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Amyloid fibril formation propensity is inherent into the hexapeptide tandemly repeating sequence of the central domain of silkmoth chorion proteins of the A-family

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Abstract

Peptide-analogues of the A and B families of silkmoth chorion proteins form amyloid fibrils under a variety of conditions [Iconomidou, V.A., Vriend, G. Hamodrakas, S.J. 2000. Amyloids protect the silkmoth oocyte and embryo. FEBS Lett. 479, 141–145; Iconomidou,V.A., Chryssikos, G.D.,Gionis, V., Vriend, G., Hoenger, A., Hamodrakas, S.J., 2001. Amyloid-like fibrils from an 18-residue peptide-analogue of a part of the central domain of the B-family of silkmoth chorion protein. FEBS Lett. 499, 268–273; Hamodrakas, S.J. Hoenger, A., Iconomidou, V. A., 2004 . Amyloid fibrillogenesis of silkmoth chorion protein peptide-analogues via a liquid crystalline intermediate phase. J. Struct. Biol. 145, 226–235.], which led us to propose that silkmoth chorion is a natural protective amyloid. In this study, we designed and synthesized two mutant peptide-analogues of the central conservative domain of the A family: (a) one, cA_m1, with a length half of that of the central domain of the A family, which folds and self-assembles, in various conditions, into amyloid fibrils very similar in properties and structure to the fibrils formed by the cA peptide, which corresponds to the entire length of the A family central domain [Iconomidou, V.A., Vriend, G. Hamodrakas, S.J. 2000. Amyloids protect the silkmoth oocyte and embryo. FEBS Lett. 479, 141–145.], in full support of our previous proposal, (b) the second, cA_m2, differing from cA_m1 at three positions, where three glutamates have replaced two valines and one alanine residues, does not form amyloid fibrils in any conditions. It appears that (a) the amyloidogenic properties of silkmoth chorion peptides are encoded into the tandemly repeating hexapeptides comprising the central domain of silkmoth chorion proteins, and, that (b) suitable mutations, properly and carefully designed, greatly affect the strong amyloidogenic properties inherent in certain aminoacid sequences and may inhibit amyloid formation.

Keywords: Amyloids; Formation; β-Pleated sheet; Electron microscopy; X-ray diffraction; ATR FT-IR; Silkmoth chorion peptides

1. Introduction

Chorion, the major part (ca. 90%) of the eggshell of many insect and fish eggs, is an important biological structure with extraordinary mechanical and physiological properties. The proteinaceous silkmoth chorion consists of more than 200 different proteins, which account for more than 95% of its dry mass (Kafatos et al., 1977). Silkmoth chorion proteins are variants of two major themes: they have been classified into two major classes the A's and the B's (Regier and Kafatos, 1985). Both families of silkmoth chorion proteins consist of three domains. The central domain is conserved in each class and contains tandemly repeating hexapeptides (Hamodrakas et al., 1985). The flanking N- and C-terminal domains are more variable and

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also contain characteristic tandem repeats different from the repeats of the central domain (Hamodrakas et al., 1982). Furthermore, the A and B central domains show distant similarities suggesting that chorion genes constitute a superfamily derived from a single ancestral gene (Lekanidou et al., 1986).

The purification of individual chorion proteins in large amounts and purity, sufficient for structural studies, is very difficult. Therefore, in order to study the structural properties of chorion proteins and their possible functional role in the formation of silkmoth chorion (Hamodrakas, 1992), we have synthesized peptide-analogues representative of parts or the entire central conservative domain of the A and B silkmoth chorion protein families (Benaki et al., 1998). Further, we have demonstrated that these peptide-analogues form amyloid-like fibrils by self-assembly mechanisms, under a great variety of conditions *in vitro* (Iconomidou et al., 2000; Iconomidou et al., 2001).These fibrils are similar in appearance and structure to those constituting silkmoth chorion (Hamodrakas, 1992; Iconomidou et al., 2000), suggesting that silkmoth chorion is a natural, protective amyloid, important for the survival and development of the oocyte and the developing embryo (Iconomidou et al., 2000; Iconomidou et al., 2001). This role of silkmoth chorion is in contrast to that of amyloids associated with serious diseases including Alzheimer's disease, the transmissible spongiform encephalopathies, type II diabetes mellitus and a number of systemic polyneuropathies (Pepys, 1996; Kelly, 1996, 1998; Dobson, 1999).

As a continuation of these studies, we have recently designed and synthesized, two peptides, mutants of the cA peptide (see Section 2). The first, called cA_m1, is approximately half the size of the cA peptide (Fig. 1), whereas the second, called cA_m2, is a variant of the first, having three hydrophobic residues (two valines,V, and an alanine, A) replaced by glutamates (E) at specific positions (Fig. 1). The logic behind this synthesis was the following: We were interested to find out (a) whether amyloid-like fibril formation is sustained by fractions of the cA peptide, which contains tandemly repeating hexapeptides, in other words to determine whether amyloid fibril formation ability is inherent into the hexapeptide repeating structure of the central domain of chorion proteins, and, (b) whether specific mutations in the model β-strands of cA, at locations where β-former residues exist, would influence the structure and assembly of the formed super-structures.

