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Analysis of Secondary Structure and Self-Assembly of Amelogenin by Variable Temperature Circular Dichroism and Isothermal Titration Calorimetry

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Abstract

Amelogenin is a proline-rich enamel matrix protein known to play an important role in the oriented growth of enamel crystals. Amelogenin self-assembles to form nanospheres and higher order structures mediated by hydrophobic interactions. This study aims to obtain a better insight into the relationship between primary-secondary structure and self-assembly of amelogenin by applying computational and biophysical methods. Variable temperature circular dichroism studies indicated that under physiological pH recombinant full-length porcine amelogenin contains unordered structures in equilibrium with polyproline type II (PPII) structure, the latter being more populated at lower temperatures. Increasing the concentration of rP172 resulted in the promotion of folding to an ordered β -structured assembly. Isothermal titration calorimetry dilution studies revealed that, at all temperatures, self-assembly is entropically driven due to the hydrophobic effect and the molar heat of assembly (ΔH_A) decreases with temperature. Using a computational approach, a profile of domains in the amino acid sequence that have a high propensity to assemble and to have PPII structures has been identified. We conclude that the assembly properties of amelogenin are due to complementarity between the hydrophobic and PPII helix prone regions.

Keywords

Amelogenin; Nanospheres; Enamel biomineralization; Modular structure; Polyproline type II; Supramolecular self-assembly

Introduction

Tooth enamel is a bioceramic with highly organized hierarchical structures that range from nano- to meso-scale levels. The mineralization of enamel takes place in the extracellular space and involves a series of complex cellular and molecular events.^{1,2} Protein secretion,

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supramolecular self-assembly, protein-mineral interactions and protein degradation are among the most studied.³⁻⁶ The critical functions of amelogenin, the major protein component of developing enamel, in matrix mediated enamel biomineralization have been well established.⁷⁻⁹ It is now well accepted that amelogenin supramolecular self-assembly into nanospheres is the key to its function in controlling crystal morphology and organization.¹⁰⁻¹² Support for the above notion has been provided by animal model studies in which blocking amelogenin gene expression resulted in complete loss of enamel rod-interrod structure.⁸⁻⁹ Mutations in human amelogenin gene lead to amelogenesis imperfecta, a hereditary disease of enamel malformation.¹³

The N- and C-termini of amelogenin are highly conserved among various species whereas small variations occur in the central region.¹⁴ Removal of the conserved domains (residues 1-42 and 157-173) in mouse amelogenin resulted in the formation of ill-defined enamel prisms highlighting the importance of these domains in the protein-protein or protein-mineral interactions.¹⁵⁻¹⁷ While numerous studies have focused on the functional importance of the N- and C- termini, little is known about the importance of the central domain in amelogenin self-assembly.^{14,17,18} The central region of amelogenin contains several PXXP/PXP repeats, the key structural motifs in polypro Type II (PPII) structure, where X represents amino acids that have high PPII helix forming propensity (Fig. 1).¹⁹⁻²⁰ We have recently reported the presence of PPII structure as a key secondary structural component in recombinant porcine amelogenin.²¹ The aim of this study was to get a better insight into the relationship between primary-secondary structure and self-assembly of amelogenin. We sought to identify the secondary structures adopted by various domains, their role in self-assembly, and finally their relevance to enamel biomineralization. We first provided direct evidence for the presence of PPII structure in rP172 and performed quantitative estimation of PPII content using variable temperature circular dichroism (VT-CD) spectroscopy. We then followed the conformational changes associated with the self-assembly as a function of concentration and temperature. By estimating the heat capacity for amelogenin self-assembly, we provided solid support for the involvement of hydrophobic interactions in assembly. Finally, by applying bioinformatics tools, we identified the domains that have high self-assembly (i.e. β -sheet or aggregation) and PPII secondary structure propensity. The results derived from these studies together with the published knowledge on amelogenin folding and self-assembly allowed us to propose a schematic modular structure for rP172 that explains possible functions of various domains in the self-assembly.

