Biophysical Chemistry 151 (2010) 155–159

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Characterization of amyloidogenic intermediate states through a combined use of CD and NMR spectroscopy

Kwang Hun Lim [a,*], Yen T.H. Le [a], Hilary H. Collver [a], Cindy Putnam-Evans [b], John M. Kenney [c]

[a] Department of Chemistry, East Carolina University, Greenville, NC, 27858, United States
[b] Department of Biology, East Carolina University, Greenville, NC, 27858, United States
[c] Department of Physics, East Carolina University, Greenville, NC, 27858, United States

ARTICLE INFO

Article history:
Received 15 January 2009
Received in revised form 10 June 2010
Accepted 14 June 2010
Available online 25 June 2010

Keywords:
SH3 domain
Amyloidogenic intermediate
Protein misfolding
NMR spectroscopy
CD spectroscopy
Amyloid

ABSTRACT

Characterization of amyloidogenic intermediate states is of central importance in understanding the molecular mechanism of amyloid formation. In this study, we utilized CD and NMR spectroscopy to investigate secondary structure of the monomeric amyloidogenic intermediate of a β-structured SH3 domain, which was induced by trifluoroethanol (TFE). The combined biophysical studies showed that the native state SH3 domain is gradually converted to the amyloidogenic intermediate state at TFE concentrations of 20–26% (v/v) and the aggregation-prone state contains substantial amount of the β-sheet conformation (~30%) with disordered (54%) and some helical characters (16%). Under weaker amyloidogenic conditions of higher TFE concentrations (>40%), the β-sheet structures were gradually changed to helical conformations and the relative content of the helical and β-sheet conformations was highly correlated with the aggregation propensity of the SH3 domain. This indicates that the β-sheet characters of the amyloidogenic states may be critical to the effective amyloid formation.

1. Introduction

Progressive accumulations of proteins into insoluble aggregates (amyloid) are implicated in diverse pathological cellular dysfunctions as well as in regular biological processes [1]. The amyloid formation involves conformational changes from native states to amyloidogenic intermediates and their self-assembly into insoluble aggregates [2–4]. Extensive biophysical studies have, therefore, been carried out on the intermediates to gain insight into structural features of the amyloidogenic state of proteins. It is widely believed that the monomeric amyloid-forming intermediate adopts partially folded molten globule states [2,4,5], which may contain extensive β-sheet conformations that can facilitate the inter β-strand aggregation. Various biophysical studies of amyloidogenic proteins have suggested the β-sheet like intermediates [6–11]. However, α-helical intermediates have also been detected for Aβ peptides and acylphosphatase [12–14]. Thus, it is of great importance to characterize the structural feature of the monomeric intermediate states in more detail, not only to understand the mechanism of amyloidogenesis, but also to develop effective therapeutic strategies.

Direct characterization of the amyloidogenic intermediate has, however, been impeded due to the fact that the intermediate state is believed to be highly flexible and might be in a dynamic equilibrium with native states and/or with soluble oligomers depending on the conditions. For example, circular dichroism (CD) spectroscopy has been predominantly used to obtain the secondary structural information since a very low protein concentration (<20 μM) can be used for the CD experiments, which is required to avoid the aggregation for most amyloidogenic proteins. However, interpretation of the CD data may not be straightforward if the monomeric intermediate is in the dynamic equilibrium, since the CD spectrum is indeed an averaged one contributed from various states in equilibrium, and thus the CD spectra cannot be properly analyzed without relative population information.

NMR spectroscopy has also been used to probe structural changes from native to the intermediate states, and provided valuable structural information of the intermediate states [11,15]. However, the urea-titration and hydrogen/deuterium exchange NMR experiments have not provided detailed secondary structural information of the amyloidogenic intermediate. In addition, most NMR characterizations of the intermediate states have been performed under close to amyloidogenic or non-amyloidogenic conditions where the non-amyloidogenic states are highly stable over a long period of time (>24 h) even at high protein concentrations (>1.5 mg/ml) [8,11,16–18], and thus the real amyloidogenic intermediate state may not be highly populated.

