J|A|C|S
COMMUNICATIONS

Published on Web 02/22/2003

# Peptides Derived from Two Dynamically Disordered Proteins Self-Assemble into Amyloid-like Fibrils

Brian Bothner,*,‡ Yves Aubin,§ and Richard W. Kriwacki*,†,‡

*Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, Department of Molecular Sciences, University of Tennessee Health Sciences Center, Memphis, Tennessee 38163, and Medicinal Chemistry Department, Merck-Frosst Centre for Therapeutic Research, Kirkland, QC, Canada, H9H 3L1*

Received August 23, 2002; E-mail: richard.kriwacki@stjude.org

Proteins that are dynamically disordered in the native state play important roles in macromolecular recognition and the assembly of functional biomolecular complexes.[1−4] In contrast, unfolding or misfolding is considered a prerequisite for the assembly of amyloid-like fibrils from proteins that are highly structured in the native state. An emerging view is that non-native, extended polypeptide conformations are required for fibril formation[5] and that protein sequences may have evolved to minimize the opportunities for their occurrence.[6] Importantly, the formation of amyloid-like fibrils, or fibril precursors, from several different proteins is associated with degenerative diseases in humans.[7,8] In apparent contradiction to this relationship between fibrils and disease, we have observed that functional domains of two proteins, Arf and Hdm2, that regulate cellular responses to stress, co-assemble into amyloid-like structures. The interacting domains of the individual proteins are highly disordered in solution and do not self-assemble.[9,10] Here we report that short peptides derived from Arf and Hdm2 co-assemble into amyloid-like fibrils. The mechanism of co-assembly and the structures formed have not been previously described for biologically derived peptides. Interestingly, nonnatural peptides have been shown to exhibit similar self-assembly phenomena.[11−14]

Short polypeptide segments within the tumor suppressor protein, $p14^{Arf}$, and the cellular oncoprotein, Hdm2, mediate their specific interaction both in vitro and in cells.[10,15,16] Two arginine-rich motifs termed A1 and A2 within the Arf N-terminus mediate binding to Hdm2, while two acidic residue-rich segments termed H1 and H2 within the central domain of Hdm2 mediate binding to Arf. In the context of 37 and 95 amino acid fragments of Arf and Hdm2, respectively, these domains were shown to be disordered in solution while retaining biological activity.[9,10] We have analyzed the structural and self-assembly properties of short peptides excised from the interacting domains of Arf and Hdm2. Circular dichroism (CD) spectropolarimetry shows that isolated peptides comprised of the A1 and H1 motifs ($p14^{Arf}$ 1−14 and Hdm2 240−254, respectively) are highly disordered in solution (Figure 1A and B, red and violet traces, respectively).[17] The sequences of the peptides are A1, MVRRFLVTLRIRRA; H1, SVSDQFSVEFEVESL. However, when combined, the components of the binary mixture self-assemble, forming $\beta$-strand-containing supramolecular structures of low solubility (Figure 1A and B, other traces). The appearance of a minimum in the CD spectra of the assemblies at 216 nm and the increase in ellipticity at 193 nm are consistent with the formation of $\beta$-strand-containing structures. The solution of binary assemblies is characterized by visible turbidity and the disappearance of $^1$H NMR resonances that, before mixing, were observed for the monodisperse, free peptides (data not shown). When either peptide

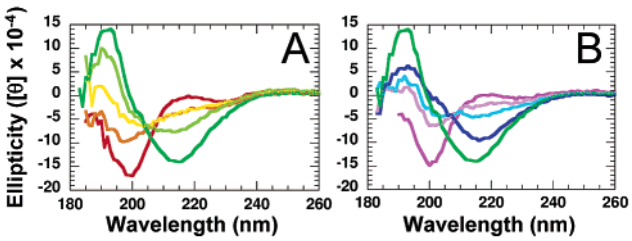


***Figure 1.*** Short peptides derived from $p14^{Arf}$ and Hdm2 self-assemble into $\beta$-strands. CD spectra of A1:H1 mixtures starting with (A) A1 and (B) H1 in excess. A1 alone (red), H1 alone (dark violet), and 1:1 A1:H1 (green). $\beta$-strand content was maximal at 1:1 A1:H1.