Materials and Methods

Protein expression and purification

Recombinant porcine amelogenin (rP172) was expressed in *E. coli* strain BL21-codon plus (DE3-RP, Strategene, La Jolla, CA), and precipitated by 20% ammonium sulfate.²² Purification of the ammonium sulfate precipitate was performed on a Varian Prostar HPLC system (ProStar/Dynamics 6, version 6.41 Varian, Palo Alto, CA, USA). The precipitate was dissolved in 0.1% TFA, loaded onto a Jupiter C4 semi-preparative column (342725-1, Phenomenex, Torrance, CA) (10 mm $\times$ 250 mm, 5 μ), and fractionated using a linear gradient of 60% acetonitrile at a flow rate of 2 mL/min. The homogeneity of the protein was confirmed by analytical chromatography using a C4 analytical column (214TP54, Vydac).

Variable temperature circular dichroism (VT-CD)

The VT-CD experiments were performed on a Jasco J-810 spectropolarimeter equipped with peltier set up. The protein was dissolved in Tris-HCl buffer at pH 7.4 $\pm$ 0.2 containing 5 mM CaCl₂. The presence of 5 mM CaCl₂ in the buffer enhances solubility of the protein and

reduces the pH sensitivity to temperature. A Suprasil quartz cell with a path length of 1 (20-45 μ M rP172) or 0.1 mm (60-90 μ M rP172) was used. For the wavelength scan, spectra were monitored over the 190-260 nm range with a resolution of 0.2 nm and a band width of 2 nm. The spectra were scanned at 5 °C intervals over a temperature range of 5 - 45 °C. Experiments at each temperature were recorded after 30 min to allow for equilibrium in the sample cell. The spectra presented are an average of 4 independent scans with a scan speed of 50 nm/min and expressed in mean residual weight ellipticity. Replicate measurements as a function of temperature reproduced the spectra within $\pm 5\%$ after the heating cycle. For the single wavelength measurements at 224 nm, the heating rate was 0.5 °C/minute over a temperature range of 5 - 45 °C and 5 - 85 °C.

Isothermal titration calorimetry (ITC) dilution experiments

ITC dilution experiments were performed on a Microcal VP-ITC (Microcal LLC, Northampton, MA). The VP-ITC measures heat by the power compensation principle and hence the output signal is opposite from that of the CSC instrument reported previously.²¹ The syringe was loaded with a concentrated solution of rP172 (250 μ M) in Tris-HCl, pH = 7.4 $\pm$ 0.2, containing 5 mM CaCl₂. Six μ L aliquots of the concentrated solution were added to the sample cell containing the same buffer, and experiments were conducted at various temperatures. The final concentration of the protein in the cell was 45 μ M. The area under each injection peak was divided by the injected mole number to obtain molar heat of disassembly and plotted against the concentration of rP172 in the cell. The enthalpy of assembly was obtained by the equation provided by Heerklotz et al.²³

Calculation of secondary structure propensity scale for rP172

For the β -sheet/aggregation propensity scale, experimental values determined for all naturally occurring amino acid residues were used.²⁴ The position sensitive preference for amino acid residues in a polyproline helix is provided in the Table 3 of ref. 25. We took the average of these values for the individual residues and estimated the PPII propensity for the whole sequence of rP172.