In this work, we report on the self-assembly properties of these mutant peptides and discuss the implications of the findings.

2. Materials and methods

2.1. Peptide synthesis

The cA_m1 and cA_m2 peptides (Fig. 1) were synthesized by the solid phase methodology and Fmoc/^{t}Bu chemistry (Fields and Noble, 1990; Miranda and Alewood, 1999), using an Advanced ChemTech Mod 396MBS automatic peptide synthesizer and 2-chlorotrityl chloride resin (Barlos et al., 1989). The removal of the amino acid side-chain protecting groups and the cleavage from the resin were accomplished by treatment with 80% trifluoroacetic acid (TFA) in dicloromethane (DCM). Purification of peptides was carried out by gel filtration chromatography and RP-HPLC. The final products were determined to be at least 97% pure by analytical HPLC and their molecular masses verified by ESI-MS.

2.2. Formation of amyloid-like fibrils—formation of oriented fibres

The cA_m1 peptide-analogue of the central domain of silkmoth chorion A proteins (Fig.1) was dissolved: (a) in doubly distilled water (pH 5.5), (b) in a 50 mM sodium acetate buffer (pH 5), (c) in a 50% (w/v) water/methanol mixture, at a concentration of 10 mg ml^{-1}. In all cases, mature

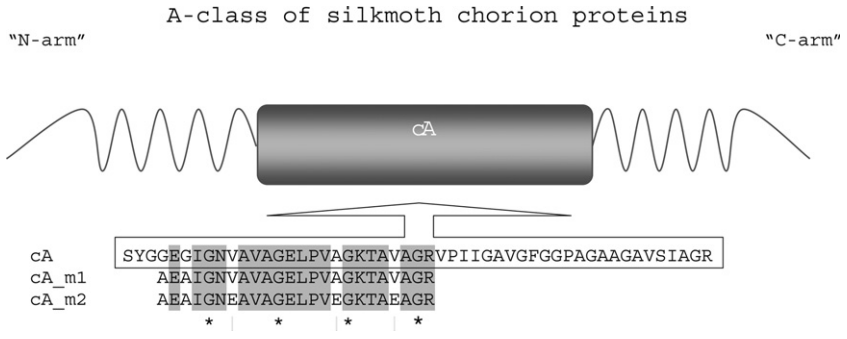

Fig. 1. A schematic representation of the tripartite structure of silkmoth chorion proteins of the A family. A highly conservative central domain of invariant length, and two more variable flanking "arms" constitute each protein. Characteristic, tandemly repeating peptides are present both in the central domain and in the "arms" (Hamodrakas et al., 1985; Hamodrakas, 1992 and references therein). The aminoacid sequence and relative position of the synthetic cA peptide (one letter code), designed to be an analogue of the entire central domain of the A family, is shown and also the sequences of the designed synthetic mutant peptides cA_m1 and cA_m2. cA_m1, 24-residues in length, has approximately half the size of the cA peptide (51 residues). Peptide cA_m2 differs from cA_m1 at three positions, where glutamates (E) have replaced two valine (V) and one alanine (A) residues. Conserved residues are shaded. Invariant glycines (G) repeating every six-residues are marked with an asterisk below the sequence.

amyloid-like fibrils were formed after 1–2 weeks incubation. Oriented fibres, suitable for X-ray diffraction, were obtained from suspensions of cA_m1 peptide amyloid-like fibrils as described in Iconomidou et al. (2000) and below. In contrast, solutions of the cA_m2 peptide, under the same conditions, did not produce amyloid-like fibrils even after incubation for several months.

2.3. X-ray diffraction

cA_m1 peptide was dissolved in a 50 mM sodium acetate buffer (pH 5) at a concentration of 10 mg ml^{-1} to produce mature amyloid-like fibrils after 1–2 weeks incubation. A droplet (~10 μl)of fibril suspension was placed between two siliconized glass rods, spaced ~2 mm apart and mounted horizontally on a glass substrate, as collinearly as possible. The droplet was allowed to dry slowly at ambient temperature and humidity for 1 h to form an oriented fibre suitable for X-ray diffraction. X-ray diffraction patterns were recorded on a Mar Research 345 mm image plate, utilizing double-mirror (Prophysics mirror system XRM-216) focused CuK_α radiation ($\lambda =$ 1.5418 Å), obtained from a GX-21 rotating anode generator (Elliot-Marconi Avionics, Hertfordshire, England) operated at 40 kV, 75 mA. The specimen-to-film distance was set at 200 mm and the exposure time was 1 h. No additional low angle reflections were observed at longer specimen-to-film distances of up to 300 mm. The X-ray patterns, initially viewed using the program MarView (MAR Research, Hamburg, Germany), were displayed and measured with the aid of the program IPDISP of the CCP4 package (Collaborative Computational Project, 1994).