In this study, we utilized CD spectroscopy to investigate the secondary structure of the amyloidogenic intermediate state for the phosphatidylinositol 3-kinase (PI3K) SH3 domain. The PI3K SH3 domain is a small 85-residue β-barrel protein interaction module implicated in

* Corresponding author. Tel.: +1 252 328 9805; fax: +1 252 328 6210.
E-mail address: limk@ecu.edu (K.H. Lim).

0301-4622/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2010.06.007

diverse signal transduction pathways (Supplementary Fig. S1) [19]. Although the SH3 domain is not associated with any amyloid disease, the small protein was shown to readily form amyloid under partly denaturing conditions [20]. In particular, the small β-structured protein effectively formed cytotoxic amyloid in the presence of ~25% (v/v) trifluoroethanol (TFE) at pH 5.5 [21]. The SH3 domain has, therefore, served as a model amyloidogenic protein for the detailed understanding of amyloid formation mechanism [21–27].

The helix-stabilizing organic solvent (TFE) has been shown to induce amyloid formation most efficiently for various types of proteins [7,9,12,13,21,28–32] including the PI3K SH3 domain with a medium range of concentrations (15–40%). The critical concentration of the proteins where oligomerization is not observed is known to be less than 25 μM at the medium TFE concentrations, indicating that amyloidogenic intermediate states are highly populated under the effective amyloidogenic condition. At lower or higher TFE concentrations, protein aggregation was not observed or aggregation rate was significantly reduced. Thus, extensive CD studies have been carried out to investigate amyloidogenic properties of various proteins using TFE, which provided valuable insight into the structural changes from native to intermediate states [7,9,12,13,28,30,31]. In conjunction with the CD studies, we employed two dimensional (2D) $^{1}H/^{15}N$ heteronuclear single-quantum coherence (HSQC) NMR experiments to probe the equilibrium between the native and non-native states of the monomeric SH3 domain at the amyloidogenic condition. The combined CD and NMR studies allowed us to obtain direct information as to the secondary structure of the amyloidogenic intermediate of the SH3 domain.

2. Experimental

2.1. Materials

A pGEX-6P1 plasmid for the PI3K SH3 domain was a gift from Prof. Okishio (Kanazawa University Faculty of Medicine, Japan). The GST fusion SH3 domain was expressed and purified according to the manufacturer's manual (Amersham Bioscience). The fusion protein was cleaved with a PreScission enzyme (Amersham Bioscience), resulting in additional five residues (GPLGS) in the N-terminus. The cleaved proteins were subsequently purified with HPLC using a Superdex-75 column (Amersham Bioscience). Protein concentration was determined by measuring the absorbance at 280 nm in 6.0 M GdnHCl with a molecular extinction coefficient of 14,500 M^{-1} cm^{-1}.

Amyloidogenic properties of the 90-residue PI3K SH3 domain were identical to the previously reported data [21], which were characterized by thioflavin T binding assays and transmission electron microscopy (Supplementary Fig. S2 and S3). The protein samples formed amyloid almost immediately in 50 mM sodium acetate (or 20 mM sodium phosphate) buffer containing 25–26% TFE (pH 5.4–5.6) at room temperature with protein concentrations of >2 mg/ml (0.2 mM). At a lower concentration of 1 mg/ml, aggregates were observed within 1 h at 25% TFE (pH 5.5), indicating that the amyloidogenic intermediate state is highly populated at the amyloidogenic condition. Thus, a very low protein concentration of 0.12–0.5 mg/ml depending on the TFE concentration was used for the CD and NMR experiments, where aggregation was not observed during the experiments.

2.2. Circular dichroism (CD) spectroscopy

All of the CD spectra were collected at 25 °C with a 0.25 mg/ml protein sample in 20 mM sodium phosphate buffer (pH 5.5) using Jasco J-810 spectropolarimeter (Easton, MD) and a 10 mm path length quartz UV cell. A CD spectrum at a lower protein concentration of 0.12 mg/ml at 26% TFE was identical to that obtained at the higher protein concentration, which indicates that the amyloid-forming states predominantly exist as monomeric forms under the experimental conditions. The experimental CD spectra were fitted with the software DICHROWEB [33] for the secondary structure analysis.

