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Short Communication

A 27-mer Tandem Repeat Polypeptide in Bovine Amelogenin: Synthesis and CD Spectra

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Abstract: CD spectra of a tandem 27-mer repeat polypeptide, Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-Met-(Gln-Pro-Leu)$_4$, from bovine amelogenin synthesized by standard solid-phase synthesis manifests an archtypical CD pattern of a β-spiral structure in phosphate buffer at pH 5.2 and trifluoroethanol (TFE), CF_3OH. β-spiral structure is unique to a class of diverse proteins including amelogenins conferring unusual physicochemical properties. Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: CD spectra; β-spiral structure; amelogenin; solid-phase synthesis; β-turns; Ca^{++} channel

INTRODUCTION

Amelogenins comprise the major protein component of the extracellular matrix of developing dental enamel[1–5] and have been implicated in enamel mineralization. The primary structures of mammalian amelogenins from bovine, porcine, human, mouse and rat have been determined, see Simmer and Snead[6] and Fincham *et al.*[7,8] The primary structure of amelogenins in the core domain of the protein appears to be well conserved. The secondary structure of amelogenin has been a challenging problem in structural biology (Renugopalakrishnan *et al.*, submitted) due to a rigid structure arising from contiguous β-turns imparting a β-spiral structure.

Amelogenin from developing bovine enamel, $M_r \sim$ 20 kDa,[9] a hydrophobic protein rich in Pro, Leu, His, Met residues has been isolated, characterized, purified, cloned and its primary structure has been determined both from gas-phase sequencing and cDNA. It has been shown to assume an unusual 3D structure characterized by β-sheets, repetitive β-turns giving rise to a β-spiral, some unordered segments, and a low α-helical content from CD and FT-IR,[2] Raman[10] studies and MMD simulation.[3] The bovine amelogenin contains a 27-residue tandem repeating sequence,Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-Met-(Gln-Pro-Leu)$_4$,(Gln-Pro-Leu)$_4$, which has been suggested as a likely candidate for the interaction of Ca^{++} ions. Earlier molecular mechanics-dynamics simulation of the 27-mer has shown that this tandem repeating polypeptide assumes an unique β-spiral structure. β-spiral structures have now been found to occur in a number of diverse protein systems conferring unique thermodynamic properties (Lagunez-Otero *et al.*, submitted from our laboratory). In this report, we present details of its solid-phase synthesis and demonstrate that the synthetic 27-residue peptides assumes a β-spiral structure in 10 mM phosphate

Abbreviations: TFE, trifluoroethanol.

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buffer, pH 5.2, and more markedly in 60 : 40 trifluoroethanol–water mixture using CD.

MATERIALS AND METHODS

Solid-Phase Polypeptide Synthesis

Peptide synthesis and HPLC purification were performed using a Vega Model 50 synthesizer, a Milton Roy LDC apparatus and a Waters Pico-Tag system, respectively. Benzhydrylamine resin (2 g, 0.64 nmol) was placed in the reaction vessel of the peptide synthesizer and the Boc-amino acid derivatives were coupled sequentially using preformed symmetrical anhydrides or HOBT esters in accordance with well-established procedures.[11,12] At the completion of synthesis, the peptide was released from the resin by a low–high HF method and purified by semipreparative HPLC and the amino acid composition confirmed by amino acid analysis. The sequence of the 27-residue was determined by using an Applied Biosystem Model 470A gas-phase sequencer.

CD Studies

CD spectra of Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-Met-(Gln-Pro-Leu)$_4$ in 10 mM phosphate buffer, pH 5.2, at a concentration of 0.5 mg ml^{-1} was obtained as a function of temperature, starting from 11 °C to 50 °C, on a JASCO 720 spectropolarimeter. A cylindrical cell with a path-length of 49 μm containing the solution was placed in a chamber flushed with N_2 gas and its temperature was regulated by a thermostat. The spectra represent the average of four repetitive scans, signal averaged and smoothed, The CD spectra were recorded from λ near the UV region, 178 nm, to the far UV region, 260 nm. Conformational analysis of CD spectra was performed in accordance with the algorithm described by Compton and Johnson.[13]

RESULTS AND DISCUSSION

CD spectra of Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-Met-(Gln-Pro-Leu)$_4$ in 10 mM phosphate buffer, pH 5.2 at a concentration of 7.33 mM at 25° and 46 °C, respectively, is shown in Figure 1. CD spectra at 25 °C show a negative trough at λ∼200 nm and a positive trough at λ ≈ 185 nm.