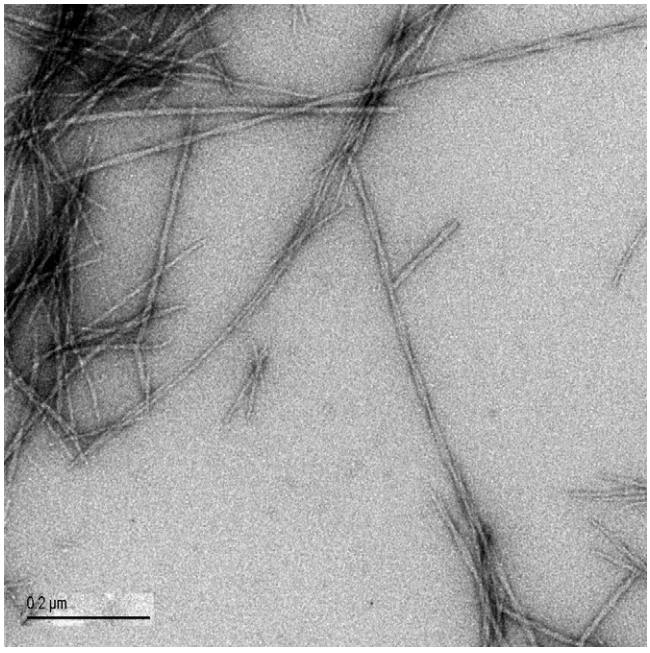

Fig. 2. Electron micrograph of amyloid-like fibrils derived by self-assembly, from a 10 mg ml^{-1} solution of the cA_m1 peptide in a sodium acetate 50 mM buffer, pH 5. Fibrils were negatively stained with 1% uranyl acetate. They are of indeterminate length (several microns), unbranched, approximately 90 Å in diameter and have a double helical structure. A pair of protofilaments each 40–50 Å in diameter are frequently wound around each other, forming the double-helical fibrils. *Bar* 0.2 μm.

2.4. Negative staining

For negative staining, the cA_m1 peptide fibril suspensions as well as the cA_m2 solutions after incubation for several months, were applied to glow-discharged 400-mesh carbon coated copper grids for 60 s. The grids were (occasionally) flash-washed with ~150 μl of distilled water and stained with a drop of 1% (w/v) aqueous uranyl acetate for 45 s. Excess stain was removed by blotting with a filter paper and the grids were air-dried. They were examined in a Philips CM120 Biotwin transmission electron microscope operated at 100 kV or in a Philips Morgagni 268D electron microscope under the same conditions. In the case of the CM120 Biotwin microscope, photographs were obtained by a retractable slow scan CCD camera (SSC™, Gatan Inc.) utilizing the program Digital Micrograph 2.5.8 (Gatan Inc.).

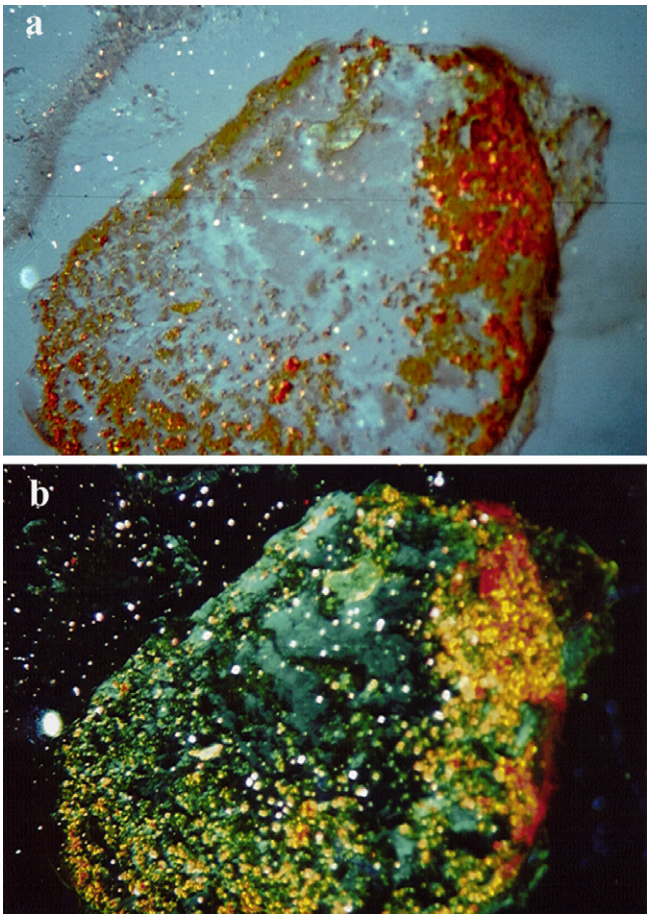

Fig. 3. Photomicrographs of cA_m1 peptide fibrils stained with Congo red: (a) Bright field illumination, (b) crossed polars. The red-green birefringence characteristic for amyloid fibrils is clearly seen.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5. Attenuated total reflectance infrared spectroscopy (ATR FT-IR)

Ten microlitres drop of the cA_m1 or cA_m2 suspensions (Section 2.2) were cast on a front coated Au mirror and left to dry slowly at ambient conditions to form thin films. Infrared spectra were obtained at a resolution of $4\,cm^{-1}$, utilizing an IR microscope (IRScope II by Bruker Optics) equipped with a Ge Attenuated Total Reflectance objective lens (20×) and attached to a Fourier-transform spectrometer (Equinox 55 by Bruker Optics). ATR spectra were also measured with a single reflection accessory (DuraSamplIR II by SensIR Technologies) equipped with a diamond element. The spectra represent averages of 100 scans at $4\,cm^{-1}$ resolution. Ca. 10 μl of a freshly made $10\,mg\,ml^{-1}$ solution of the cA_m1 peptide in D_2O was brought in contact with the diamond element. The solvent was then evaporated under a flow of N_2 gas, and the spectrum of the formed thin film was measured.