2.3. NMR spectroscopy

All spectra were obtained at 25 °C on a 500 MHz Varian Inova spectrometer equipped with a *z*-axis gradient double resonance NMR probe and 600 MHz (Varian Inova) with a cold probe (Duke University NMR center). Protein concentrations of 0.12–0.5 mg/ml in 50 mM sodium acetate buffer (pH 5.5) were used for the NMR studies. At the amyloidogenic conditions (22–26% TFE) where the equilibrium is observed, the HSQC spectra were obtained using a cold probe with a 0.25 mg/ml protein concentration, which took a couple of hours of experimental time. One dimensional NMR experiments were also performed before and after the 2D NMR experiments and the NMR spectra were identical, suggesting that the SH3 domain remains as monomeric forms during the experiments.

3. Results

3.1. Conformational transition to amyloidogenic intermediate states

CD experiments were carried out on the SH3 domain as a function of the TFE concentration to investigate structural changes under amyloidogenic conditions (Fig. 1). A very low protein concentration of 0.25 mg/ml (25 μM) was used to probe the conformational changes between the monomeric native and amyloidogenic states. The CD signals remained almost unchanged up to 20% TFE. At slightly higher TFE concentrations (21–26%), changes in the CD signals were observed, suggesting a conformational transition of the SH3 domain at the moderate TFE concentrations. With increased TFE concentrations (>40%), the SH3 domain adopted extensive helical conformations.

The amyloid formation property of the SH3 domain as a function of the TFE concentration has been well characterized. For example, the SH3 domain protein at higher concentrations (>0.2 mM) almost immediately aggregated at moderate TFE concentrations of 22–26%, and its aggregation propensity is decreased at higher TFE concentrations (>40%). Secondary structural analyses of the CD spectra at the different TFE concentrations may, therefore, provide useful information as to correlation between the secondary structural features and the aggregation propensity. However, the aggregation-prone intermediate state may be in a dynamic equilibrium with the native state, which limits unambiguous analyses of the secondary structure using

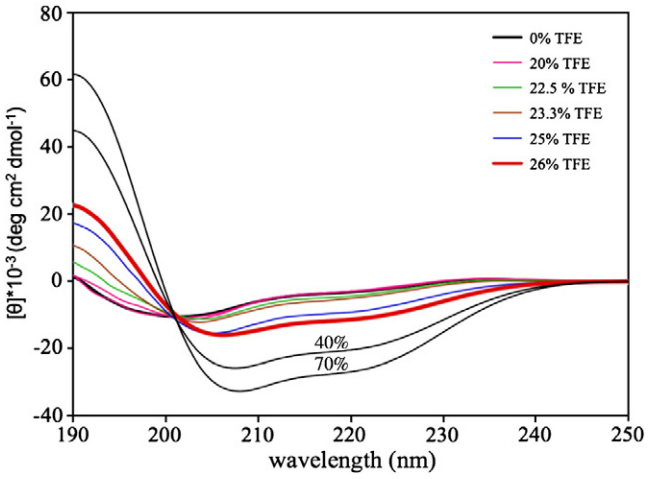

Fig. 1. Far-UV CD spectra of the SH3 domain (0.25 mg/ml) in 20 mM sodium phosphate buffer (pH 5.5) with various TFE concentrations (v/v).

the CD data alone. For the structural studies for the intermediate state, it is necessary to characterize the presumable equilibrium between the native and intermediate states.

In order to investigate the structural changes in more detail, 2D $^{1}H/^{15}N$ HSQC NMR experiments were carried out at the same conditions as used for the CD experiments (Fig. 2). The NMR spectra demonstrate that the protein undergoes at least two steps of conformational transitions depending on the TFE concentration. At low TFE concentrations of up to 20% (Fig. 2A), the protein remains folded based on the well-dispersed NMR resonances [34]. At 22.5% TFE where the CD signals started changing, new NMR resonances appeared mainly in the middle of the NMR spectrum (Fig. 2B). Interestingly, the NMR resonances were observed within 1 ppm in the ^{1}H dimension. The minimal dispersion observed in the ^{1}H dimension suggests that the tertiary structure of the SH3 domain is disrupted under these conditions, indicating that the folded proteins undergo a drastic conformational transition to unfolded states [34]. At higher TFE concentrations, the native state gradually disappeared (more dispersed black and blue peaks at 8.6–9.6 ppm in the ^{1}H dimension), while new resonances (red and green peaks) grew in

