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Secondary Structure in Solution of an Analog of Salmon Calcitonin: [Val^1, Ala^7]sCT

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Abstract: Secondary structure of [Val^1, Ala^7]sCT, an analog of salmon calcitonin (sCT) not containing an N-terminal disulfide bridge, was investigated by circular dichroism (CD) and Fourier-transform infrared spectroscopy (FTIR) methods. Both CD and FTIR results show that the main conformational structure of [Val^1, Ala^7]sCT in aqueous solution is random coil structure, while in trifluorethanol (TFE) it displays a strong α-helical structure. The relationship between the biological activity and the conformational structure of [Val^1, Ala^7]sCT is also discussed.

Key words: Salmon calcitonin analog, CD, FTIR, peptide secondary structure.

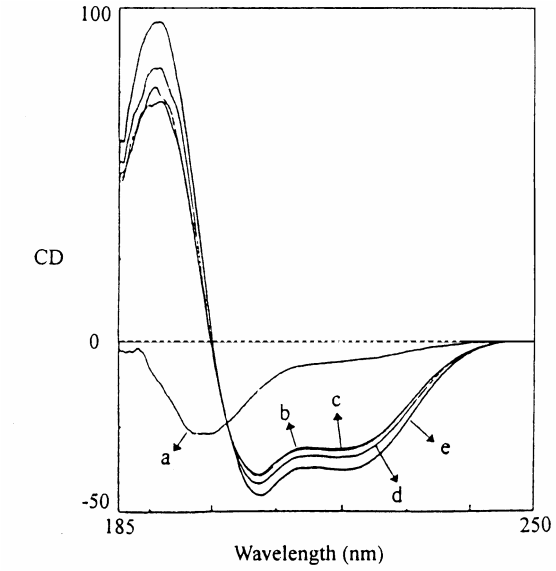
Calcitonin is a cyclic peptide with 32 residues. Although there are many differences in individual amino acids in calcitonins from different species, they all have a disulfide bridge between cysteine residues at positions 1 and 7, and the C-terminus is always proline amide. Salmon calcitonin (sCT) is known mainly for its highest hypocalcemic effect among calcitonins. Although the disulfide bridge in the N-terminal region of different calcitonins is essential for the biological activity of human calcitonin, it is not required for that of eel and salmon calcitonin. Many salmon calcitonin analogs not containing an N-terminal disulfide bridge have been reported[1]. [Val^1, Ala^7]sCT, a salmon calcitonin analog not containing an N-terminal disulfide bridge, was synthesized and proved to have full biological activity as salmon calcitonin[2].

Conformational structure studies have suggested that calcitonins assume a structure containing a loop, involving disulfide bridge between N-terminal cysteine residues at positions 1 and 7, an amphiphilic α-helix from residues 8 to 22, and a hydrophilic random coil sequence comprising C-terminal region[3]. It has also been proposed that the specific structure features are necessary for hypocalcemic activity of calcitonins, especially the amphiphilic α-helical structure plays a very important role in keeping biological activity[4]. For example, human calcitonin tends to form a lower degree of amphiphilic α-helical structure, as a result, the hypocalcemic effect of human calcitonin is 30 times less than that of salmon calcitonin. To better understand the relationship between the activity and the conformational features of salmon calcitonin analogs, we studied the secondary structure of [Val^1, Ala^7]sCT in aqueous solutions in the presence and absence of a structure - promoting solvent TFE by CD and FTIR investigations.

CD studies were performed on a JASCO 270 CD spectropolarimeter in aqueous solutions containing 0-100% TFE. The sample concentration was about 95~100 umol/L. Spectra were recorded at 25°C, using a 1.0 nm bandwidth, 0.2 nm step, a 1.0 s intergradation time, circular cells with a 1.0 mm path length. The average of 6 scans in the range of 185-250 nm was collected for both sample and solvent. The estimation of the secondary structure was made using the PROSEC program [5].

CD results (**Figure 1**) show that in aqueous solution, [Val[1], Ala[7]]sCT assumes a nearly random coil conformation as shown by the main negative absorbance at 198 nm, and some β-structure indicated by small positive absorbance in the far UV bands. In aqueous solutions containing more than 15%TFE, [Val[1], Ala[7]]sCT displays a strong α-helical character as shown by the two negative bands at 222 nm and 207 nm, and a positive band at 194 nm.

Figure 1 CD spectra of [Val[1], Ala[7]]sCT in: a) H_2O, b) 30%TFE, c) 50%TFE, d)75%TFE, e) TFE



FTIR spectra were scanned on a Bio-Rad FTS-65A spectrometer in aqueous solution containing 0-100% TFE. The sample concentration was about 8~10 mmol/L. Spectra were recorded at 25°C at a resolution of 2 cm^{-1} by scanning 128 double-sided interferograms which were Fourier-transformed by using a triangular apodization function. Secondary structure contents were obtained by the curve-fitting method, which were performed with the Bio-Rad Win-IR CUVEFIT.AB program[6].