Results

Estimation of PPII content in rP172 by VT-CD spectra

The most convincing evidence for the presence of PPII structure in a protein or a polypeptide is the observation of an iso-elliptic point that ranges from 205-213 nm in a VT-CD spectrum.²⁶⁻³² Therefore, we monitored CD for rP172 from 5 °C to 45 °C. At 5 °C, the CD spectrum was typical of β_{II} proteins described by Sreerama and Woody, with a strong negative minimum around 200 nm and a weak shoulder in the $n-\pi^*$ region indicating the existence of unordered structure (Fig. 2A).²⁷ As the temperature was increased, the CD intensity in the 215-230 nm region decreased, whereas the CD intensity at 202 nm increased. The spectra exhibited a well defined iso-elliptic point around 211 nm indicating a two state equilibrium between unordered and PPII conformations. Such iso-elliptic points have been observed for several proteins and polypeptides that exhibit unordered-PPII equilibrium at low temperature ranges.²⁸⁻³² The CD difference spectrum ($[\theta]_{5^\circ\text{C}} - [\theta]_{45^\circ\text{C}}$ and $[\theta]_{10^\circ\text{C}} - [\theta]_{45^\circ\text{C}}$) indicates a negative minimum around 197 nm and a positive maximum in the 215-230 nm range, a characteristic CD spectra observed for PPII that is indicative of a higher population of PPII structure at low temperature in rP172 (Fig. 2B). Plotting the differences in CD spectra of amelogenin between 45 °C and 5 °C revealed a negative band around 221 nm and a positive band around 195 nm (data not shown). This indicates that with increasing temperature β -sheet-like conformation was stabilized at the expense of PPII structure.

A characteristic feature of a protein that exhibits a conformational equilibrium between the left-handed PPII conformation and a truly unordered conformation is the linear decrease in the ellipticity values as a function of temperature.^{26,32,33} As shown in Fig. 2C, a linear dependency of the ellipticity at 224 nm on temperature has been observed for both heating and cooling with a slope of $-29.7.0 \pm 0.4$ (for heating) and 28.7 ± 0.3 (for cooling) $\text{deg cm}^2 \text{ dmol}^{-1} \text{ C}^{-1}$. These values are close to those for polypeptides that exhibit unordered-PPII equilibrium. At higher temperatures, the increase in CD intensity in the 215-230 nm range indicated that the unordered amelogenin molecules become partially folded. The linear dependency further indicates less energy difference between the two states.²⁸ We have also followed the change in ellipticity at 224 nm over a temperature range of 5 - 85 °C in PBS buffer at pH 7.4 (Fig. 2D). A linear increase in ellipticity with slope values -24.1 ± 0.3 and $-23.3 \pm 0.3 \text{ deg cm}^2 \text{ dmol}^{-1} \text{ C}^{-1}$ have been observed for heating and cooling cycles, respectively. These results confirm the presence of PPII structure in rP172. Using the thermal dependency of ellipticity values we estimated the PPII content by the method reported by Park et al.³⁴ Table I lists the %PPII content at various temperatures. The PPII content in rP172 varies from 20.2% to 42.6%, depending on the temperature. The estimated PPII content at 37 °C (22.3%) is in good agreement with the estimated values obtained by other methods (Table I). Thus, VT-CD results demonstrate that PPII is more populated at low temperatures when amelogenin is in its lower assembly form and decreases at higher temperatures following folding or assembly where the presence of β -sheet structures is more apparent.

Concentration-dependent conformational changes in rP172

To monitor the conformational changes following self-assembly, we performed CD experiments by diluting a concentrated solution (90 μM) of rP172 at two different temperatures. At 10 °C, the concentrated solution of rP172 exhibited CD spectra that were characteristic of a β -sheet conformation, with a strong negative π - π^* minimum around 215 nm and a weak positive maximum around 200 nm. Following dilution to low concentration (20 μM), an unordered structure was observed at this temperature (Fig. 3A). At 40 °C, the negative minimum observed at 215 nm was considerably red-shifted to 221 nm in concentrated solution, indicating a β -turn structure, while the unordered structure was retained at low concentration (Fig. 3B). A plot of $[\theta]_{224}$ against concentration at these two temperatures shows that the formation of a β -structured assembly is rapid at higher temperatures (Fig. 3C). These experiments collectively suggest that at higher concentrations, and in assembled form (90 μM), rP172 contains predominantly β -sheet or β -turn conformations.^{5,6,11} Such behavior has been observed for a number of proteins associated with neurodegenerative diseases, silk fibroin, and elastin-like peptides.³⁵⁻⁴¹