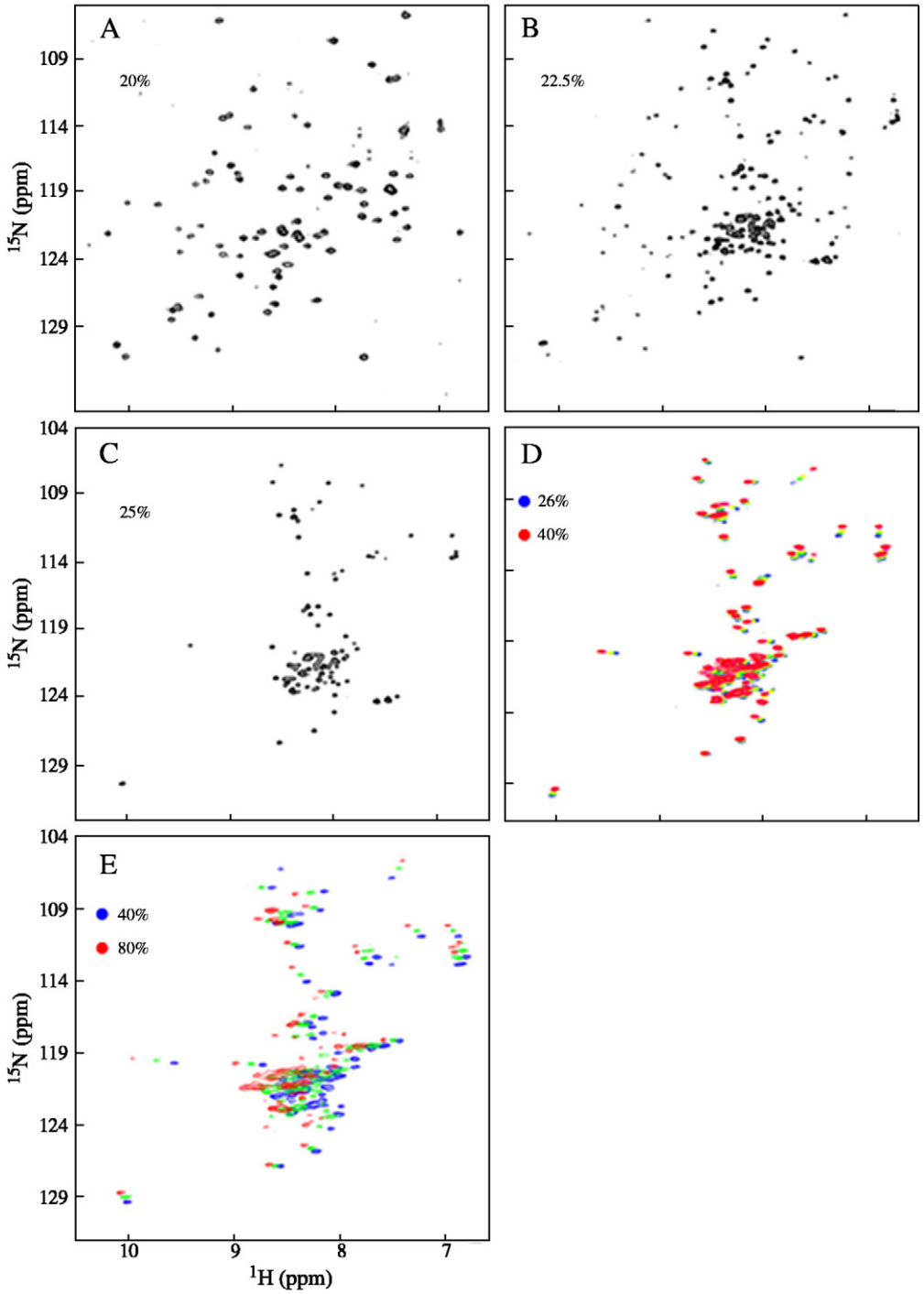

Fig. 2. $^{1}H/^{15}N$ HSQC NMR spectra of the SH3 domain in 50 mM sodium acetate buffer (pH 5.5) with (A) 20%, (B) 22.5%, (C) 25%, (D) 26–40%, and (E) 40–80% TFE concentrations. At the 26% TFE, 75 peaks were resolved in the HSQC spectrum, and more than 20 peaks were significantly broadened or disappeared at 80% TFE.

intensity (Supplementary Fig. S4). The NMR resonances for the two states in equilibrium were clearly resolved, indicating that the conformational transition occurs on slow time scales (>ms), as was observed for that between folded and unfolded Drosophila signaling adapter protein drk SH3 domain [35]. In addition, the chemical shifts for the native and unfolded states remained identical during the transition, consistent with the two-state transition probed by CD spectroscopy (Fig. 1).

