrapeptide Ala-Thr-Ala-Ala [7]. Such similarity indicates that the steric effect by proline on the antecedent amino acid residue [5] is not present in the Pro-Thr-Pro-Ser sequence. The chemical shifts of Pro^1, Pro^3 and Ser^4 (table 5) in peptide IV (Pro-Thr-Pro-Ser-NH_2) are almost the same as in peptide Pro-Ser-Pro-Ser-NH_2 investigated earlier [4]. The Δ_h coefficients of Thr^2 in

Pro-Thr-Pro-Ser-NH_2 and Ser^2 in Pro-Ser-Pro-Ser-NH_2 differ significantly (2.64–2.87 for Thr^2 and 1.53 for Ser^2) indicating the difference in mean conformation of residue 2 in both peptides.

The comparison of Δ_h coefficients of the Pro^2-Ser^3 sequence in the peptides I–III with those of the Thr^2-Pro^3 sequence in peptides IV–VI shows that the conformation of the peptide chain must be different in both cases. The difference consists of the closing of the θ angle of residue 2 in the series IV–VI (in comparison with the situation in series I–III) with the simultaneous opening of the θ angle of residues 3. Both changes act against the β-turn formation.

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