On increasing the temperature to 46 °C there was a slight red shift of the negative trough and the positive trough manifested a noticeable positive hump with little or no change in the wavelength. CD spectra of Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-Met-(Gln-Pro-Leu)$_4$ in 60 : 40 trifluoroethanol (TFE) at the same concentration (7.33 mM) as in 10 mM phosphate buffer at 4°, 25° and 46 °C, respectively, is shown in Figure 2.

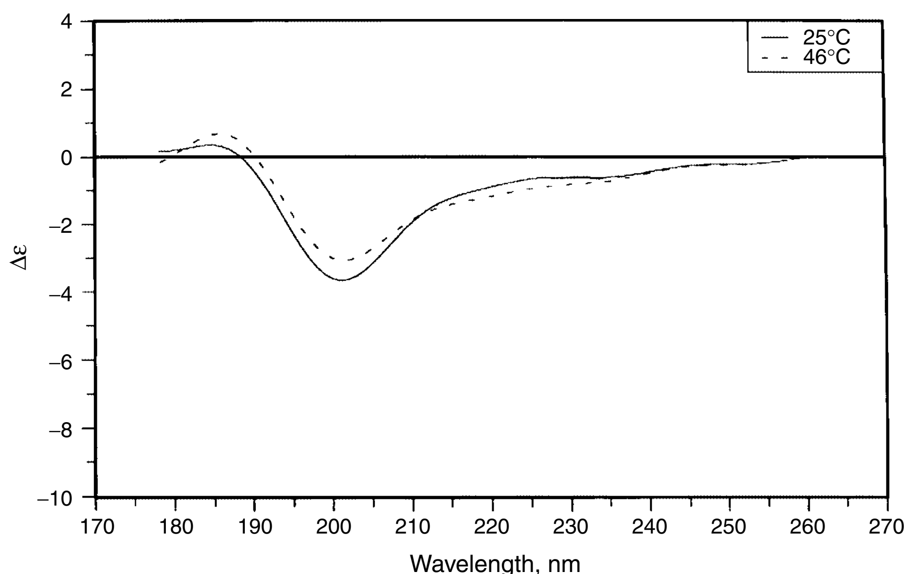

Figure 1 CD spectra of Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-Met-(Gln-Pro-Leu)$_4$ in 10 mM phosphate buffer, pH 5.2 at a concentration of 7.33 mM at 25° and 46 °C, respectively.

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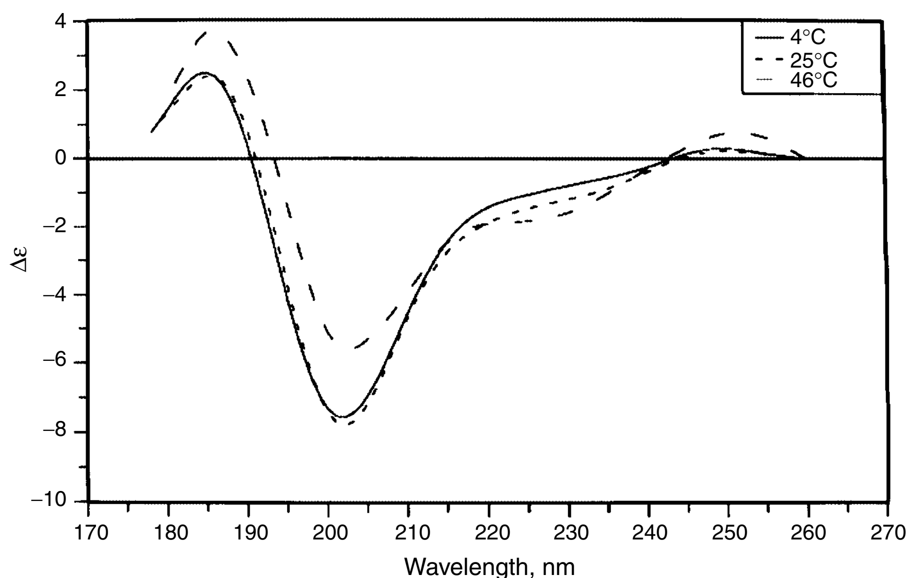