The native state is almost completely converted to the amyloidogenic unfolded state at 25–26% TFE concentration (Fig. 2C–D), which is the most effective amyloidogenic condition for the SH3 domain. This clearly indicates that the changes in the CD signals with the increased TFE concentration at 20–26% were caused by the shift of the equilibrium from the native state to the monomeric intermediate rather than by gradual structural changes. As the TFE concentration is increased further up to ~40%, the NMR spectra remain almost unchanged except for the several resonances that are linearly shifted (Fig. 2D). At higher TFE concentrations, almost all of the NMR resonances in the HSQC spectra were slightly affected. In addition, more than 20 resonances became broader and many of those disappeared (Fig. 2E), presumably due to slow protein motions on ms time scales characteristic of the partly structured molten globule state of proteins [34,36]. This indicates that the SH3 domain becomes more structured with more stable helical conformations at the higher TFE concentrations, as was observed in the CD experiments (Fig. 1).

3.2. Secondary structural analyses

The NMR studies showed that the native state is almost fully converted to the amyloidogenic unfolded state at the 25–26% TFE. The CD spectra at different TFE concentrations were analyzed to investigate the secondary structural changes. The natively β-structured SH3 domain (Supplementary Fig. S1) contains extensive β-sheet characters (~30%) even in the helix-stabilizing organic solvent, TFE, of 26% (Table 1). The relative content of the β-sheet conformation significantly decreased to 14% at the higher TFE concentration, while the SH3 domain adopts more helical conformation (Table 1). More disordered regions including turns were not affected by the TFE, suggesting that the β-sheet conformations were mainly converted to the helical structures. The changes in the secondary structures are highly correlated to the amyloidogenic properties of the SH3 domain. The small protein with substantial β-sheet characters at the 26% TFE forms amyloid even at a low protein concentration (40 μM), as demonstrated in the CD studies (Fig. 3 and Supplementary Fig. S5). After 52 h of incubations, the SH3 domain (40 μM, 26% TFE) was almost fully converted to β-structured oligomers. The ThT fluorescence assays confirmed that the SH3 domain aggregates possess amyloid properties (Supplementary Fig. S2). However, it appears that the SH3 domain forms less ordered aggregates at 26% THE than amyloid fibril based on the TEM image (Supplementary Fig. S3). At higher TFE concentrations (>40%), the SH3 domain was not oligomerized even at higher concentrations. This strongly indicates that the β-sheet characters at the 26% TFE are critical to effective amyloid formation.

Table 1
Relative content of the secondary structures at different TFE concentrations calculated by using the software DICHOWEB [33].

TFE concentration (v/v)	α-helix	β-sheet	β-turn	Random coil
0%	11.9%	23.5%	21.1%	43.5%
26%	15.9%	28.7%	19.4%	36.0%
40%	30.0%	19.4%	20.5%	30.1%
70%	37.6%	13.8%	18.3%	30.3%

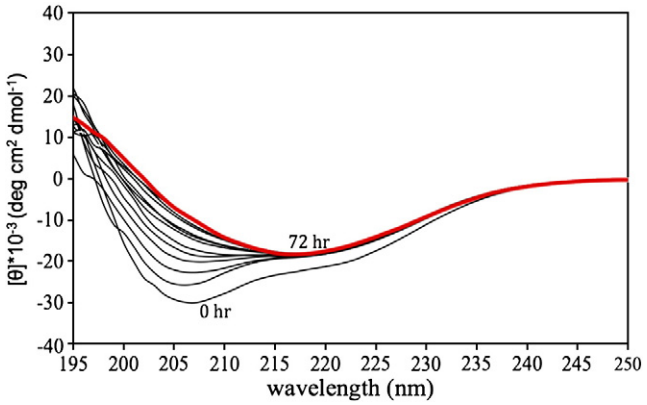

Fig. 3. Far-UV CD spectra of the SH3 domain (0.40 mg/ml) in 50 mM sodium acetate buffer (pH 5.5) with 26% TFE as a function of the incubation time. The spectra were collected every 4 h at 25 °C.