Internal reflection spectroscopy has several advantages compared to the more common KBr dispersion technique (De Jongh et al., 1996). The choice of ATR was dictated by the need to exclude any possible spectroscopic and chemical interactions between the sample and the dispersing medium. Having a penetration depth of less than 1 μm ($1000\,cm^{-1}$, Ge), ATR is free of saturation effects, which may be present in the transmission spectra of thicker samples. Moreover, the use of a single reflection accessory facilitates the acquisition of data from small samples. Ten 32-scan spectra were collected from each sample and averaged to improve the S/N ratio. The spectra are shown in the Absorption mode after correction for the wavelength-dependence of the penetration depth ($d_p \propto \lambda$). Absorption peak maxima were determined from the minima in the 2nd derivative of the corresponding spectra computed by the Savitzky–Golay algorithm over a $\pm 8\,cm^{-1}$

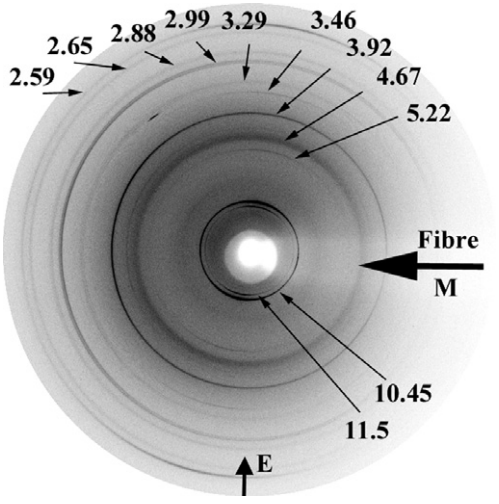

Fig. 4. X-ray diffraction pattern from an oriented fibre of cA_m1 peptide amyloid-like fibrils. The meridian, M (direction parallel to the fibre axis) is horizontal and the equator, E, is vertical in this display. The X-ray diffraction pattern resembles a "cross-β" pattern showing a 4.67 Å reflection as a ring and 10.45 and 11.5 Å reflections on the equator. This indicates a regular structural repeat of 4.67 Å and structural spacings of 10.45 and 11.5 Å perpendicular to the fibre axis. These two equatorial reflections usually merge into one, in other diffraction patterns from oriented fibres of cA_m1 peptide amyloid-like fibrils. The structural repeat of 4.67 Å corresponds to the spacing of adjacent β-strands and the 10.45 Å spacing parallel to the fibre axis corresponds to the face-to-face separation (packing distance) of the β-sheets. Possible origin and measured spacings of the other reflections have been discussed previously (Iconomidou et al., 2000).