Figure 2 CD spectra of Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-Met-(Gln-Pro-Leu)$_4$ in 60 : 40 trifluoroethanol (TFE) at the same concentration (7.33 mM) as in 10 mM phosphate buffer at 4°, 25° and 46 °C.

TFE is a solvent known to promote helicity[14] and in this solvent the polypeptide exhibits similar CD patterns as in 10 mM phosphate buffer with a deeper negative trough at $\lambda = 202$ nm and a larger ellipticity value, θ, the positive hump, while occurring at approximately the same position as in Figure 1, is much more marked. The CD spectra at 4° and 25 °C respectively bear similarities whereas the CD spectra at 46 °C is red shifted with a negative trough and positive hump. CD spectra of Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-His-Gln-Pro-Leu-Gln-Pro-Met-(Gln-Pro-Leu)$_4$ in 10 mM phosphate buffer and TFE are temperature sensitive and are archtypical of type I β-turns.[15] The CD spectra of the tandem repeat polypeptide does not bear a resemblance to the CD spectra of bovine amelogenin,[2] the CD spectra of porcine amelogenin and fragments,[16] or the recombinant mouse amelogenin (M179) (Renugopalakrishnan *et al.*, submitted; Oobatake *et al.*, submitted) because the integral protein also contains β-sheet regions, rigid poly Pro segments, and sparse α-helical segments in addition to isolated β-turns and β-spiral segments.

Quantitative analysis of CD spectra of peptides and proteins rich in β-turns and β-spiral has been a contentious issue.[17] Nevertheless, the CD spectra were analysed for secondary structural composition using a variable selection (VS) algorithm.[13] The VS algorithm relies on the basis set of observed CD spectra of 15 proteins with a wide range of secondary structural motifs and an α-helical polypeptide, poly (L-Glu), and is also based on the concept of removing proteins from the basis set that do not correspond to the CD spectrum of the protein being analysed in a systematic, self-consistent manner. The CD spectra of the 27-mer in 60 : 40 trifluoroethanol : water contains in excess of 75% β-turns, whereas in 10 mM phosphate buffer it contains slightly reduced amounts of β-turns. A discrimination of the type of β-turn based on CD data is quite difficult. The percentage of β-turns is temperature dependent with the percentile increasing with temperature, typical of hydrophobic polypeptides and proteins exhibiting inverse temperature transition.[18,19]

β-spiral structure offers an ideal structure for facilitating the passage of Ca^{++} ions in amelogenin, a hydrophobic protein (80% non-polar residues), very sparse in traditional Ca^{++} chelators, e.g. negatively charged residues Asp, Glu, Gla and yet participates in the early events of biomineralization of mammalian tooth enamel. Amelogenin is closely associated with lipids in its natural habitat and lipid encapsulation studies of amelogenin in our laboratory have revealed that the protein spans a phosphatidylcholine bilayer[20] and shows Ca^{++} channel activity.

Tandem repeats have been found to occur in a number of protein sequences spread across eukaryotic and prokaryotic systems (mammalian tropoelastin,[21,22] mammalian tooth enamel protein — amelogenin,[23] RNA polymerase II,[24–26] prolamin storage proteins of wheat and related

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cereals,[27] titin,[28] mucins,[29,30] and flagelliform silk cDNA).[31] They impart important structural features and confer specialized functional roles for this diverse group of proteins.

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