Circular Dichroism Spectra of Synthetic Steroidogenesis-Activator Polypeptide and Its Fragments

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Synopsis. Circular dichroism spectroscopic analysis of synthetic steroidogenesis-activator polypeptide (SAP) and its fragments in 2,2,2-trifluoroethanol-containing media suggests that SAP holds two folding sites in the N- and C-terminal regions. The N-terminal folding shows some vulnerability toward acidic conditions, while the C-terminal folding prefers to acidic circumstances.

There have been few studies on the conformational properties of steroidogenesis-activator polypeptide (SAP) (**1**)[1] except for recent one by Glass et al.,[2] who concluded that **1** showed little evidence of preferred structure in aqueous solution. Our preliminary study[3] showed, however, that **1** contains some ordered structure in 2,2,2-trifluoroethanol (TFE)-buffer systems, suggesting that if **1** locates in hydrophobic circumstances in cells it folds into some restricted structure.

The amino acid sequence of **1** (Fig. 1)[4] is rather unique; the N-terminal region is hydrophobic, while the C-terminal one is composed of amino acids with charged side chains and is highly hydrophilic. The two domains are bridged by the middle region, which is abundant in glycyl and prolyl residues.

In the present study, we synthesized **1**[5] and the three fragment peptides, **2**, **3**, and **4** (Fig. 1), using the solid-phase technique,[6] and investigated their circular dichroism (CD) spectra in buffer solutions with or without addition of TFE. To avoid the effects of ionized additional terminals on the conformation, **3** and **4** hold acetylated N-terminals and **2** and **3** end with carboxamides.

The CD spectra of **1** in aqueous buffer solutions (pH 3.10, 7.20, and 8.00) showed a deep negative trough near 199 nm and a shallow negative shoulder around 220 nm, indicating that random structure is predominant in the peptide (Fig. 2).[7] However the spectra also implyed that **1** contains some ordered structure in the solvents, since random coil usually gives no negative ellipticity near 220 nm. Addition of TFE to the aqueous solutions induced red-shift of the negative trough and increase in negative ellipticity around 223 nm. The spectra of **1** in the TFE-abundant solvents closely resemble the spectra simulated for poly-L-lysine with random structure partially including α-helical and β-sheet domains,[7] suggesting **1** holds a few types of folding in the solvents. The spectra of **1** observed in the acidic media containing TFE closely resembled those in the neutral media, while the spectra obtained at pH 8.00 showed slight decrease in negative ellipticity in the region of 200—230 nm (the values of $[\theta]_{210}$ and $[\theta]_{222}$ in 25% TFE at pH 8.00 are −5600 and −3600, respectively, while −7600 and −4400 at pH 3.10, and −7000 and −4400 at pH 7.20). In the solvents without TFE, **2** prefers to random conformation, while in ones containing TFE, the spectra accompanying the characteristic double negative troughs near

Ile-Val-Gln-Pro-Ile-Ile-Ser-Lys-Leu-Tyr-Gly-Ser-Gly-Gly-Pro-Pro-Pro-Thr-Gly-Glu-Glu-Asp-Thr-Asp-Glu-Lys-Lys-Asp-Glu-Leu : **1**

Ile-Val-Gln-Pro-Ile-Ile-Ser-Lys-Leu-Tyr-Gly-NH_2 : **2**

Ac-Tyr-Gly-Ser-Gly-Gly-Pro-Pro-Pro-Thr-Gly-NH_2 : **3**

Ac-Thr-Gly-Glu-Glu-Asp-Thr-Asp-Glu-Lys-Lys-Asp-Glu-Leu : **4**

Fig. 1. Amino acid sequences of **1**—**4**.

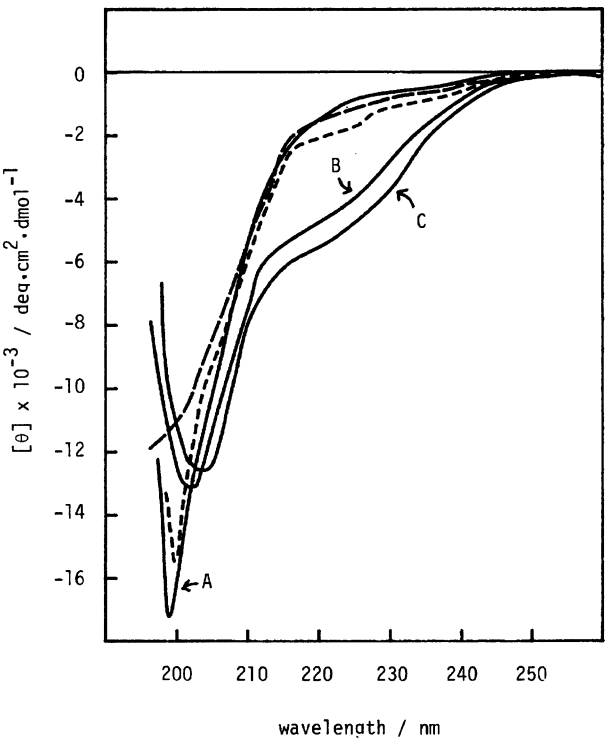

Fig. 2. CD spectra of **1** in TFE (%)-aqueous buffers. pH 3.10, 0% (····); pH 7.20, 0% (—A), 25% (—B), and 50% (—C); pH 8.00, 0% (- - -).