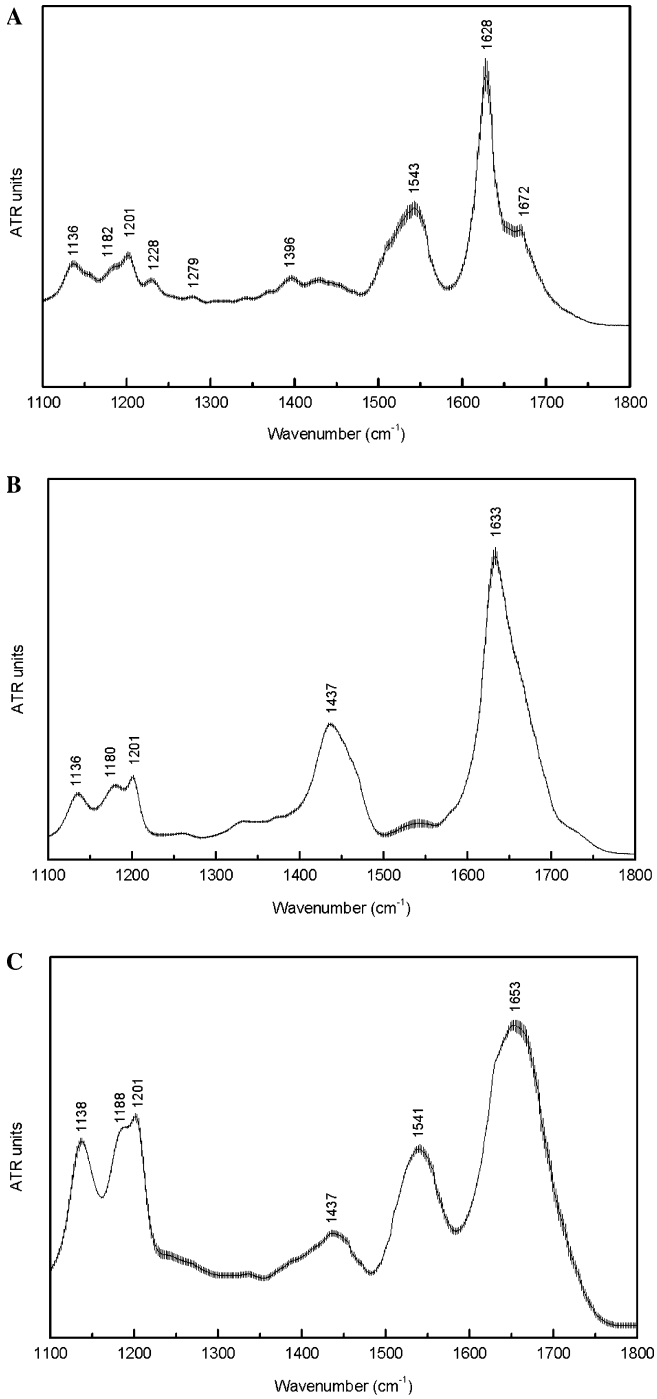

Fig. 5. ATR FT-IR ($1100–1800\,cm^{-1}$) spectrum of: (A) cA_m1 peptide amyloid fibrils, cast as a thin film on a Au mirror, (B) a freshly made solution of cA_m1 peptide in D_2O ($10\,mg\,ml^{-1}$) cast as a thin film on a diamond ATR element, and (C) a cA_m2 peptide solution, cast as a thin film on a Au mirror (see Section 2). Errorbar equals σ in the IR spectrum.

range, around each data point (Savitsky and Golay, 1964). Smoothing over narrower ranges resulted to a deterioration of the *S*/*N* ratio and did not increase the number of minima that could be determined with confidence.

2.6. Modelling

Modelling was performed following procedures described in detail earlier (Iconomidou et al., 2000).

3. Results and discussion

cA_m1 peptide (Fig. 1) folds and self-assembles, forming mature amyloid-like fibrils (Fig. 2) after 1–2 weeks incubation, in a variety of solvents and conditions (see Section 2). In contrast, solutions of cA_m2 (Fig. 1), under the same conditions and after incubation for several months, do not form amyloid-like fibrils and only very rarely show some tubular-like structures (data not shown). The fibrils formed by the cA_m1 peptide are very similar in structure and properties to the fibrils formed by the cA peptide (Iconomidou et al., 2000). They are of indeterminate length, they have a thickness of ca. 100 Å and they appear to be double helical in structure (Fig. 2). They also bind Congo red showing the characteristic red-green birefringence when seen under crossed polars (Fig. 3), and they give characteristic "cross-β"-like X-ray diffraction patterns (Fig. 4) from oriented fibres (see Section 2). Therefore, they display all the features that characterize amyloid fibrils.

The X-ray diffraction patterns of oriented fibres from cA_m1 exhibit several reflections (Fig. 4). Most of these reflections appear as rings due to poor alignment of the constituent fibrils. The strong reflections corresponding to periodicities of 4.67 Å and 10.45 Å may be attributed to the inter-strand and inter-sheet distances of β-sheet arrangements, respectively. These reflections are characteristic of the "cross-β" conformation (Geddes et al., 1968), and are observed for several amyloid-like fibrils (Iconomidou et al., 2000; Sunde and Blake, 1997 and references therein), in which the β-strands (if oriented) are perpendicular to the fibre axis and the sheets are packed parallel to the fibre axis. This conformation would produce oriented patterns from oriented samples with a meridional reflection at ca. 4.7 Å and the corresponding equatorial reflection at ca. 10 Å, i.e., very similar to the patterns obtained from fibres of the chorion cA peptide (Iconomidou et al., 2000). As seen in Fig. 4, only the 10.45 Å reflection of the oriented fibres produced from the amyloid fibrils of the cA_m1 peptide shows preferred orientation. Similarly, preferred orientation is observed for the very sharp 5.22 Å and 3.46 Å reflections, which are the second (10.45/2) and third (10.45/3) orders of the 10.45 Å reflection. This probably indicates that the packed β-sheets exhibit long range order in a direction perpendicular to the fibre axis, that is the sheets are packed parallel to the fibre axis. The fact that the 4.67 Å reflection is not preferentially meridional (perpendicular to the fibre axis) but instead is a ring, indicates a more random orientation of the packed β-sheets (and concomitantly of the β-strands) with respect to the fibre axis.

Attempts to obtain oriented fibres from solutions of the cA_m2 peptide were not successful since this peptide did not form amyloid fibrils or suitable viscous solutions for fibre formation. Furthermore, solutions of this peptide do not bind Congo red exhibiting the characteristic for amyloids red-green birefringence.

The ATR FT-IR spectra of the cA_m1 and cA_m2 peptides are compared in Fig. 5A–C and Table 1. The spectrum from cA_m1 peptide amyloid fibrils (Fig. 5A and Table 1) shows one prominent band at 1628 cm^{-1} in the amide I region and an amide III component at 1228 cm^{-1}, which are definitely assigned to β-sheet (Krimm and Bandekar, 1986; Surewicz et al., 1993; Haris and Chapman, 1995; Jackson and Mantsch, 1995; Cai and Singh, 1999). The low frequency of these amide I and III components results from the strong hydrogen bonds in the β-sheets, whereas the very narrow width of the amide I band at 1628 cm^{-1}(ca. 20 cm^{-1}) suggests that the distribution of the phi and psi angles in the sheets is narrow and implies a very uniform structure. Thus, ATR FT-IR supports the presence of uniform β-sheets in the structure of cA_m1 peptide fibrils, in agreement with the existence of a β-sheet structure suggested by X-ray diffraction.

