Notes

Novel Secondary Structure of Calcitonin in Solid State as Revealed by Circular Dichroism Spectroscopy

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The solid-state circular dichroic study reveals that salmon calcitonin presents a typical α-helical structure while human calcitonin appears to form a β-sheet in solid state, although both of them adopt random coil structures in aqueous solution.

Keywords calcitonin, circular dichroism, solid state, secondary structure

Introduction

Calcitonin (CT) is a small peptide hormone functioning in calcium phosphorus metabolism and used as treatment for various diseases, such as osteoporosis. Human calcitonin (hCT) easily aggregates into fibrils that are thought to be the pathogenesis of medullary carcinoma of the thyroid, which limits its clinical usage.[1] Interestingly, the CT homologue from salmon (sCT) shows more potent than mammalian CTs in reducing calcium concentration in the blood stream, and preferably it is not an aggregation-prone peptide.[2] To insight into the polymorphism and aggregation mechanism of the two CT homologues of different origins, the secondary structures of these peptides in solid state were studied by CD spectroscopy.[3] The primary sequences of the three peptides are shown as follows.

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     1         10        20        30
sCT  CSNLSTCVLG KLSQELHKLQ TYPRTNTGSG TP-NH2
hCT  CGNLSTCMLG TYTQDFNKFH TFPQTAIGVG AP-NH2
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Experimental

The dry thin film for solid-state measurement was made by peptide sample (0.8 mg/mL, 150 μL) associated from a solution of Tris-HCl buffer (25 mmol/L Tris, 50 mmol/L NaCl, pH 7.4). The samples were prepared by casting a peptide solution onto a 2-cm diameter cylindrical quartz glass for evaporating overnight (16 h) at room temperature. The CD parameters for measuring the solid sample were the same as the solution except an unidentified film thickness.[3]

Results and discussion

Fig. 1 shows the far-UV CD spectra of sCT in solution (dotted) and in solid state (solid). As expected, the solution sCT shows a strong negative peak at 202 nm indicating a random coil structure. The peptide in solid state, however, gives a double-peak spectrum with two negative peaks at 222 nm and 209 nm and a strong positive peak at 193 nm, suggesting that it forms a typical α-helical structure. The helical structure of sCT was also reported in organic solvent.[1] Salmon CT has a propensity to α-helix formation, but it is still flexible and unstructured in solution. In the solid state, however, sCT becomes less flexible that favors forming a more compact helical structure. This study reveals, for the first time, that a peptide forms a novel helical structure in solid state while a random coil in solution.

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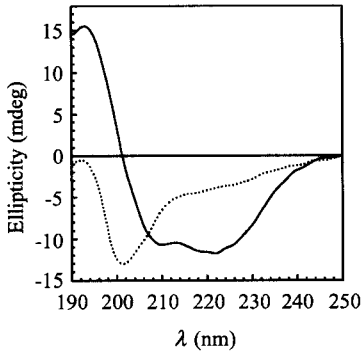

Fig. 1 CD spectra of salmon calcinotin (sCT) in solid state (solid) and in aqueous solution (0.1 mg/mL) (dotted).

The hCT analogues also present compact secondary structures in solid state as revealed from CD spectra associated in films albeit they are unstructured in solution (Fig. 2). The solid-state CD spectrum of LCT (addition of Leu residue at the *N*-terminus of hCT) also shows a negative peak at 209 nm and a broad peak shifting to around 225 nm (A), suggesting that it contains a helical structure and indicatively some amount of aggregates. The CD spectrum of LCTG (addition of Gly residue at the *C*-terminus of LCT) gives a broad negative peak at around 227 nm (B), demonstrating that it forms β-sheet aggregation. The presence of β-sheet structure was also confirmed by FTIR in the gel derived from hCT but not from sCT[4] and by ^{13}C NMR during the fibril formation of hCT.[5]

Some amyloidogenic peptides present random coil structures in solution, but they readily aggregate into fibrils with β-sheet-dominant structures.[3,6] The solubility of CT is relevant to its structures both in solution and in solid state. Two types of CT with different origins show different secondary structure in solid state, α-helix of sCT and β-sheet aggregation of hCT. The *N*- and central regions of the two CTs share a similar hydrophobicity, but the *C*-terminus of hCT appears to be more hydrophobic and also higher propensity of β-structure formation. Addition of Gly residue and blockage of the *C*-terminal amide group seem to increase β-sheet aggregation and decrease the solubility. This finding provides information for developing potent analogues of calcitonin by reducing aggregation and prompting biological activity for clinical usage.[2,7]

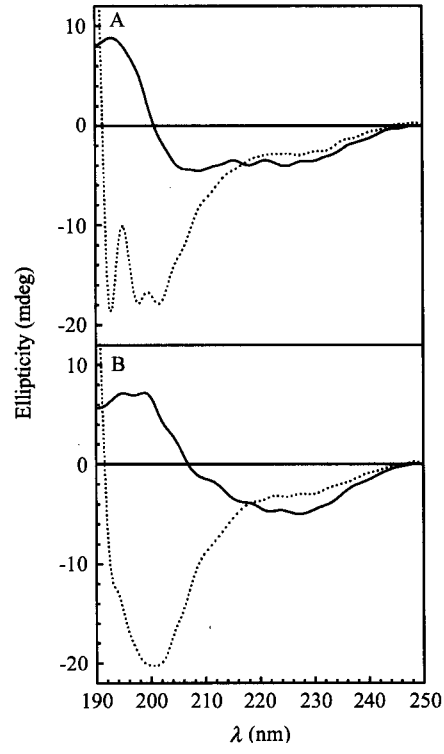

Fig. 2 CD spectra of LCT (addition of Leu residue at the *N*-terminus of hCT) (A) and LCTG (addition of Gly residue at the *C*-terminus of LCT) (B) in solid state (solid) and hCT in aqueous solution (0.2 mg/mL) (dotted).

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