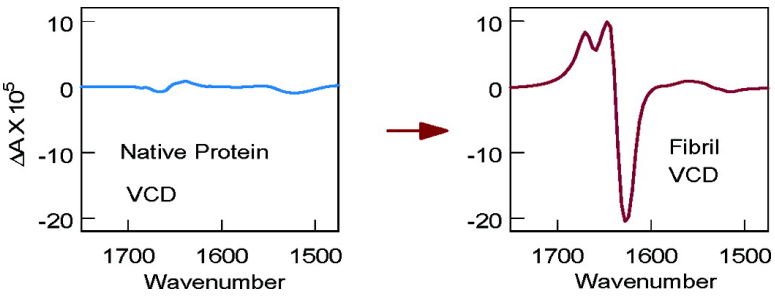
Communication

Vibrational Circular Dichroism Shows Unusual Sensitivity to Protein Fibril Formation and Development in Solution

Shengli Ma, Xiaolin Cao, Mimi Mak, Adeola Sadik, Christoph Walkner, Teresa B. Freedman, Igor K. Lednev, Rina K. Dukor, and Laurence A. Nafie

J. Am. Chem. Soc., **2007**, 129 (41), 12364-12365• DOI: 10.1021/ja074188z • Publication Date (Web): 26 September 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 09/26/2007

Vibrational Circular Dichroism Shows Unusual Sensitivity to Protein Fibril Formation and Development in Solution

Shengli Ma,[†] Xiaolin Cao,[†,¶] Mimi Mak,[†] Adeola Sadik,[†] Christoph Walkner,[†] Teresa B. Freedman,[†] Igor K. Lednev,[‡] Rina K. Dukor,[§] and Laurence A. Nafie*[,†,§]

Department of Chemistry, Syracuse University, Syracuse, New York 13244, Department of Chemistry, University at Albany, SUNY, Albany, New York 12222, and BioTools, Inc., 17546 Bee Line Highway, Jupiter, Florida 33458

Received June 8, 2007; E-mail: lnafie@syr.edu

Protein fibril formation is of considerable interest due to the association of fibrils with many neurodegenerative diseases.[1,2] The details of the fibrillation process in solution are not yet understood in detail, owing in part to insufficient sensitivity to fibril structural changes in situ. We report vibrational circular dichroism (VCD) spectra of aqueous solutions of two proteins, hen egg white lysozyme and bovine insulin, that exhibit unusually large spectral features in the amide I and II regions under conditions for which protein fibrillation is known to occur. It is found further that changes in the enhanced VCD occur as protein fibrils form and develop. This demonstrates that VCD is a unique probe of protein fibrillation dynamics in the solution state.

Previous solution studies of fibril formation in a variety of proteins and peptides have utilized electronic circular dichroism (ECD) in the ultraviolet (UV) region,[3,4] infrared absorption in the amide I and II regions,[5−8] Raman,[9] Raman optical activity (ROA),[10] and deep UV resonance Raman[11] scattering across a wider region of vibrational modes. In these studies, spectral changes as a function of conditions such as pH, solvent, heating, and concentration have been explored that lead to changes in frequency distributions of intensities but not to large changes in the magnitude of the observed intensities. By contrast, VCD spectra show dramatic increases in spectral intensities by more than an order of magnitude as fibrils form, and show further changes as they develop with time.

The infrared (IR) absorbance and VCD spectra of lysozyme and insulin solutions are presented in Figures 1 and 2, respectively. All spectra were measured using a Chiral*IR* Dual-PEM VCD spectrometer from BioTools, Inc. with 8 cm^{-1} resolution, for aqueous samples in a 6 μm path length cell, followed by subtraction of solvent spectra. Native protein was measured as a solution of 100 mg/mL, pH 2.0, for 12 h. Fibril formation was initiated by heating the solution at 60 °C for 2 days for lysozyme and 2 h for insulin. Samples were then centrifuged at 10 000 rpm for 10 min. The VCD spectra of the supernatant phase and gel phase were measured for 12 and 1 h, respectively. Spectra have been normalized to approximately equal amide I absorbance.

