Molten Globule Precursor States Are Conformationally Correlated to Amyloid Fibrils of Human β-2-Microglobulin

Lukasz Skora,† Stefan Becker,† and Markus Zweckstetter*†,‡

Department of NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Goettingen, Germany, and DFG Center for the Molecular Physiology of the Brain, 37073 Goettingen, Germany

Received January 18, 2010; E-mail: mzwecks@gwdg.de

Abstract: Misfolding intermediates play a key role in defining aberrant protein aggregation and amyloid formation in more than 15 different human diseases. However, their experimental characterization is challenging due to the transient nature and conformational heterogeneity of the involved states. Here, we demonstrate that direct carbon-detected NMR experiments allow observation, assignment, and structural analysis of molten globule amyloid intermediates that are severely broadened by conformational exchange. The method is used to characterize the structure and dynamics of partially unfolded intermediates of the 99-residue protein β-2-microglobulin, which is the major component of insoluble aggregates occurring in dialysis-related amyloidosis. Comparison of the conformational properties of the molten globule-like intermediates with levels of deuterium incorporation into amyloid fibrils of β-2-microglobulin revealed a close relationship between the conformational properties of the metastable intermediates and the β-sheet-rich insoluble aggregates of β-2-microglobulin.

Misfolding intermediates play a key role in defining aberrant protein aggregation and amyloid formation in more than 15 different human diseases.1-2 However, the experimental characterization of the conformation of amyloid intermediates is challenging due to their transient nature and conformational heterogeneity.3,4 This severely limits our knowledge about the relation of the conformation of the precursor states to the structure of amyloid fibrils.

Dialysis-related amyloidosis is a protein misfolding disease resulting from deposition of amyloid aggregates in skeletal tissue that contain fibrils of the 99-residue-long protein β-2-microglobulin (β2m).5 Amyloid formation of β2m is strongly enhanced in conditions that destabilize its globular structure.6 This can be achieved in vitro by acid denaturation, with two distinct intermediate states being formed under acidic conditions. The highest population of the partially unfolded intermediate occurs at pH 3.6, where also the rate of fibril formation reaches a maximum.7 However, long and straight amyloid fibrils resembling those extracted from patients are formed from a precursor state formed at pH 2.5.8,9 Here, we compare the conformational properties of these two partially unfolded intermediates with single-residue resolution and demonstrate that a close relationship exists between the structure and dynamics of the amyloid precursor species and the morphology of mature fibrils of β2m.

At pH 2.5 β2m is highly unfolded, the majority of resonances are observed in 1H-15N HSQC spectra (Supporting Information, Figure 1) and can be assigned to individual residues.10 In contrast, only about 40–60 resolved peaks on top of a broad hump of unresolved signal intensity (visible at lower contour levels) could be observed at pH 3.6 (Figure 1a).11 To obtain sequence-specific information about the pH 3.6 intermediate, we tested direct 13C detection.12-14 Direct carbon detection was previously successfully used for the study of unfolded proteins and to alleviate problems of exchange.15-18 Strikingly, out of 84 nonproline residues, about 90 resolved signals were observed in a two-dimensional (2D) 13C CON spectrum (Figure 1b). Resonances in this spectrum had very different line-widths (Figure 1), suggesting that the pH 3.6 intermediate contains nonrandom structure. Assignment of resonances was achieved by a combination of 1H- and 13C-detected experiments: backbone resonances were assigned at pH 2.5 using 1H-detected triple-resonance NMR experiments, the assignment was transferred to the 2D 13C CON spectrum at pH 2.5, followed by a series of CON and CACO spectra from 2.5 to 3.6, in which chemical shift changes were monitored (Supporting Information, Figure 2). Using this strategy >75% of backbone resonances of the intermediate at pH 3.6 were assigned (Supporting Information, Figure 2).

