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pH-Regulated formation of amyloid-like β-sheet assemblies from polyglutamate grafted polyallylamine

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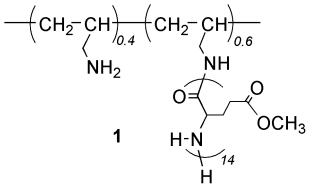
A novel artificial protein with simple primary structure, poly(γ-methyl-L-glutamate)-grafted polyallylamine, has been prepared and the resultant peptide has shown a unique property of pH-regulated conformation and morphology.

The self-assembly of peptides and proteins into β-sheet-rich quaternary structures has attracted attention[1] because of the characteristic structure of these assemblies and because of their association with neurodegenerative diseases,[2] such as Alzheimer's and Creutzfeldt-Jacob's. In order to understand the mechanisms by which proteins misfold and amyloid fibrils form in disease states, it is important to elucidate the atomic-level structure of these fibrils and how amyloid fibrils form. In previous studies, several proteins have been identified in amyloid diseases,[3] and these aggregates were found to have a common core structure. Recently, Dobson's group found that two non-pathogenic proteins also form amyloid fibrils *in vitro* under particular conditions[4] and suggested that the formation of amyloid fibrils is a property common to many of the proteins. In synthetic systems, various peptides that have designed sequences for β-sheet formation through the interaction of side chains have been prepared as a structural model of amyloid.[5] In these systems, the correlation between peptide sequence and amyloid formation has been investigated.

Here, we describe the first example of an amphiphilic artificial protein with an α-helical homopeptide, polyglutamate-grafted polyallylamine, being converted into amyloid-like fibrils with a structural transition from α-helix to β-sheet under certain appropriate conditions. Amyloid fibril formation seems to occur if peptide chains are folded appropriately, even for simple synthetic peptides which have no specific protein sequences.

A recent approach to artificial protein design is to use template molecules,[6] since protein tertiary structures can be considered assemblies of secondary structure elements. Many artificial proteins have been prepared in aqueous solutions by attaching peptide blocks to templates that direct the component helices into protein-like packing arrangements. We have chosen a poly(γ-methyl-L-glutamate) (PMLG) as a structural element because of its simple structure and well-defined conformational characteristics in solution, and we have chosen a polyallylamine (PAA) as both a template for assembly and an initiator for graft-polymerization.[7] First, the PAA copolymer whose amino groups (40%) were protected with BOC groups was obtained by reacting the PAA (Mw = 10000) with di-*tert*.-butyl dicarbonate in water–dioxane (1 : 1). Next, the graft-polymerization of MLG–NCA was carried out from free amino groups of this PAA copolymer in $CHCl_3$. The number-average degree of polymerization of the PMLG chain was determined to be 14 from ^1H NMR analysis. Finally, the desired PMLG grafted PAA (**1**) was obtained by removal of the BOC groups in trifluoroacetic acid solution.

The secondary structure of **1** was investigated by means of circular dichroism (CD)† and FTIR spectroscopies. At pH 4.0



(Fig. 1*a*), **1** showed a negative maximum at 222 and 208 nm shortly after preparation of the sample solution, indicating the existence of a right-handed α-helix structure. The helicity of the PMLG units was calculated to be about 60% from the observed molar ellipticity at 222 nm. This helicity is reasonable taking account of the relatively short segment length of $n = 14$. The CD measurements revealed a gradual change in a spectrum typical for a β-sheet structure, with a single negative maximum at 218 nm and a positive maximum at 197 nm after incubation for 30 h. Generally, PMLG has been known to form a stable α-helix structure, and the β-sheet formation would require a particular, harsh condition (*e.g.* high temperature, mechanical stress). Thus, the β-sheet structure for PMLG may not be primarily so stable. In our case, however, the amphiphilic property of **1** presumably causes tight packing of hydrophobic PMLG chains under acidic conditions and then the intramolecular hydrogen bonding is likely to rearrange to the intermolecular one, which induces the structural change of the PMLG chain from α-helix to β-sheet. On the other hand, at pH 9.2 (Fig. 1*b*), the CD spectra showed the typical α-helix pattern (negative maximums at 222 and 208 nm) even after 30 h, although the molar ellipticity was reduced to half its initial value. This reduction in ellipticity was probably caused by the

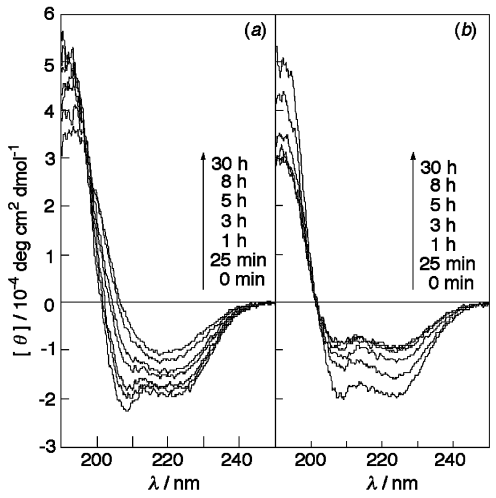


Fig. 1 CD spectral changes of **1** in water–TFE (8 : 2 v/v) at pH 4.0 (*a*) and pH 9.2 (*b*). The peptide **1** was incubated for the indicated periods (0–30 h) at room temperature. [MLG] = 1.1×10^{-4} unit M.