The IR and VCD spectra in Figures 1 and 2 exhibit interesting similarities. On the VCD intensity scale, appropriate to the fibril gel spectra, the VCD of native proteins are difficult to see, yet they exhibit normal levels of VCD intensity. The IR spectra of native lysozyme and insulin are typical of proteins with predominantly PPII and α-helix structure, although they differ in relative amounts as indicated by their differing VCD spectra. As fibrils, these two proteins exhibit similar enormous VCD intensities with peaks at five closely correlated frequencies with the sign pattern

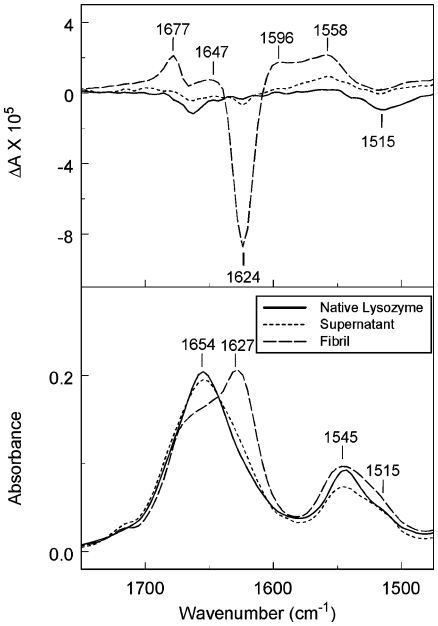


Figure 1. VCD and IR spectra of native lysozyme, centrifuged lysozyme supernatant, and centrifuged lysozyme fibril gel at pH 2. The supernatant and the gel were obtained after heating at 60 °C for 2 days.

(+ + − + +). The frequencies for lysozyme are 1558, 1596, 1624, 1647, and 1677 cm^{-1}, while for insulin they are 1554, 1593, 1627, 1647, and 1670 cm^{-1}. Since these bands are not fully resolved, the values of the peak frequencies are affected by the relative magnitudes of the overlapped bands, which are somewhat different for the two proteins. Of these five bands, the most dramatic is the negative VCD band near 1625 cm^{-1}, which is larger by nearly a factor of 2 for insulin compared to lysozyme. The VCD spectra from the supernatant also exhibit interesting features. For both proteins, the supernatant VCD shows small but distinct departures from the spectra of the native protein in the direction of the full fibril VCD spectrum. In particular, the development of the negative VCD band near 1625 cm^{-1} can be seen in both cases and most easily in the case of insulin. The appearance of fibril-like VCD features is an indication that small proto-filament structures are present that are not large enough to be removed from the supernatant by centrifugation. Finally, the normalized IR spectra in Figures 1 and 2 show only a shift in the frequency of the main absorption band from 1654 to near 1625−1630 cm^{-1}, which has been shown to be characteristic of fibril formation in previous IR studies.[5−8]

The same measurements of IR and VCD of insulin fibrils grown at a reduced concentration of 10 mg/mL were carried as shown in the Supporting Information and found to yield the same spectral

[†] Syracuse University.
[‡] University at Albany, SUNY.
[§] BioTools, Inc.
[¶] Current address: Department of Global Cellular and Analytical Resources, Amgen Inc., Thousand Oaks, CA 91320.

10.1021/ja074188z CCC: $37.00

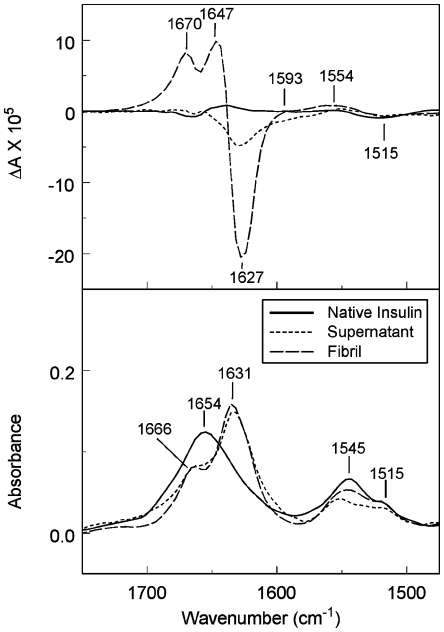


Figure 2. VCD and IR spectra of native insulin, centrifuged insulin supernatant, and centrifuged insulin fibril gel at pH 2. The supernatant and the gel were obtained after heating at 60 °C for 2 h.