Determination of thermodynamic parameters of assembly by ITC

ITC dilution has been successfully used to investigate the thermodynamic driving force for supramolecular self-assembly.^{21,23,42-48} In this approach, a concentrated solution of the protein in the syringe is added stepwise into a calorimetric cell that contains the same buffer and the heat of dilution is measured. The sign of heat of dilution determines the nature of self-assembly. A negative sign indicates hydrophobic interactions that mainly drive the self-assembly, whereas a positive value indicates other non-covalent interactions such as hydrogen bonding that drive the self-assembly.⁴⁸ Here we studied the effect of temperature on the thermodynamic driving force for self-assembly of rP172. At all temperatures, dilution causes dissociation/disintegration of the assembled rP172 with exothermic heat flow (Fig. 4 A and B). The measured heat flow is related to the self-assembly (ΔH_A) but with an opposite sign (i.e., heat of dilution, $\Delta H_{\text{dil}} = -\Delta H_A$). The dynamic nature of the disassembly was inferred from the fact that the heat flow reaches baseline within few seconds. As the concentration of rP172 increases in the calorimetric cell, the heat flow decreases and

remains constant at higher injections. The area under the curve for each injection (δq_i), divided by injected mole number (δn_i), gives molar heat of disassembly. A quasi-sigmoidal curve with an inflection point at the critical aggregation concentration (CAC) was obtained when $\delta q_i/\delta n_i$ was plotted against total rP172 concentration in the cell (Fig. 4C). The enthalpy of self-assembly was obtained as reported by Heerklotz et al.¹⁸ The enthalpy of self-assembly at 30° C (+ 26.3 kCal/mole) and 37° C (+17.8 kCal/mole) were considerably lower than the values obtained at 25 °C.²¹

The total enthalpy of dilution of the assembled protein was contributed by three major terms: i) solvation enthalpy (ΔH_{solv}), ii) conformational enthalpy (ΔH_{conf}), and iii) interaction enthalpy ($\Delta H_{\text{interaction}}$); whichever dominates the assembly process will determine the sign of ΔH_A ($\approx \Delta H_{\text{solv}} + \Delta H_{\text{conf}} + \Delta H_{\text{interaction}}$). For self-assembly of rP172, $\Delta H_{\text{interaction}}$ and ΔH_{conf} are exothermic (negative) since the assembled structure is stabilized by non-covalent interactions and an unordered to an ordered β -sheet transition takes place upon self-assembly. The overall large endothermic (positive) enthalpy observed for rP172 indicates that solvation enthalpy contributes significantly (because $\Delta H_{\text{solv}} \gg -\Delta H_{\text{conf}} - \Delta H_{\text{interaction}}$) and suggests that considerable dehydration occurs upon self-assembly. At all temperatures examined, the driving force for the self-assembly is therefore to remove the ordered water molecules from the hydrophobic interface of monomer/oligomer, so as to create a hydrophobic phase even though the conformational entropy (ΔS_{conf}) opposes such transformation. However, the formation of a hydrophobic core increases entropy contribution due to the hydrophobic effect (ΔS_{HE}), which in turn increases the overall entropy of the assembly (ΔS_A). As a result, even at 37 °C and physiological pH, self-assembly of rP172 is endothermic ($\Delta H > 0$) and driven by a large change in entropic contribution due to the hydrophobic effect. Previously described cases of self-assembly driven by the hydrophobic effect are characterized by large negative changes in heat capacity.^{23,46} A plot of ΔH_A against temperature for rP172 gives a linear decrease (negative heat capacity), indicating that the assembly of rP172 is driven by the hydrophobic effect (Fig. 4D).