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large aggregation[8] of **1** with the α-helical form owing to the deprotonation of amino groups of PAA units. To obtain more quantitative information on the secondary structure, transmission FTIR spectra were measured. The peptide **1** was adsorbed onto a CaF_2 plate after incubation for 30 h in water–TFE (8 : 2) at pH 4.0 and 9.2, respectively. In the amide I region,[9] characteristic absorptions of an antiparallel β-sheet structure were observed at 1695 and 1627 cm^{-1} at pH 4.0, whereas **1** took mainly the α-helix structure (peak maximum at 1656 cm^{-1} and shoulder at 1627 cm^{-1}) at pH 9.2. The β-sheet contents were evaluated to be 77 and 34% at pH 4.0 and 9.2, respectively, by peak resolution of the spectra obtained. It is clear from these spectral data that the pH of the solution was responsible for regulating the self-conformational change of PMLG graft chains. In addition, such a structural transition of PMLG units was found to proceed only at pH 7.8 and below. The pK_a value of allylamine moiety was estimated to be 8.0 from potentiometric analysis.[7] On the other hand, the peptide chains involved have no pH-responsive side chains. Therefore, it can be assumed that the protonation of amino groups of PAA units affected the three-dimensional packing arrangement of PMLG chains; as a result, the structural transition of **1** was observed only at pH < 8.

Subsequently, the amyloid-like fibril formation of **1** with an α-to-β structural transition was observed directly by using atomic force microscopy (AFM).‡ Fig. 2 shows the time dependence of AFM images (3 × 3 μm^2) for **1** at pH 4.0. An AFM image obtained just after the sample solution was prepared (0 h), in which the PMLG chain took mainly the α-helical form, revealed the presence of only nonfibrillar, globular aggregates (Fig. 2A). The average height of the globular species was determined to be 6.0 ± 1.0 nm, and the width was typically 40–70 nm. It is important to note that AFM provides accurate measurements of a sample′s height above the substrate, but the well-known convolution of the scanning tip leads to an overestimation of the sample width.[10] After incubation for 5 h, protofibrils of **1** (average height 4.5 ± 1.0 nm) were observed together with the globular species (Fig. 2B). Harper *et al.* reported recently that the amyloid β-peptide (Aβ) protofibril elongation involved both the incorporation of monomers and the association of immature protofibrils.[11] In our case, the protofibril was characterized by a clearly periodic substructure, as they were spaced at 30 ± 10 nm intervals along the protofibrils axis (Fig. 2C). Therefore, this observation, namely the similarity of shape and size between the substructures of protofibrils and globular species, supports the idea that protofibrils of **1** are also formed through the association of globular species. For 48 h after incubation, the major portion of **1** was found to form amyloid-like fibril structures with an average height of 4.0 ± 1.0 nm and a length of 0.3–1 μm (Fig. 2D). These dimensions were in fair agreement with those of typical amyloids.[3] In addition, when Congo Red binding studies of these fibrillar assemblies of **1** were examined, the stained peptide assemblies exhibited a yellow birefringence under cross-polarized light. Although the birefringence differs somewhat from the green birefringence of amyloid,[4] it is a clear indication of their anisotropy and therefore indicates the presence of a regular quaternary structure. On the other hand, this amyloid-like structure was not observed for **1** at pH 9.2, *i.e.* at a pH at which no structural transition of the PMLG graft chain was observed. In conclusion, it should be noted that this purely synthetic homo-polypeptide formed an amyloid-like fibril structure with an α-to-β structural transition under appropriate conditions (pH < 8) as did the other proteins associated with neurodegenerative diseases. These results strongly suggest that the formation of fibril structure is not restricted to specific protein sequences but is, instead, a common property of polypeptides, although the appropriate packing arrangement of peptide chains is required. We believe that this kind of work should be helpful in studies of the mechanisms underlying the misfolding of proteins and amyloid fibril formation.