4. Discussion

Structural features of the amyloidogenic intermediate states of various amyloidogenic proteins have been extensively investigated. The structural properties of the intermediate state at the amyloid-forming conditions have been explored mainly by using CD spectroscopy, which have provided useful secondary structural information of the monomeric intermediate states. The CD experiments of various amyloidogenic proteins suggested that the monomeric amyloidogenic states adopt β-sheet conformations [7–10]. However, similar CD studies showed that α-helical conformations are involved in the amyloid-forming process of the Aβ peptides and acylphosphatase [12–14]. In addition, it was recently shown that myoglobin forms amyloid through highly unfolded amyloidogenic states rather than β-sheet like molten globules [37]. In those extensive CD studies, the probable equilibrium between the native polypeptides and amyloidogenic states was, however, not characterized to interpret the CD spectra, which limits quantitative secondary structural analyses of the CD spectra.

In this study, the structural transition between the monomeric native and amyloidogenic intermediates is probed by using NMR spectroscopy (Fig. 2). Although the CD experiments suggested a two-state transition from the native (0% TFE) to molten globule state (70% TFE), the TFE titration NMR experiments revealed at least three distinct states of the SH3 domain, folded native state, amyloidogenic intermediate, and partly structured molten globule depending on the TFE concentration. The single isodichroic point in the CD spectra (Fig. 1) may be presumably due to the overlap of the isodichroic points for the two structural transitions. This clearly demonstrates that the NMR experiments are critical to properly interpret experimental CD data. The CD spectra for the amyloidogenic intermediate and non-amyloidogenic molten globule states of the SH3 domain could be analyzed for the secondary structure determination with the aide of the NMR experiments. The secondary structure analyses of the CD data showed that the monomeric amyloidogenic intermediate state contains considerable β-sheet conformations (~30%) with extensive turns and disordered regions (~54%) and some helical characters (16%). Interestingly, the amount of the β-sheet conformation at the 26% TFE remained almost unchanged compared to that in the native state (Table 1), suggesting that the amyloidogenic state contains extensive native β-sheet character.

At the higher TFE concentrations (>40%), the β-sheet like structures gradually disappeared and changed to helical conformations, while the other structural features remained unchanged. Amyloid formation of the SH3 domain was not observed at the higher TFE concentrations, and thus the conformational changes are closely related to the aggregation property of the SH3 domain. In addition, the

SH3 domain with extensive other secondary structural characters including helical conformation (~70%) was completely converted to β-sheet structured oligomers at the 26% TFE (Fig. 3). These suggest that the β-sheet structures in the amyloidogenic intermediate state are critical to initiating and promoting effective amyloid formation.

In summary, we demonstrated unambiguous secondary structural analyses of the monomeric amyloidogenic intermediate state of the SH3 domain with a combined use of the CD and NMR spectroscopy. Structural conversions of the SH3 domain from the native to monomeric amyloidogenic intermediate and to the non-amyloidogenic states were analyzed by 2D NMR experiments, which allowed for the secondary structural analyses of the CD data. The combined biophysical methods can also be applied to other amyloidogenic proteins.

Acknowledgements

We thank Prof. Okishio for providing a plasmid vector for the PI3K SH3 domain. We are grateful to Drs. Ventors and Rebeiro for technical assistance to NMR experiments at Duke University NMR Center and Mr. Tim Charles for his help with the measurements of EM images of amyloids. This work was supported by the Research Corporation (CC6165, K.H.L.) and the ECU Research Creative Activity Grant (K.H.L.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2010.06.007.