210 and 220 nm indicated that α-helical structure exists in the peptide (Fig. 3).[7] If we assume that **2** contains only α-helical and random structures, the helix content of **2** in 50% TFE (pH 7.20) is estimated to be 40%.[8] A similar content (39%) was observed for **2** in the basic conditions (50% TFE, pH 8.00), but in the acidic ones (50% TFE, pH 3.10) the content considerably decreased (28%). The helical folding is obviously disturbed in the acidic media (Table 1). Considering that **2** has a prolyl residue, the helix breaker residue,[9] at position 4, the helix in **2** probably located in the C-terminal half of the molecule. The spectrum of **3** in a TFE-deficient solvent at pH 7.20 (Fig. 3) showed a deep negative trough near 203 nm and a weak positive peak around 225 nm, whose feature rather resembles those of the spectrum of poly-L-proline (type II).[10] Alteration of the pH-conditions or addition of TFE to the solution of **3** induced little change in the spectra. The C-terminal peptide, **4**, prefers to random structure in the TFE-deficient media but still contains some folding especially in the acidic conditions (Fig. 3). The pH-dependence of the ellipticities of **4** at different TFE concentrations indicated that the TFE-induced folding in **4** is stabilized in the acidic circumstances (Table 2). On assumption that **4** contains no β-sheet structure, the helix contents of **4** are estimated to be 11% (50% TFE, pH 3.10) and 14% (75% TFE, pH 3.10).[8]

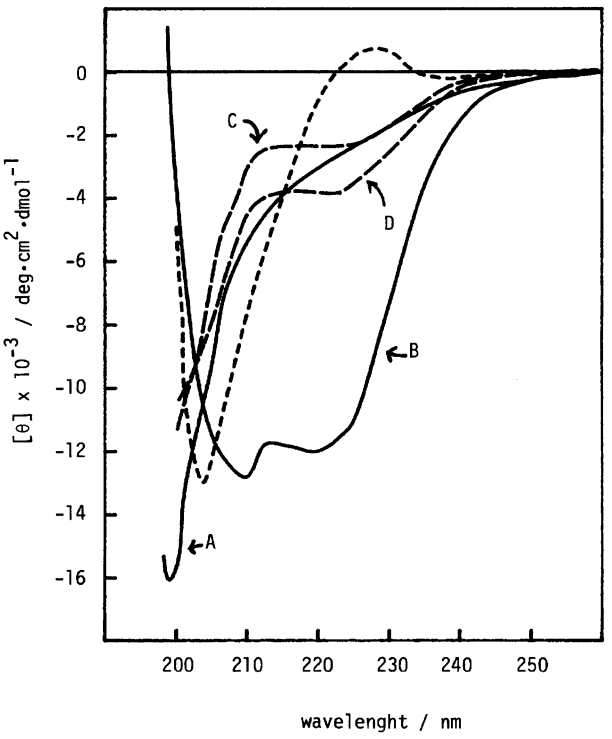

Fig. 3. CD spectra of **2**—**4** in TFE (%)-aqueous buffers. **2**: pH 7.20, 0% (—A) and 50% (—B); **3**: pH 7.20, 0% (····); **4**: pH 3.10, 0% (---C) and 50% (---D).

The CD spectra of equimolar mixtures of **2**, **3**, and **4** in the solvents at pH 7.20 with or without TFE closely resemble the spectra of **1** in the corresponding media, although the intensities of the spectra of the mixtures are lower than those of the spectra of **1** (the values of $[\theta]_{210}$ and $[\theta]_{222}$ in 50% TFE are −7800 and −5300 for **1**, respectively, while −6700 and −4300 for the mixture). The spectra of the equimolar mixtures are nearly superimposable on the corresponding simulated spectra for the mixtures which were obtained according to the following equation:

$$[\theta]^{\text{simulated}} = [\theta]^{\mathbf{2}} \times 11/34 + [\theta]^{\mathbf{3}} \times 10/34 + [\theta]^{\mathbf{4}} \times 13/34,$$

where $[\theta]^{\mathbf{2}}$, $[\theta]^{\mathbf{3}}$, and $[\theta]^{\mathbf{4}}$ are mean residue ellipticities of **2**, **3**, and **4**, respectively. These facts strongly suggest that the conformational properties of the N-terminal, central, and C-terminal domains of **1** are fairly well retained in the smaller peptides corresponding to the domains, respectively, and interactions between the domains little influence the conformations.