It is interesting to note that, the ATR FT-IR spectrum of the cA_m1 peptide obtained immediately after dissolving the peptide in D_2O (Fig. 5B and Table 1), before any formation of amyloid fibrils, exhibits components characteristic of β-sheet structure as well: an amide I′ band at 1633 cm^{-1}

Table 1
Main ATR FT-IR (1100–1800 cm^{-1}) peak maxima of: (a) cA_m1 peptide amyloid fibrils cast as a thin film on a Au mirror, (b) soluble cA_m1 peptide in D_2O cast as a thin film on a diamond ATR element and (c) cA_m2 peptide solution in water cast as a thin film on a Au mirror (Fig. 5)

cA_m1 Fibrils		cA_m1 in D_2O		cA_m2 Solution in H_2O	
Peak (cm^{-1})	Assignments	Peak (cm^{-1})	Assignments	Peak (cm^{-1})	Assignments
1228	Amide III (β-sheet)				
1279	Amide III (β-turns?)				
				1437	CH_2-deformation
1543	Amide II	1437	Amide II' (β-sheet)	1541	Amide II
1628	Amide I (β-sheet)	1633	Amide I' (β-sheet)	1630 (sh)	Amide I (β-sheet?)
				1653	Amide I (Unordered structure)
1672	Amide I (β-turns?) (TFA?)				

Tentative assignments are included. For details see text.

and an amide II′ band at 1437 cm^{-1}(Haris and Severcan, 1999). Formation of mature amyloid fibrils occurs after a period of approximately 4–5 days, as we have shown previously rather conclusively for solutions of the cA peptide (Hamodrakas et al., 2004) and for solutions of the cA_m1 peptide (our unpublished data) as well. Therefore, in this case, it appears that β-sheet structure dictates formation of amyloid fibrils in a rather decisive way, since β-sheet structure is the structure that the peptide cA_m1 adopts, directly after solution.

On the contrary, thin films cast from cA_m2 peptide solutions on front-coated gold mirrors produce ATR FT-IR spectra characteristic of an unordered conformation of the peptide in these solutions (Fig. 5C). The infrared band at 1653 cm^{-1} in the amide I region and the absence of any features in the amide III region are indicative of unordered structure (Krimm and Bandekar, 1986; Surewicz et al., 1993; Haris and Chapman, 1995; Jackson and Mantsch, 1995; Cai and Singh, 1999). A shoulder at ca. 1630 cm^{-1} may indicate a small fraction of β-sheet structure.

Taking into account all experimental and theoretical evidence accumulated previously for silkmoth chorion proteins (Hamodrakas, 1992) and their synthetic peptide-analogues (Iconomidou et al., 2000; Iconomidou et al., 2001; Hamodrakas et al., 2004) and the hexapeptide periodicities present in the central domain of the A and B families of chorion proteins (see also Fig. 1), we propose the models shown in Fig. 6a and b for the structure of the