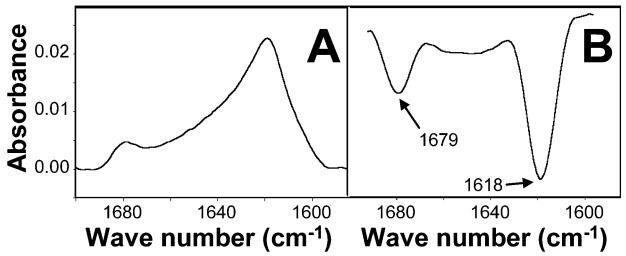


***Figure 2.*** The FT-IR spectrum of A1/H1 assemblies suggests antiparallel $\beta$-strand secondary structure. (A) Absorbance maxima in the amide I region were observed at 1618 and 1679 $cm^{-1}$. (B) Second-derivative analysis of spectrum in (A).

was titrated into a solution of the other, both NMR and CD spectra revealed that binding was saturable. Maximal $\beta$-strand character was observed when the molar ratio of A1:H1 was 1:1 (Figure 1A and B, green trace versus others). The Fourier transform infrared (FT-IR) spectrum of the co-assemblies confirms the CD results and suggests that the $\beta$-structures may be classified as antiparallel $\beta$-strands on the basis of the absorbances at 1618 and 1679 $cm^{-1}$ (Figure 2).[18−20] In summary, the individually disordered A1 and H1 peptides self-assemble into supramolecular structures comprised of $\beta$-strands.

Interestingly, the FT-IR spectrum of the A1/H1 co-assembly bears a striking resemblance to those of amyloid-like fibrils.[21−24] To extend our structural analysis, we used electron microscopy to determine whether the supramolecular structure of A1/H1 co-assemblies was similar to that of amyloid fibrils. Figure 3A shows that A1/H1 co-assemblies are networks of short, thin fibrils that are 10−20 nm long and less than 5 nm wide. These structures are morphologically similar to amyloid protofibrils derived from the $\beta$-amyloid peptide,[24] insulin,[23] lysozyme,[25] and an SH3 domain.[22] Denaturing conditions promote the conversion of many proteins from the native state to amyloid-like fibrils. To test their stability and potential for further self-assembly, A1/H1 protofibrils were heated. The structure of A1/H1 co-assemblies became more

† St. Jude Children's Research Hospital.
‡ University of Tennessee Health Sciences Center.
§ Merck-Frosst Centre for Therapeutic Research.


10.1021/ja028265w CCC: $25.00 

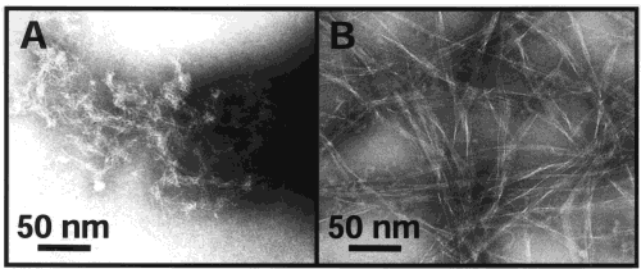


***Figure 3.*** Electron microscopy reveals that A1/H1 peptide co-assemblies adopt structures similar to amyloid protofibrils and fibrils. (A) Protofibrils were prepared by combining 1 mg/mL solutions of the A1 and H1 peptides in 20 mM Tris, pH 7.0 at 23 °C. (B) After heating at 70 °C, pH 3.5 for 100 h. Samples were dried onto freshly glow-discharged, carbon-coated, EM grids and negatively stained with 2% phosphotungstic acid (pH 6.4).

organized after treatment (Figure 3B), appearing as distinct fibrils that were hundreds of nanometers in length. The long fibrils appeared to be bundled together into parallel, flat ribbons comprised of 3−6 individual fibrils. Heat treatment was performed at pH values from 2.0 to 8.5, and the structural transition from protofibrils to fibrils was observed only at pH 3.0 and 3.5.