Analysis of regions with propensity to associate and to adopt PPII structure

The experimental results demonstrate the presence of PPII structure in the monomer/oligomer of rP172 and that the assembly is driven by hydrophobic interactions with concomitant conversion to an ordered β -sheet structure. Amelogenin is enriched in proline, a well-known α -helix/ β -sheet breaker that decreases the aggregation rate.⁴⁹ We next sought to understand how regions with a tendency to assemble and a tendency to adopt PPII structure are distributed within the amino acid sequence and how such regions complement the self-assembly characteristics of rP172. It has been well established that a small stretch of amino acid residues can promote a disorder to order structural transition. Such stretches are characterized by high hydrophobicity, high propensity to form β -sheet structure, and low net charge. For an unordered protein, self-assembly is driven by both internal factors (hydrophobicity, propensity to form β -sheet structure, and net charge) and external factors (pH, ionic strength, and protein concentration) that control the assembly into an organized structure.⁵⁰ By considering the internal factors, a scale of β -sheet propensity for all 20 naturally occurring amino acids has been developed to predict the regions in a primary sequence that transform into a β -sheet structure upon self-assembly.²⁴ Using this empirical scale, a β -sheet/aggregation propensity profile was obtained for rP172 over a sliding window of 5 contiguous amino acid residues and plotted against the residue number (Fig. 5A). Two main regions with high β -sheet propensity above the mean propensity of the whole sequence (indicated by the horizontal broken line) can be distinguished. We interpret these regions as the protein-protein interacting domains for amelogenin self-assembly. Interestingly, these two regions lie close to the A- and B-domains (underlined in the Fig. 5C) defined by Paine

and Snead for mouse amelogenin.¹⁷ Amelogenins that lack these domains have defective self-assembly and therefore poor mineral orchestrating properties, validating the importance of the two domains in enamel formation.^{7,15,16}

In globular proteins, PPII helix occurs in small regions (around 3–4 amino acid residues in length) and are present even in regions that do not have proline residues.^{51,52} Stapley and Creamer analyzed about 274 high resolution crystal structures and estimated the preference for each amino acid in a PPII helix.²⁵ We applied this scale to obtain a profile of regions that are dominated by high PPII helix propensity in rP172 (Fig. 5B). Note that the short N-terminal region and the major central regions have average PPII helix propensities above the mean PPII propensity (indicated by the horizontal broken line) of the whole sequence. Interestingly, the two properties (viz., aggregation or assembly propensity and PPII propensity) complement each other in the rP172 amino acid sequence, except for a slight overlap close to the B-domain (Fig. 5C). The charged C-terminal 12 amino acid residues have low PPII and β -sheet propensities confirming the presence of random-coil structure.⁵³ From Fig. 5B, we estimated a maximum PPII content of 43.6% (assuming that the residues close to the horizontal dotted line may contribute to the PPII structure) and a minimum of 34.8%. The average value (39.2%) is in excellent agreement with the experimentally observed values at low temperatures (Table I). We further estimated the %PPII content in rP172 using the propensity scales reported by Chen et al.,⁵⁴ and Eker et al.,⁵⁵ and in order to put these results into context we compared the estimated PPII content for rP172 and other peptides with their experimentally determined values (Table II). While reasonable agreement between the three scales was observed for short peptides, Schweitzer-Stenner scale predicted a higher PPII content for rP172. We observed such a high value only in the presence of 4M guanidine hydrochloride (data not shown). Disagreement in rP172 PPII content estimation may be the result of differences in the experimental conditions under which rP172 was analyzed.

The ubiquitous presence of PPII structure renders an unfolded protein resistant to aggregation and to extensive hydration by increasing the solvent accessibility of the peptide backbone through coordinated water molecules.⁵⁶ The presence of unordered structure exposes the assembly prone regions to the solvent and makes them available for intermolecular contacts leading to self-assembly upon an increase in protein concentration. It has been established that a few short regions in a primary sequence can act as a facilitator or an inhibitor of assembly. Interestingly, the propensity scales indicate that the amino acid sequence of rP172 is finely adapted to counterbalance the solubility and assembly characteristics.

Fig. 6 is a schematic model that explains the modular structure for rP172 proposed based on the above analysis. The structure contains independently foldable domains that are separated by extended flexible (or unfolded) linker regions. At low protein concentration the dominant presence of PPII structure provides an extended structure that confers high water/buffer solubility with ordered water molecules around the hydrophobic assembly-prone regions. The presence of amphiphilic structure is important for the concentration dependent conformational changes. At higher concentrations, the disorder to order transformation depends on the availability of a number of non-covalent interactions sufficient to compensate for the conformational entropy loss. Under these conditions, the hydrophobic and hydrophilic domains phase separate to form an assembled structure (i.e. nanospheres) with a hydrophobic core and hydrophilic corona. The presence of domains with competing physico-chemical properties is critical to control the intermolecular interactions and phase segregation upon self-assembly. As the hydrophobic domains interact, the ordered water molecules around the monomer or oligomer structure are expelled (breaking of water molecules, hence overall endothermic enthalpy) and the entropy of these domains increases.