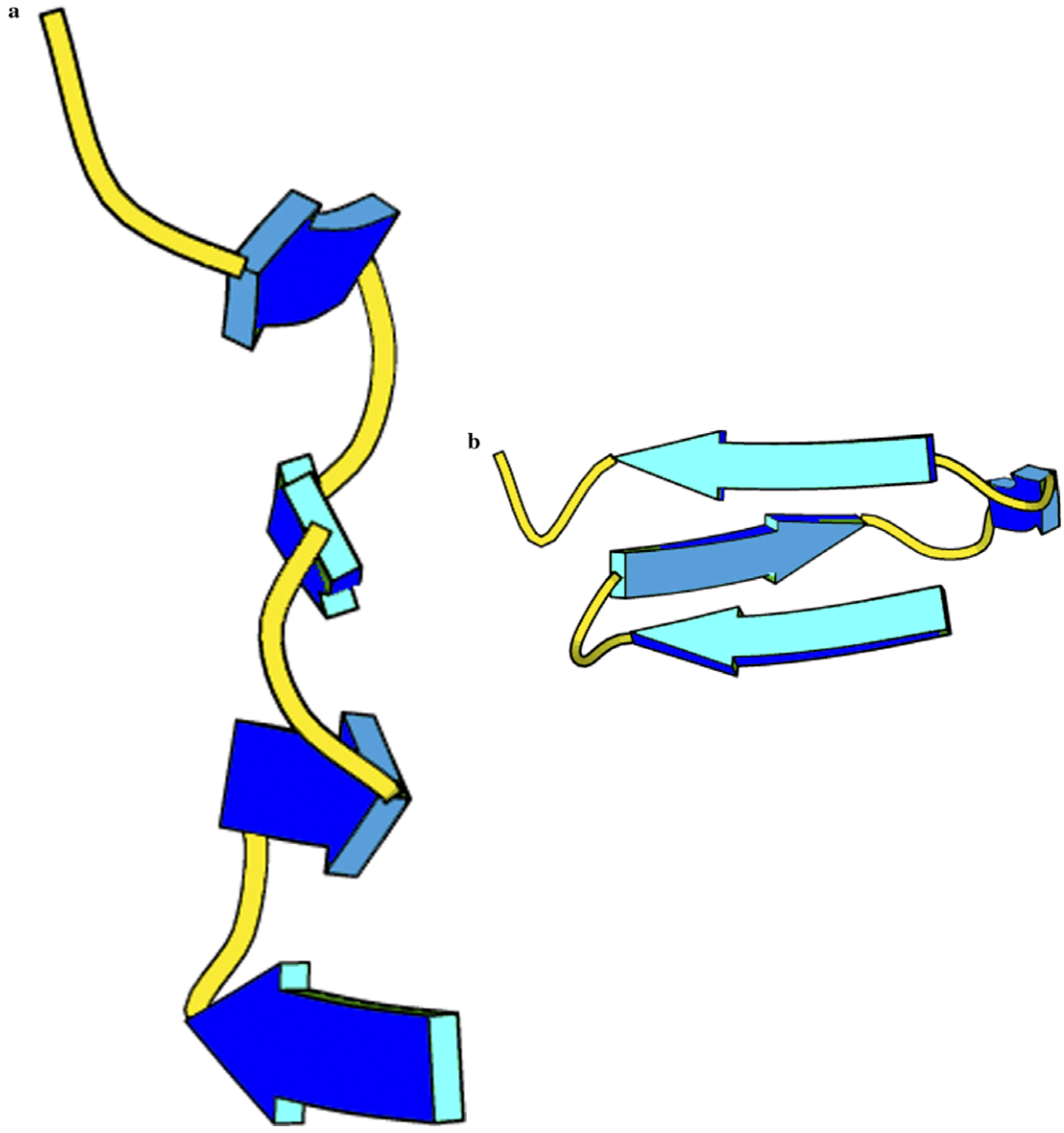

Fig. 6. (a) Antiparallel twisted β-sheet model ("cross-β" structure) proposed for the cA_m1 peptide ribbon representation (Kraulis, 1991), without side-chains. Arrows represent β-strands. Four-residue β-strands alternate with tentative type II' β-turns (Iconomidou et al., 2000). View along the β-strands. (b) A ribbon representation of the cA_m1 peptide in a left-handed parallel β-helix conformation (ca. one and a half turn of the helix). Four-residue β-strands alternate with tentative type II β-turns (the two middle residues of the β-turns comprise the Gly (G) residues, tandemly repeating every six-residues in Fig. 1, and the subsequent, usually polar or charged residue). Arrows represent β-strands. View perpendicular to the axis of the helix.

cA_m1 peptide by homology modelling to the structure of the cA peptide (Iconomidou et al., 2000).

The data presented here are clearly in favour of the antiparallel β-pleated sheet model shown in Fig. 6a, but the left-handed parallel β-helix model of Fig. 6b has attractive features as well. Most interesting among these is the hydrophobic core and hydrophobic faces of the triangular prism-like β-helix. Nevertheless, the "edges" of this prism are occupied by charged, polar residues and glycines and this makes 3-D packing difficult, unless there are very specific interactions. On the contrary, the hydrophobic faces of the antiparallel β-sheet structure shown in Fig. 6a facilitate uniform 3-D packing of the β-sheets, leaving the polar and charged residues on both lateral "edges" of the sheet for favourable lateral interactions. Although we were the first, to our knowledge, to propose a detailed left handed parallel β-helix structural model at atomic resolution, as a possible structure underlying amyloid fibrils (Iconomidou et al., 2000), this publication remained unnoticed. However, several groups also proposed recently that β-helices may dictate amyloid fibrillar structure (Wille et al., 2002; Wetzel, 2002; Pickersgill, 2003; Williams et al., 2004; Kishimoto et al., 2004; Govaerts et al., 2004).

Khurana and Fink (2000) studied and concluded that proteins adopting a parallel β-helix structure do not exhibit a unique infrared signature. As mentioned above, although we do not have evidence from ATR FT-IR spectroscopy, as we had for the cA and B peptide (Iconomidou et al., 2000; Iconomidou et al., 2001) that the antiparallel β-sheet model of Fig. 6a is favoured compared to the left-handed parallel β-helix model of Fig. 6b, all other evidence is in favour of the ladder model presented in Fig. 6a.