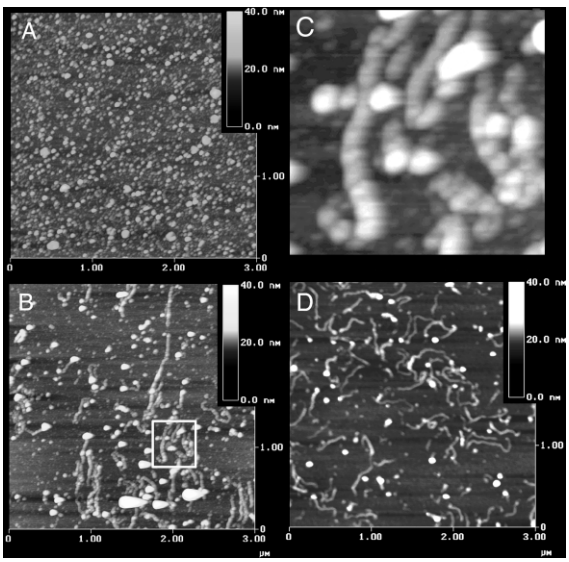

Fig. 2 Time dependence of tapping mode AFM images (3 × 3 μm^2) for **1**. The peptide **1** was incubated for 0 h (A), 5 h (B, C), and 48 h (D) in water–TFE (8 : 2 v/v) (pH 4.0) at room temperature. The image C corresponds to the area marked with a rectangle in image B. [MLG] = 1.1 × 10^{-4} unit M.

Notes and references

† Sample solutions for CD measurements were prepared by diluting the 2,2,2-trifluoroethanol (TFE) stock solution of **1** with purified water (final peptide concentration 1.1 × 10^{-4} glutamate unit M, TFE content 20%). The pH of the solution was adjusted with 0.1 M HCl or 0.1 M NaOH.

‡ The AFM images were recorded in tapping mode by placing 10 μL of **1** in water–TFE (8 : 2, pH 4.0) onto freshly cleaved mica. After adsorption for 3 min, the excess solution was removed by absorption onto filter paper.

1 D. W. Walsh, A. Lomakin, G. B. Benedek, M. M. Condron and D. B. Teplow, *J. Biol. Chem*., 1997, **272**, 22364; N. Yamada, K. Ariga, M. Naito, K. Matsubara and E. Koyama, *J. Am. Chem. Soc*., 1998, **120**, 12192; K. Janek, J. Behlke, J. Zipper, H. Fabian, Y. Georgalis, M. Beyermann, M. Bienert and E. Krause, *Biochemistry*, 1999, **38**, 8246.

2 G. G. Glenner and C. W. Wong, *Biochem. Biophys. Res. Commun*., 1984, **122**, 1131; J. D. Sipe, *Crit. Rev. Clin. Lab. Sci*., 1994, **31**, 325; P. T. Lansbury, *Acc. Chem. Res*., 1996, **29**, 317.

3 J. C. Rochet and P. T. Lansbury, *Curr. Opin. Struc. Biol*., 2000, **10**, 60.

4 J. I. Guijarro, M. Sunda, J. A. Jones, I. D. Campbell and C. M. Dobson, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 4224; F. Chiti, P. Webster, N. Taddei, A. Clark, M. Stefani, G. Ramponi and C. M. Dobson, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 3590.

5 H. A. Lashuel, S. R. LaBrenz, L. Woo, L. C. Serpell and J. W. Kelly, *J. Am. Chem. Soc*., 2000, **122**, 5262; Y. Takahashi, A. Ueno and H. Mihara, *Chem. Eur. J*., 1998, **12**, 2475; Y. Takahashi, A. Ueno and H. Mihara, *Structure*, 2000, **8**(9), 915.

6 M. Mutter and S. Vuilleumier, *Angew. Chem., Int. Ed. Engl*., 1989, **28**, 535; T. Sasaki and E. T. Kaiser, *J. Am. Chem. Soc*., 1989, **111**, 380; R. M. Ghadiri, C. Soares and C. Choi, *J. Am. Chem. Soc*., 1992, **114**, 825; D. W. Choo, J. P. Scheider, N. R. Graciani and J. W. Kelly, *Macromolecules*, 1996, **29**, 355; P. Wallimann, R. J. Kennedy and D. S. Kemp, *Angew. Chem., Int. Ed*., 1999, **38**, 1290; N. Higashi, T. Koga, N. Niwa and M. Niwa, *Chem. Commun*., 2000, 361.

7 M. Higuchi, J. P. Wright, K. Taguchi and T. Kinoshita, *Langmuir*, 2000, **16**, 7061.

8 D. J. Gordon, *Biochemistry*, 1972, **11**, 413.

9 T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc*., 1961, **83**, 712.

10 K. A. Ramirez-Aguilar and K. L. Rowlen, *Langmuir*, 1998, **14**, 2562.

11 J. D. Harper, S. S. Wong, C. M. Lieber and P. T. Lansbury, *Biochemistry*, 1999, **38**, 8972.