References

[1] F. Chiti, C.M. Dobson, Protein misfolding, functional amyloid, and human disease, Ann. Rev. Biochem. 75 (2006) 333–366.
[2] J.W. Kelly, The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways, Curr. Opin. Struct. Biol. 8 (1998) 101–106.
[3] C.M. Dobson, Protein folding and misfolding, Nature 426 (2003) 884–890.
[4] J.C. Rochet, P.T. Lansbury, Amyloid fibrillogenesis: themes and variations, Curr. Opin. Struct. Biol. 10 (2000) 60–68.
[5] D.R. Booth, M. Sunde, V. Bellotti, C.V. Robinson, W.L. Hutchinson, P.E. Fraser, P.N. Hawkins, C.M. Dobson, S.E. Radford, C.C.F. Blake, M.B. Pepys, Instability, unfolding and aggregation of human lysozyme variants underlying amyloid fibrillogenesis, Nature 385 (1997) 787–793.
[6] F. Chiti, N. Taddei, F. Baroni, C. Capanni, M. Stefani, G. Ramponi, C.M. Dobson, Kinetic partitioning of protein folding and aggregation, Nat. Struct. Biol. 9 (2002) 137–143.
[7] I. Pallares, J. Vendrell, F.X. Aviles, S. Ventura, Amyloid fibril formation by a partially structured intermediate state of alpha-chymotrypsin, J. Mol. Biol. 342 (2004) 321–331.
[8] G.S. Jackson, L.L.P. Hosszu, A. Power, A.F. Hill, J. Kenney, H. Saibil, C.J. Craven, J.P. Waltho, A.R. Clarke, J. Collinge, Reversible conversion of monomeric human prion protein between native and fibrilogenic conformations, Science 283 (1999) 1935–1937.
[9] S. Srisailam, T. Krishnaswamy, T.K.S. Kumar, D. Rajalingam, K.M. Kathir, H.S. Sheu, F.J. Jan, P.C. Chao, C. Yu, Amyloid-like fibril formation in an all beta-barrel protein — partially structured intermediate state(s) is a precursor for fibril formation, J. Biol. Chem. 278 (2003) 17701–17709.
[10] Z.H. Lai, W. Colon, J.W. Kelly, The acid-mediated denaturation pathway of transthyretin yields a conformational intermediate that can self-assemble into amyloid, Biochemistry 35 (1996) 6470–6482.
[11] V.J. McParland, A.P. Kalverda, S.W. Homans, S.E. Radford, Structural properties of an amyloid precursor of beta(2)-microglobulin, Nat. Struct. Biol. 9 (2002) 326–331.
[12] Y. Fezoui, D.B. Teplow, Kinetic studies of amyloid beta-protein fibril assembly — differential effects of alpha-helix stabilization, J. Biol. Chem. 277 (2002) 36948–36954.
[13] F. Chiti, P. Webster, N. Taddei, A. Clark, M. Stefani, G. Ramponi, C.M. Dobson, Designing conditions for in vitro formation of amyloid protofilaments and fibrils, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 3590–3594.
[14] M.D. Kirkitadze, M.M. Condron, D.B. Teplow, Identification and characterization of key kinetic intermediates in amyloid beta-protein fibrillogenesis, J. Mol. Biol. 312 (2001) 1103–1119.
[15] K. Liu, H.S. Cho, H.A. Lashuel, J.W. Kelly, D.E. Wemmer, A glimpse of a possible amyloidogenic intermediate of transthyretin, Nat. Struct. Biol. 7 (2000) 754–757.
[16] G.W. Plattt, V.J. McParland, A.P. Kalverda, S.W. Homans, S.E. Radford, Dynamics in the unfolded state of beta(2)-microglobulin studied by NMR, J. Mol. Biol. 346 (2005) 279–294.
[17] S. Srisailam, H.M. Wang, T.K.S. Kumar, D. Rajalingam, V. Sivaraja, H.S. Sheu, Y.C. Chang, C. Yu, Amyloid-like fibril formation in an all beta-barrel protein involves the formation of partially structured intermediate(s), J. Biol. Chem. 277 (2002) 19027–19036.
[18] R. Bussell, D. Eliezer, Residual structure and dynamics in Parkinson's disease-associated mutants of alpha-synuclein, J. Biol. Chem. 276 (2001) 45996–46003.
[19] J. Liang, J.K. Chen, S.L. Schreiber, J. Clardy, Crystal structure of P13K SH3 domain at 2.0 angstrom resolution, J. Mol. Biol. 257 (1996) 632–643.