Figure 1. (a) Two-dimensional (2D) 1H-15N HSQC and (b) direct carbon-detected CON spectra of β2m at pH 3.6. (c) Secondary 13C (gray) and 13Cα (black) chemical shifts of β2m at pH 3.6; exchange-broadened resonances are marked by open circles; filled circles indicate resonances observable at pH 2.5 but not at pH 3.6; black bars above the plot indicate the location of β-strands in the native fold.
downfield shifts (less extended or more
addition, comparison of peak intensities in the $^{13}$C CON experiment
between the two intermediate states are present in this region. In
affected by chemical exchange, suggesting that structural differences
were seen for residues 63 and 79 located in the region most strongly
the amide hydrogen/deuterium (H/D) exchange profile of amyloid
disulfide-bridged strands B and F in the native structure that were
allowed characterization of the secondary structure of the amyloid
precursor of b2m at pH 3.6 with single-residue resolution. Regions
1–33, 43–53, and 82–99 have a propensity for extended structure, while residues 40–42 and 57–63 preferentially populate turn-
or helix-like conformations (Figure 1c). In the region 65–82 chemical
shift information could only be gained for residues 74, 78, and 79
due to severe exchange broadening even in $^{13}$C-detected experi-
ments. A comparison between chemical shifts at pH 3.6 and 2.5
(Supporting Information, Figure 4) reveals a higher propensity for
extended structure for residues 90–96 at pH 3.6, while it is reduced
for residues 34–40, 45–48, 53–63, and 79–87. Interestingly,
downfield shifts (less extended or more $\alpha$-helical or turn propensity)
were seen for residues 63 and 79 located in the region most strongly
affected by chemical exchange, suggesting that structural differences
in addition, comparison of peak intensities in the $^{13}$C CON experiment
between the two precursors (Supporting Information, Figure 5)
shows an overall decrease of signal intensity in the pH 3.6
intermediate, especially in the C-terminal region 82–98, in agree-
ment with the highest population of the intermediate at pH 3.6.
Noteworthy, truncation of b2m after residue 83 impairs aggrega-
tion.19

To gain insight into the correlation between the conformational
properties of the precursors with the morphology of resulting
amyloid fibrils (Supporting Information, Figure 6), we determined
the amide hydrogen/deuterium (H/D) exchange profile of amyloid
fibrils of b2m at pH 2.5 using NMR spectroscopy. Fibrils formed in
H$_2$O were subjected to solvent exchange in D$_2$O for 7 days at 4
$^\circ$C, pD 2.5. Accordingly, regions protected from solvent exchange
are expected to have lower levels of deuterium incorporation.
Importantly, dissolution of the amyloid fibrils by 4 M guanidinium
thiocyanate in 50% H$_2$O/D$_2$O enabled observation of almost all residues
(Figure 2), including residues which are part of the disulfide-bridged strands B and F in the native structure that were
not detected in previous H/D exchange experiments.20 Our data
show that parts of strands B and F stay protected from solvent
exchange and are buried in the fibrillar core. Importantly, reduction
of the disulfide bridge leads to formation of fibrils with different
morphology.10

A very good correlation was found between the solvent protection pattern of the fibrils and the exchange broadening of the molten
globule-like intermediate represented by signal intensities in the
carbon-detected spectra (Figure 2): (i) residues 1–20 and 88–99 are least protected in amyloid fibrils of b2m and show the highest signal
intensities in the pH 2.520 as well as pH 3.6 precursor (Figures 1 and 2); (ii) residues 29–34 and 38–60 have intermediate solvent exposure in the fibrils and have intermediate signal intensities in the spectrum of the intermediate; (iii) residues 22–25,
35–37, 61–68 and 72–78 show the lowest levels of deuterium incorporation in the fibrils and have low signal intensities or are
broadened beyond detection in the $^{13}$C CON experiment. Increased
chemical exchange was previously reported for residues 20–80 in the
b2m intermediate at pH 2.5.10 Many hydrophobic residues are found in these regions, and mutagenesis experiments designed to
decrease the hydrophobicity resulted in slower aggregation kinetics,19,21 suggesting the formation of hydrophobic clusters. Two peptides (residues 21–40, the so-called K3 peptide, and residues 59–71), which overlap with the regions that are strongly exchange broadened in the pH 3.6 intermediate, were reported to self-associate
in vitro.22,23 The two $\beta$-strands (residues 21–28 and 33–40) observed in amyloid fibrils of the K3 peptide24 match the two
minima in the H/D exchange profile (Figure 2), suggesting that
amyloid fibrils of full-length b2m may also contain two $\beta$-strands
in this region.

We demonstrated that signals of partially unfolded intermediate
ensembles broadened due to conformational exchange can be
structurally characterized using direct carbon-detected NMR experi-
ments. Comparison between the structural and dynamic properties
of the amyloid intermediate of b2m with H/D exchange measure-
ments on amyloid fibrils revealed a close relationship between the
conformational properties of the metastable partially unfolded
precursor and the $\beta$-sheet-rich insoluble aggregates of a disease-
relevant protein that assumes a rigid 3D fold in its native state.

Acknowledgment. We thank S. Xiang for HSQC measurements
and Max Planck Society and DFG (ZW 71/2-2 and 3-2 to M.Z.).

Supporting Information Available: $^1$H—$^{15}$N HSQCs, signal intensi-
ties, and secondary chemical shifts at pH 3.6 and 2.5; electron
micrographs of b2m fibrils. This material is available free of charge
via the Internet at http://pubs.acs.org.

References