In the FTIR spectra of [Val[1], Ala[7]]sCT (**Figure 2**), the amide I band at 1645 cm^{-1} in water indicates main random coil structure, while at 1658 cm^{-1} in pure TFE shows a strong α-helical structure. FTIR studies did not show an obvious α-helical structure in aqueous solutions containing TFE less than 50%, which was different from the CD results. The Fourier deconvoluted amide I band spectrum (**Figure 3**) of the [Val[1], Ala[7]]sCT in

TFE reveals more structural features such as peak around 1658 cm^{-1} represents α-helix, peaks around 1626 cm^{-1} , 1635 cm^{-1} and 1683 cm^{-1} indicate β-structure , peak around 1672 cm^{-1} indicates turn structure, and peak around 1645 cm^{-1} is the character of random coil.

Figure 2 FTIR amide I band spectra of [Val^1, Ala^7]sCT: a) absorption in H_2O, b) absorption in TFE, c) deconvoluted in H_2O, d) deconvoluted in TFE

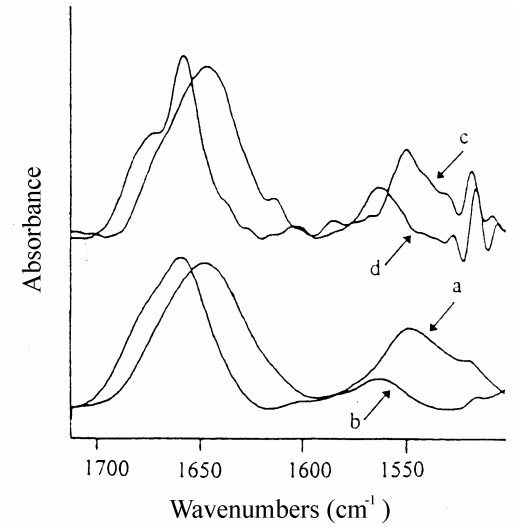


Figure 3 The deconvoluted amide I spectra and the fitted individual component bands of [Val^1, Ala^7]sCT in TFE

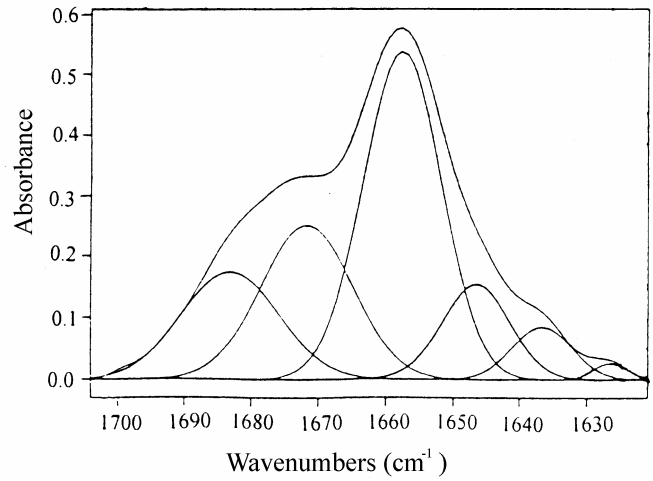


Table 1 The secondary structure contents (%) of [Val[1], Ala[7]]sCT in aqueous and TFE solutions

	CD					FTIR
	H_2O	30%TFE	50%TFE	75%TFE	TFE	TFE
Random coil	53.6	37.0	37.8	36.6	36.8	9.3
α-helix		37.0	39.3	41.8	48.6	48.0
β-sheet	32.6	26.0	22.9	21.6	14.6	22.2
Turns	13.7					20.5

The secondary structure contents of [Val[1], Ala[7]]sCT in aqueous solutions and TFE are shown in **Table 1**. The secondary structural features of [Val[1], Ala[7]]sCT in aqueous solutions and TFE measured by CD and FTIR are generally consistent, only the random coil structure in TFE measured by CD includes turns. The α-helical content of [Val[1], Ala[7]]sCT in TFE measured by CD or FTIR is about 48%, higher than that of salmon calcitonin, which is about 40%[2, 3]. The reason is that the N-terminal disulfide sequence in salmon calcitonin tends to form β-structure (loop)[3], while the N-terminal sequence in [Val[1], Ala[7]]sCT is probably involved in the central sequence to form α-helix.

The present results indicate that the main conformational structure of [Val[1], Ala[7]]sCT in aqueous solution is random coil, while in TFE it displays a strong α-helix, which is a very similar structural feature to that of salmon calcitonin, although there is not an N-terminal disulfide bridge in [Val[1], Ala[7]]sCT. The previous CD and HNMR studies of salmon calcitonin have demonstrated that it displays a main random coil in aqueous solution, while it turns to a strong α-helical structure at the presence of TFE, methanol and dimyristoylphosphatidylglycerol[3, 7]. The fact that [Val[1], Ala[7]]sCT and other open chain salmon calcitonin analogs have full hypocalcemic activity as salmon calcitonin suggests that their conformational structure might be not greatly changed after the molecular modifications.

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