Discussion

Identifying the driving forces and the mechanism of self-assembly is a prerequisite to understanding the function of amelogenin and its cleavage products during enamel formation. Combined analysis of CD and bioinformatics suggests the existence of a strong correlation between secondary structure and self-assembly of amelogenin. The functional relevance of unordered structures with exposed hydrophobic domains presumably resides in an increased propensity to undergo unordered (unfolded) to ordered (folded) transformation. The important findings presented here are 1) that there is PPII structure present in amelogenin under physiological conditions, 2) that there is a disorder to order transition upon self-assembly, and 3) that the self-assembly is entropically driven due to the hydrophobic effect. Collectively, these results allow us to conclude that self-assembly of amelogenin is derived from the intrinsic propensities of the polypeptide chain. Support for the present CD data that amelogenin has an extended PPII conformation within certain regions in the sequence, and lacks apparent tertiary structure is reported elsewhere by structural determination of amelogenin monomer using 2D NMR.⁵⁷ Our recent NMR studies together with an analysis of amelogenin sequences from various species have established a link between amelogenin and “intrinsically disordered proteins”⁵⁷.

In vivo, the full length amelogenin is cleaved into various fragments by matrix proteases. An elongated bundle like structure has also been reported for 20 kDa fragment of porcine amelogenin.⁵⁸ According to our data, removal of the C-terminal 24 amino acid residues from rP172 does not affect the PPII content. We observed that rP148, the C-terminal truncated variant of rP172, had a PPII content of 26.1% at 37 °C confirming the validity of the proposed model (see Supporting Information). As isolated entities, the “13k” amelogenin (residue 46 – 148 in native porcine amelogenin) and the N-terminal TRAP domains are predicted to have PPII and β -sheet structures, respectively. The validity of our structural prediction is supported by reported findings on the N-terminal region and “13k” amelogenin secondary structure by Goto et al.⁵⁹ These authors have shown that the N-terminal region of the porcine amelogenin has a β -sheet structure and the “13k” amelogenin exhibits a well-defined iso-elliptic point around 211 nm providing evidence that this central region possesses an equilibrium between PPII structure and unstructured conformations. The central region in particular, provides good water solubility and extended structure due to the presence of high PPII propensity in this region.⁶⁰ Our prediction is further supported by a recent demonstration that stretches of proline attached to polyglutamine retain the PPII structure in an otherwise aggregated β -sheet structured polyglutamine domain and increase the threshold for amyloid fibril formation to ~15 glutamine residues.⁶¹ Recently, Fukae et al. have shown that the central domain (“13k” amelogenin) assumes a cylindrical structure, consistent with the morphology exhibited by proline-rich proteins.²⁰ Thus, we suggest that the proline-rich central domain confers solubility to the monomeric amelogenin and prevents excessive aggregation. We therefore conclude that the assembly properties of amelogenin are due to complementarity between the hydrophobic and PPII helix prone regions. The presence of alternate arrangements of these domains with a predominantly unordered and charged C-terminal region provide the tendency to segregate into separate regions in the molecule during self-assembly.⁶² Such structural arrangement may act as a precursor to the hierarchically organized structures observed in the enamel matrix.¹¹

While much remains to be investigated about the mechanism of amelogenin self-assembly, understanding of the influence of various domains will undoubtedly provide further insight into the relationship between secondary structure and self-assembly properties.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Amino acid sequence of porcine amelogenin. The recombinant amelogenin used in this study lacks the first methionine and a phosphate group on serine16. The PXP/PXXP motifs that play an important role in imparting the PPII structure are highlighted in grey.

Fig. 2.