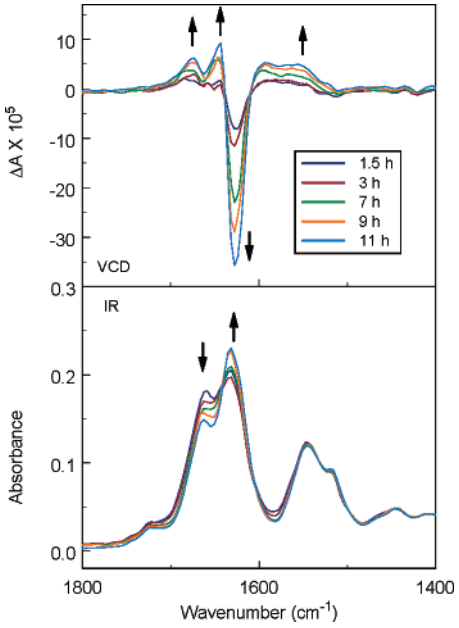


Figure 3. VCD and IR spectra of insulin in H_2O (70 mg/mL) at pH 2, heated 45 min at 65 °C, measured at room temperature at the times indicated after heating was complete and solvent baseline corrected.

features, confirming that the spectra presented here are not the result of starting from high-concentration protein solutions.

Centrifugation is not necessary to follow the progression of fibril formation using VCD, as shown in Figure 3, where the evolution of the fibril state occurs after heating for 40 min at 65 °C at pH 2. IR and VCD spectra were measured at room temperature at the time intervals indicated following the completion of heating. The same five VCD peaks are present with different relative intensities compared to those in Figure 2 and which also change with time and likely correspond to changing states of fibril development. The positive VCD bands develop slowly at first and increase more rapidly and differentially later. The intensity of the negative VCD band near 1625 cm^{-1} steadily increases over the measurement period despite only small changes in the IR intensity distribution without an increase in intensity.

These spectra demonstrate the sensitivity of VCD to fibril formation and growth. The origin of the unusually intense VCD is currently under investigation. Scattering from large fibril particles is a possibility, but evidence does not point in this direction since the hallmark of scattering in the infrared is asymmetric band shapes in the parent IR spectra, while the band shapes here are symmetric. Most likely, the enhanced VCD originates in long-range vibrational coupling from inter-β-sheet contacts as well as possible twists of single β-sheet fibrils or braiding of multiple β-sheet proto-fibrils.[12] In future research, we will seek to connect VCD measurements in solution to correlated images of the same fibril samples as static, dried films using atomic force or scanning electron microscopy.

Acknowledgment. We thank the National Science Foundation for support of this work through the REU (A.S.) and iRES (C.W.) programs at Syracuse University. The assistance of Ludmila Popova and Ming Xu for sample preparation is acknowledged.

Supporting Information Available: AFM images of fibrils of lysozyme and insulin, comparison of secondary structure content of lysozyme and insulin, expanded views of the VCD of native lysozyme and insulin, and IR and VCD of insulin fibrils grown from lower concentration solution. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chiti, F.; Dobson, C. M. *Annu. Rev. Biochem.* **2006**, *75*, 333−366.
(2) Ellis, J. R.; Pinheiro, T. J. T. *Nature* **2002**, *416*, 483−484.
(3) Schmittschmitt, J. P.; Scholtz, J. M. *Protein Sci.* **2006**, *12*, 2374−2378.
(4) Arnaudov, L. N.; de Vries, R. *Biophys. J.* **2005**, *88*, 515−526.
(5) Hiramatsu, H.; Kitagawa, T. *Biochim. Biophys. Acta* **2005**, *1753*, 100−107.
(6) Paul, C.; Axelsen, P. H. *J. Am. Chem. Soc.* **2005**, *127*, 5754−5755.
(7) Zandomeneghi, G.; Krebs, M. R. H.; McCammon, M. G.; Fandrich, M. *Protein Sci.* **2004**, *13*, 3314−3321.
(8) Petty, S. A.; Decatur, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 14272−14277.
(9) Haston, J. L.; Engelsen, S. B.; Roessle, M.; Clarkson, J.; Blanch, E. W.; Baldock, C.; Kielty, C. M.; Wess, T. J. *J. Biol. Chem.* **2003**, *42*, 41189−41197.
(10) Blanch, E. W.; Morozova-Roche, L. A.; Cochran, D. A. E.; Doig, A. J.; Hecht, L.; Barron, L. D. *J. Mol. Biol.* **2000**, *301*, 553−563.
(11) Xu, M.; Ermolenkov, V. V.; He, W.; Uversky, V. N.; Fredricksen, L.; Lednev, I. K. *Biopolymers* **2005**, *79*, 58−61.
(12) Dobson, C. M. *Trends Biochem. Sci.* **1999**, *29*, 329−332.

JA074188Z