The chemical dyes, Congo red[26] (CR) and thioflavin T[27] (ThT), bind to amyloid fibrils generated from a wide variety of proteins, and binding is often associated with characteristic changes in absorbance and fluorescence spectra of the dyes. Surprisingly, the A1/H1 co-assemblies, before and after heat treatment, failed to bind CR and ThT. While the spectral and structural characteristics of these assemblies are very similar to those of classical amyloid fibrils, their binary nature seems inconsistent with Congo red and ThT dye binding.

We have shown that peptides derived from two different proteins, Arf and Hdm2, self-assemble to form amyloid-like protofibrils comprised of $\beta$-strands. Larger fragments of Arf and Hdm2 that encompass the A1 and H1 peptides, respectively, were previously shown to self-assemble into $\beta$-strand-containing structures. These structures, however, were less ordered than those described here.[10] Further, the larger A1- and H1-containing protein fragments were shown to interact in cells,[10] emphasizing the biological relevance of our structural observations. The A1 peptide from Arf contains five Arg residues (positions 3, 4, 10, 12, and 13), two of which (positions 3 and 10) are found within an evolutionarily conserved motif referred to as the Arf motif.[10] In contrast, the H1 peptide from Hdm2 is oppositely charged, containing one Asp residue (position 4) and three Glu residues (positions 9, 11, and 13). Additionally, both peptides contain several bulky, hydrophobic residues. It is likely that the process of co-assembly for A1/H1 and Arf/Hdm2 is mediated by attractive electrostatic interactions between Arg and Asp/Glu residues from different peptide molecules which ultimately may be found on the same face of an antiparallel $\beta$-sheet. The hydrophobic residues, which are found between the charged residues in both A1 and H1, are likely to further stabilize A1/H1 co-assemblies through intermolecular hydrophobic interactions. Repulsive electrostatic interactions between like-charged side chains in the individual peptides may favor extended conformations that promote self-assembly. How the $\beta$-strands are arranged within the amyloid-like protofibrils and fibrils is unknown at this time. To our knowledge, this is the first example of self-assembly of amyloid-like fibrils involving peptides from two proteins and represents a novel mechanism for the formation of biomolecular complexes. In contrast to the pathogenic properties of fibrils formed from pure proteins, the A1/H1 co-assemblies described here are associated with the interaction of Arf and Hdm2, important components of the p53 tumor suppressor pathway.[28,29] These findings suggest that amyloid-like fibrilar structures may play functional roles in human cells, as has been demonstrated for the Sup35 protein in *S. cerevisiae*.[30] Further, these peptides could be used as tags to direct the assembly of biomaterials.

**Acknowledgment.** This work was supported by the American Lebanese Syrian Associated Charities, National Cancer Institute, and a Cancer Center (CORE) Support Grant CA 21765. The authors thank Drs. C. Schultz and B. Jun of Bruker Optics for insightful discussion on the use of FT-IR, Ms. C. Ashley and Dr. C. Galea for assistance with peptide purification and fluorescence studies, respectively, and Dr. G. Murti for the EM images.