(A) VT-CD spectra of rP172 in Tris-HCl buffer (pH = 7.4) containing 5 mM CaCl₂. The protein concentration used was 20 μ M. The inset shows the iso-elliptic point around 211 nm. (B) Difference spectra obtained by subtracting the CD spectra at 5 °C and 10 °C from the CD spectra at 45 °C. (C) Changes in CD intensity at 224 nm as a function of temperature. (D) Thermal dependency of CD intensity at 224 nm as a function of temperature in PBS buffer (pH 7.4). The black open circles and grey open circles indicate heating and cooling cycles, respectively. Note that the ellipticity is linear over a broad temperature range.

Fig. 3.

Concentration dependent conformational changes in rP172 (A) CD spectra of various concentrations of rP172 at 10 °C (A) and 40 °C (B). (C) The ellipticity values at 224 nm as a function of concentration at 10 °C (solid circles) and at 40 °C (open circles). Note that the β -structured assembly is rapidly reached at elevated temperature.

Fig. 4. ITC dilution studies of assembled rP172 at various temperatures. (A, B) Raw ITC data for the dilution of rP172 at 30 °C and 37 °C. The temperature is indicated in each enthalpogram. (C) Heat of dilution (δ_{qi}) per mole of rP172 injected (δ_{ni}) as a function of concentration of rP172 in the cell at 30 °C and 37 °C. (D) The molar heat of assembly as a function of temperature (based on three temperatures).

Fig. 5.

Predicting the propensities for primary structures of rP172. (A) β -sheet or aggregation propensity (red) and (B) PPII propensity (blue) for rP172. Each point represents average propensity of 5 contiguous residues. The broken line in the figure indicates mean propensity of the whole protein. The regions higher than this line are the stretches that, when compared to the whole sequence, have high aggregation, or β -sheet, and PPII propensity. (C) Amino acid sequence of rP172 with the regions having high PPII (blue letters) and aggregation (red letters) propensities. The overlapping and unknown regions are labeled in green and black, respectively. The A- and B- domains that were shown to play an important role in the self-assembly and protein-protein interactions are indicated by the horizontal lines.

Fig. 6.

Proposed modular structure for rP172 with alternate PPII and hydrophobic domains. The domains with high β -sheet propensity are involved in protein-protein interactions and aid the self-assembly whereas PPII domains provide water solubility and prevents excessive aggregation.

Table I

Estimation of PPII content in rP172

Temperature	%PPII estimated by		
	Our method ^a	Kelly et al. ^b	Bienkiewicz et al. ^c
5	42.6	38.4	31.2
10	35.8	35.6	28.6
15	33.4	34.6	27.8
20	30.7	33.5	26.8
25	28.4	32.6	25.9
30	26.8	31.9	24.3
35	23.4	30.5	25.3
37	22.3	30.3	24.8
40	22.0	29.9	23.5
45	20.2	29.2	22.8

^a Limiting ellipticity values were obtained by fitting the curve in Fig. 2C to a two state transition. The %PPII is estimated by using the equations, $[\theta]_{224} = +2791f_{\text{PPII}} - 3522f_{\text{U}}$ (for the heating cycle) and $[\theta]_{224} = +2086f_{\text{PPII}} - 3013f_{\text{U}}$ (for the cooling cycle), where f_{PPII} and f_{U} are, respectively, fractions of polyproline and unordered conformations. The average value is reported in the table.

^b Kelly et al. Ref.19

^c Equation 1 from Bienkiewicz et al. Ref. 26

Table II

Comparison of %PPII content in rP172, amyloid polypeptides and calcitonin as estimated by three different propensity scales.

Proteins/peptides	Experimentally Determined PPII content	Predicted PPII content by		
		Stapley-Creamer	Kallenbach	Schweitzer-Stenner
rP172	^a 42.6%	39.2%	49%	59%
A β (1-40)	^b 45%	50%	50%	42.5%
A β (1-28)	^b 54% ^c (65%)	64.3%	41%	48.2%
Salmon Calcitonin	^c 43%	37.5%	37.5%	43.8%

^aThis article at 5 °C

^bRef.30

^cRef.63