The data so far obtained suggest that **1** in the aqueous media predominantly prefers to random structure but still holds some ordered conformation. In the TFE-containing media, the folding was obviously enhanced. It would be natural to assume that the type of the folding is α-helix and the folding locates in the N-terminal region of **1**, since the corresponding peptide, **2**, folds easily into α-helical structure in the TFE-containing media. However, the helix contents of **2** in the acidic media are considerably lower than those in the neutral ones, while the spectra of **1** in the acidic conditions and those in the neutral ones closely resemble. If the conformational properties of a fragment peptide are similar to those of the corresponding region in a longer peptide, this contradiction shows that **1** holds another folding site that is enhanced in the acidic media. The features of **4**, whose folding prefers to acidic conditions, seem to be quite favorable to the assumption that **1** in hydro-

Table 1. Ellipticities of **2** at 222 nm in Different Conditions

Concentration of TFE/%	$[\theta]_{222}$/deg cm^2 dmol^{-1}		
	pH 3.10	pH 7.20	pH 8.00
0	−2200	−2600	−3000
25	−6600	−8300	−9000
50	−8200	−11700	−11300
75	−8200	−11000	−10000

Table 2. Ellipticities of **4** at 222 nm in Different Conditions

Concentration of TFE/%	$[\theta]_{222}$/deg cm^2 dmol^{-1}		
	pH 3.10	pH 7.20	pH 8.00
0	−2300	−1000	−1400
25	−3200	−1300	−1300
50	−3800	−1800	−1800
75	−4700	−3200	−3000

phobic circumstances holds two folding sites on its N- and C-terminal regions; the N-terminal folding is stable in neutral or basic conditions but is partially disturbed in acidic ones, while the C-terminal folding prefers to acidic conditions and is rather vulnerable in neutral or basic ones. Chou–Fasman's prediction[11] shows the C-terminal region of **1** favors α-helical structure with high probability. If the folding induced in this region is indeed α-helix, it seems reasonable to assume that the folding prefers to acidic conditions, because the amino acid sequence of this region is abundant in aspartyl and glutamyl residues and contains adjacent alignments of the acidic amino acid residues and hence pH-dependent properties of the folding must be similar to those of the α-helical structure composed of poly-L-glutamic acid.[12,13] The feature of the central region of **1** is still puzzling. Chou–Fasman's prediction also shows that the central region extensively prefers to β-turn structure. However, the spectra of **3** resemble those of helical poly-L-proline, although the alignment of three prolyl residues in **1** or **3** seems too short to construct the helix, and do not suggest the potential β-turn structure.

Further spectroscopic analyses should be focused on the conformation of the unique triacontapeptide in hydrophobic circumstances.

Experimental

The procedures for the peptide synthesis were similar to those adopted in preparation of **1**.[5] **2**; Yield, 65%. Found: C, 49.37; H, 6.99; N, 12.94%. M^+, 1228. Calcd for $C_{59}H_{100}O_{14}N_{14}\cdot 2CF_3COOH\cdot 4H_2O$: C, 49.47; H, 7.25; N, 12.82%. **3**; 63%. Found: C, 48.96; H, 6.72; N, 15.73%. MH^+, 930. Calcd for $C_{41}H_{59}O_{14}N_{11}\cdot 4H_2O$: C, 49.14; H, 6.74; N, 15.38%. **4**; 64%. Found: C, 41.99; H, 5.53; N, 10.63%. MH^+, 1550. Calcd for $C_{62}H_{99}O_{31}N_{15}\cdot 4CF_3COOH$: C, 41.90; H, 5.17; N, 10.47%. Amino acid analysis of the peptides gave reasonable data. The CD spectra were measured at room temperature on a JASCO J-500A spectropolarimeter equipped with a calibrated 0.74 mm quartz cell. For the measurement, the peptides were dissolved in 1 volume of an appropriate buffer solution (50 mM glycine–HCl, pH 3.10; 50 mM Tris (hydroxymethyl) aminomethane–HCl, pH 7.20 or 8.00. Concentrations of the peptides; 0.23×10^{-6}—1.34×10^{-6} mol dm^{-3}) and then diluted with total 3 volumes of the buffer and/or TFE. The concentration of peptides in the buffer solutions were determined on quantitative amino acid analysis. The data of CD spectra are reported using mean residue ellipticity, $[\theta]$.

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