**References**

(1) Dunker, A. K.; Obradovic, Z. *Nat. Biotechnol.* **2001**, *19*, 805−806.
(2) Dunker, A. K.; Brown, C. J.; Lawson, J. D.; Iakoucheva, L. M.; Obradovic, Z. *Biochemistry* **2002**, *41*, 6573−6582.
(3) Dyson, H. J.; Wright, P. E. *Curr. Opin. Struct. Biol.* **2002**, *12*, 54−60.
(4) Dyson, H. J.; Wright, P. E. *Nat. Struct. Biol.* **1998**, *5*, 499−503.
(5) Chiti, F.; Taddei, N.; Baroni, F.; Capanni, C.; Stefani, M.; Ramponi, G.; Dobson, C. M. *Nat. Struct. Biol.* **2002**, *9*, 137−143.
(6) Bucciantini, M.; Giannoni, E.; Chiti, F.; Baroni, F.; Formigli, L.; Zurdo, J.; Taddei, N.; Ramponi, G.; Dobson, C. M.; Stefani, M. *Nature* **2002**, *416*, 507−511.
(7) Dobson, C. M. *Biochem. Soc. Symp.* **2001**, 1−26.
(8) Koo, E. H.; Lansbury, P. T., Jr.; Kelly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9989−9990.
(9) DiGiammarino, E. L.; Filippov, I.; Weber, J. D.; Bothner, B.; Kriwacki, R. W. *Biochemistry* **2001**, *40*, 2379−2386.
(10) Bothner, B.; Lewis, W. S.; DiGiammarino, E. L.; Weber, J. D.; Bothner, S. J.; Kriwacki, R. W. *J. Mol. Biol.* **2001**, *314*, 263−277.
(11) Kisiday, J.; Jin, M.; Kurz, B.; Hung, H.; Semino, C.; Zhang, S.; Grodzinsky, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9996−10001.
(12) Takahashi, Y.; Ueno, A.; Mihara, H. *ChemBioChem* **2002**, *3*, 637−642.
(13) Takahashi, Y.; Ueno, A.; Mihara, H. *ChemBioChem* **2001**, *2*, 75−79.
(14) Takahashi, Y.; Ueno, A.; Mihara, H. *Struct. Fold Des.* **2000**, *8*, 915−925.
(15) Weber, J. D.; Kuo, M. L.; Bothner, B.; DiGiammarino, E. L.; Kriwacki, R. W.; Roussel, M. F.; Sherr, C. J. *Mol. Cell Biol.* **2000**, *20*, 2517−2528.
(16) Midgley, C. A.; Desterro, J. M.; Saville, M. K.; Howard, S.; Sparks, A.; Hay, R. T.; Lane, D. P. *Oncogene* **2000**, *19*, 2312−2323.
(17) CD spectra were recorded with an Aviv 62DS instrument in 5 mM potassium phosphate (pH 7.0) using a 1 mm cuvette. In Figure 1A and B, the total peptide concentration was 4.5 $\mu$M. (A) A1:H1 molar ratios were 1:0.0, 1:0.07, 1:0.15, 1:0.36, and 1:1. (B) Molar ratios were 0.07:1, 0.15:1, 0.36:1, and 1:1. Data were collected for 4 s in 1 nm steps, and four scans were averaged for each spectrum.
(18) Krimm, S.; Bandekar, J. *Adv. Protein Chem.* **1986**, *38*, 181−364.
(19) Casal, H. L.; Kohler, U.; Mantsch, H. H. *Biochim. Biophys. Acta* **1988**, *957*, 11−20.
(20) FT-IR data were recorded using a Bruker Vector 22 instrument equipped with an attenuated total reflectance (ATR) cell. Resolution was 2 $cm^{-1}$, and 2000 scans were recorded for both background and sample. The concentration of each peptide was 1 mg/mL in 10 mM Tris, pH 7.0 in $^2H_2O$.
(21) Zurdo, J.; Guijarro, J. I.; Dobson, C. M. *J. Am. Chem. Soc.* **2001**, *123*, 8141−8142.
(22) Zurdo, J.; Guijarro, J. I.; Jimenez, J. L.; Saibil, H. R.; Dobson, C. M. *J Mol. Biol.* **2001**, *311*, 325−340.
(23) Bouchard, M.; Zurdo, J.; Nettleton, E. J.; Dobson, C. M.; Robinson, C. V. *Protein Sci.* **2000**, *9*, 1960−1967.
(24) Soto, C.; Castano, E. M.; Frangiones, B.; Inestrosa, N. V. *J. Biol. Chem.* **1995**, *270*, 3063−3067.
(25) Krebs, M. R.; Wilkins, D. K.; Chung, E. W.; Pitkeathly, M. C.; Chamberlain, A. K.; Zurdo, J.; Robinson, C. V.; Dobson, C. M. *J. Mol. Biol.* **2000**, *300*, 541−549.
(26) Klunk, W. E.; Pettegrew, J. W.; Abraham, D. J. *J. Histochem. Cytochem.* **1989**, *37*, 1273−1281.
(27) Levine, H. *Amyloid* **1995**, *2*, 1−6.
(28) Sherr, C. J. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 731−737.
(29) Sherr, C. J. *Cancer Res.* **2000**, *60*, 3689−3695.
(30) Serio, T. R.; Cashikar, A. G.; Kowal, A. S.; Sawicki, G. J.; Moslehi, J. J.; Serpell, L.; Arnsdorf, M. F.; Lindquist, S. L. *Science* **2000**, *289*, 1317−1320.

JA028265W