[20] J.I. Guijarro, M. Sunde, J.A. Jones, I.D. Campbell, C.M. Dobson, Amyloid fibril formation by an SH3 domain, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 4224–4228.
[21] M. Bucciantini, E. Giannoni, F. Chiti, F. Baroni, L. Formigli, J.S. Zurdo, N. Taddei, G. Ramponi, C.M. Dobson, M. Stefani, Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases, Nature 416 (2002) 507–511.
[22] N. Carulla, G.L. Caddy, D.R. Hall, J. Zurdo, M. Gairi, M. Feliz, E. Giralt, C.V. Robinson, C.M. Dobson, Molecular recycling within amyloid fibrils, Nature 436 (2005) 554–558.
[23] J.L. Jimenez, J.L. Guijarro, E. Orlova, J. Zurdo, C.M. Dobson, M. Sunde, H.R. Saibil, Cryo-electron microscopy structure of an SH3 amyloid fibril and model of the molecular packing, EMBO J. 18 (1999) 815–821.
[24] J. Zurdo, J.I. Guijarro, J.L. Jimenez, H.R. Saibil, C.M. Dobson, Dependence on solution conditions of aggregation and amyloid formation by an SH3 domain, J. Mol. Biol. 311 (2001) 325–340.
[25] J. Zurdo, J.I. Guijarro, C.M. Dobson, Preparation and characterization of purified amyloid fibrils, J. Am. Chem. Soc. 123 (2001) 8141–8142.
[26] S. Ventura, J. Zurdo, S. Narayanan, M. Parreno, R. Mangues, B. Reif, F. Chiti, E. Giannoni, C.M. Dobson, F.X. Aviles, L. Serrano, Short amino acid stretches can mediate amyloid formation in globular proteins: the Src homology 3 (SH3) case, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 7258–7263.
[27] P.P. de Laureto, N. Taddei, E. Frare, C. Capanni, S. Costantini, J. Zurdo, F. Chiti, C.M. Dobson, A. Fontana, Protein aggregation and amyloid fibril formation by an SH3 domain probed by limited proteolysis, J. Mol. Biol. 334 (2003) 129–141.
[28] G. Plakoutsi, N. Taddei, M. Stefani, F. Chiti, Aggregation of the acylphosphatase from Sulfolobus solfataricus — the folded and partially unfolded states can both be precursors for amyloid formation, J. Biol. Chem. 279 (2004) 14111–14119.
[29] T. Cellmer, R. Douma, A. Huebner, J. Prausnitz, H. Blanch, Kinetic studies of protein L aggregation and disaggregation, Biophys. Chem. 125 (2007) 350–359.
[30] N. Rezaei-Ghaleh, A. Ebrahim-Habibi, A.A. Moosavi-Movahedi, M. Nemat-Gorgani, Role of electrostatic interactions in 2, 2, 2-trifluoroethanol-induced structural changes and aggregation of alpha-chymotrypsin, Arch. Biochem. Biophys. 457 (2007) 160–169.
[31] Y.R. Chen, H.B. Huang, C.L. Chyan, M.S. Shiao, T.H. Lin, Y.C. Chen, The effect of A beta conformation on the metal affinity and aggregation mechanism studied by circular dichroism spectroscopy, J. Biochem. 139 (2006) 733–740.
[32] K. Yamaguchi, H. Naiki, Y. Goto, Mechanism by which the amyloid-like fibrils of a beta(2)-microglobulin fragment are induced by fluorine-substituted alcohols, J. Mol. Biol. 363 (2006) 279–288.
[33] L. Whitmore, B.A. Wallace, DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data, Nucleic Acids Res. 32 (2004) W668–W673.
[34] H.J. Dyson, P.E. Wright, Unfolded proteins and protein folding studied by NMR, Chem. Rev. 104 (2004) 3607–3622.
[35] O.W. Zhang, L.E. Kay, J.P. Olivier, J.D. Formankay, Backbone H-1 and N-15 resonance assignments of the N-terminal Sh3 domain of Drk in folded and unfolded states using enhanced-sensitivity pulsed-field gradient NMR techniques, J. Biomol. NMR 4 (1994) 845–858.
[36] C. Redfield, Using nuclear magnetic resonance spectroscopy to study molten globule states of proteins, Methods 34 (2004) 121–132.
[37] M. Fandrich, V. Forge, K. Buder, M. Kittler, C.M. Dobson, S. Diekmann, Myoglobin forms amyloid fibrils by association of unfolded polypeptide segments, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15463–15468.