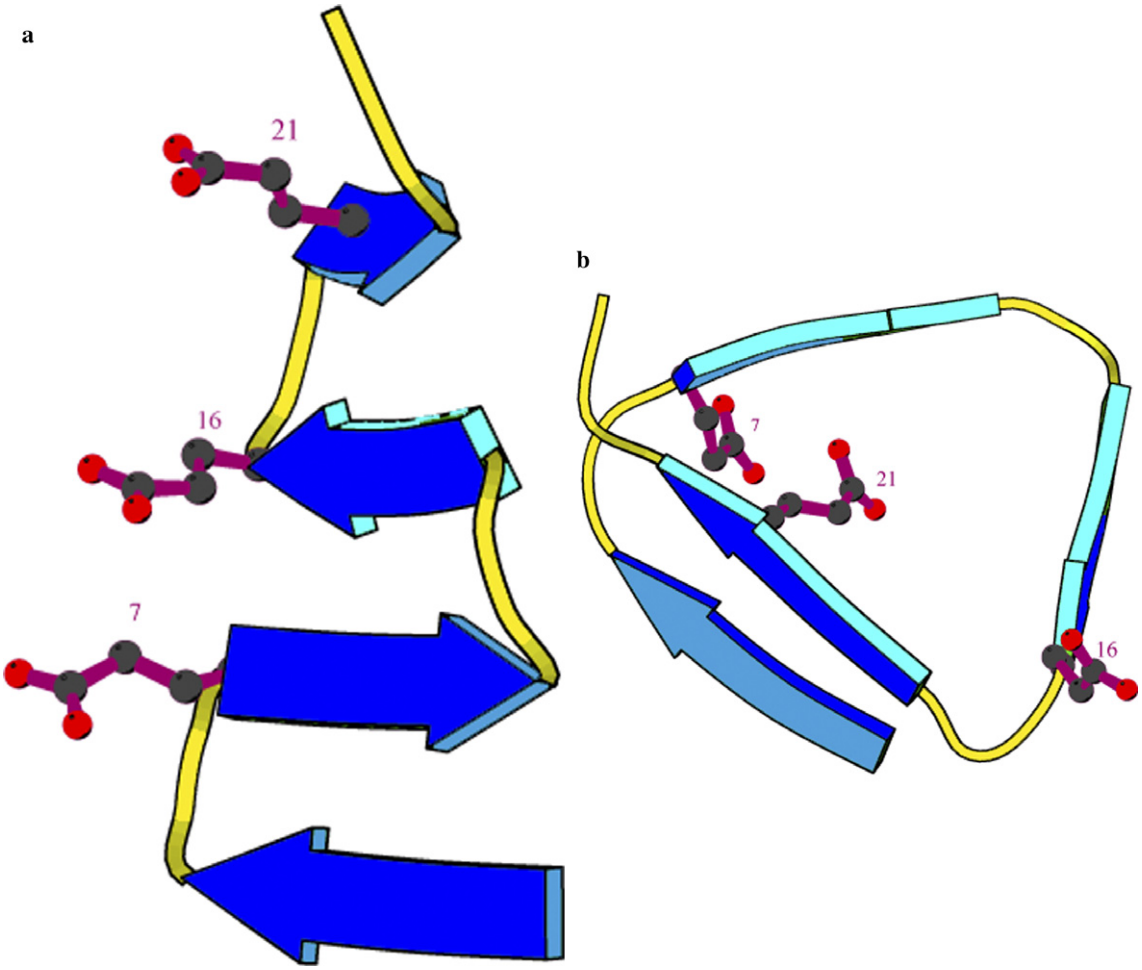

Fig. 7. (a) A schematic antiparallel twisted β-sheet model ("cross-β" structure) for the cA_m2 peptide ribbon representation (Kraulis, 1991), with the side-chains of the three glutamate (E) residues that have replaced two valines (V) and one alanine (A) residues, in the cA_m2 peptide (cf. Fig. 1) as ball and sticks. Arrows represent β-strands. Four-residue β-strands alternate with tentative type II' β-turns (Iconomidou et al., 2000). View along the β-strands. It is clear that this structure is not favoured because of strong repulsive electrostatic interactions of the glutamate side chains in close proximity. (b) A ribbon representation of the cA_m2 peptide in a left-handed parallel β-helix conformation with the side-chains of the glutamates (as in (a) above) added as balls and sticks. Four-residue β-strands alternate with tentative type II β-turns (the two middle residues of the β-turns comprise the Gly (G) residues, tandemly repeating every six-residues in Fig. 1, and the subsequent, usually polar or charged residue). Arrows represent β-strands. View parallel to the axis of the helix. It is evident that this structure is not favourable because the side chains of two glutamate residues are packed in the hydrophobic interior of the left-handed parallel β-helix.

In contrast, none of the models shown in Fig. 6 seem to be a favourable structure for peptide cA_m2. As shown in the ladder model of Fig. 7a, glutamates would be very close to each other leading to unfavourable electrostatic interactions. Similarly, in the left-handed helix model of Fig. 7b, two glutamates would occupy the hydrophobic interior of the β-helix producing a very unstable structure. These results are clearly compatible with the fact that the cA_m2 peptide does not form amyloid fibrils even after very long incubation periods.

In conclusion, this work presents convincing evidence that a peptide with a length half of that of the central domain of the A family of silkmoth chorion proteins folds and self-assembles into amyloid fibrils, which are very similar in properties to those of the cA peptide (which corresponds to the entire length of the A family central domain), which probably implies that the underlying molecular substructure that dicates proper folding and self-assembly of chorion fibrils into the superstructure of silkmoth chorion is encoded into the tandem hexapeptide repeats present in the aminoacid sequences of the central domain of silkmoth chorion proteins. This, apparently, is in strong support of our previous proposal that silkmoth chorion is a natural protective amyloid (Iconomidou et al., 2000; Hamodrakas et al., 2004). Furthermore, we have demonstrated that carefully designed mutations on the sequence of these amyloidogenic peptides can inhibit self-assembly and amyloid formation. It remains to be seen which is the shortest possible peptide from the sequences of chorion proteins that folds and self-assembles forming fibrils similar to those appearing *in vivo* in the structure of silkmoth chorion.

Acknowledgments

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