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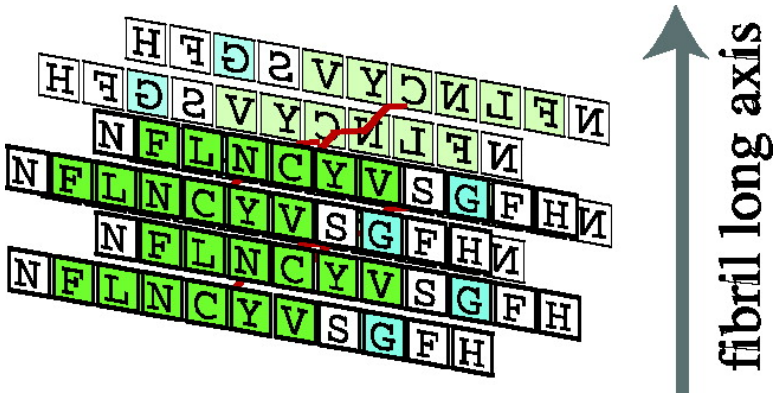

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Structural Model of the Amyloid Fibril Formed by β_2-Microglobulin #21−31 Fragment Based on Vibrational Spectroscopy

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Native structures of peptides in proteins have been thought to represent the most stable among the possible conformations that a peptide can take. However, amyloid fibrils are now considered to be stable structures for peptide aggregates. The relationship between the secondary structural preferences of individual residue native proteins[1] and amyloid fibrils has not been fully addressed because only fibril structures of short peptides have been examined so far.[2,3] Accordingly, in the current study, we carried out a detailed analysis of peptide structures of fragments in fibril and the native states.

For this purpose, we employed vibrational spectroscopy, which can be used to study the structures of small peptides and full-length proteins in both fibril and native solution states. The secondary structures of component residues in the peptide were deduced from the shifted amide I frequencies of peptides specifically labeled with ^{13}C on the carbonyl group of the main chain.[4−7] The IR spectra for these analyses were obtained with an FT spectrometer in conjunction with a microscope.[8]

The IR absorption spectrum for the fibril of the #21−31 fragment of β_2-microglobulin [^{21}NFLNCYVSGFH^{31}] is shown in Figure 1a. The fibril was prepared at pH 7.5 and in the presence of 100 mM NaCl.[9] The spectrum was similar to that of the full-length amyloid fibril,[8] and this fragment corresponds to strand B (#22−28) of β_2-microglobulin.[10] On the basis of the spectrum shown in Figure 1a, it was previously deduced that five of the residues in the #21−31 fibril are in a β-sheet conformation, four are in a random conformation, and two are in a non-α/non-β structure.[8] The error for these values was ±1 residue, mainly due to the uncertainty of the analysis. The absence of a band at >1680 cm^{-1} suggests the parallel stacking of β-strands.

To determine the location of each secondary structure, isotope substitution was carried out. The spectra of four ^{13}C-substituted compounds are shown in Figure 1b−e. The ^{13}C substitution is located on F22 in b, L23 in c, G29 in d, and both F22 and V27 in e. For ^{12}C═O amide I modes, the transition dipoles of nearby amide groups couple strongly with each other (transition dipole coupling [TDC]), resulting in variable changes in frequency. The empirical rule predicts that the amide I frequencies of peptides are located around >1660, 1660−1640, and 1640−1615 cm^{-1} for non-α/non-β, random, and β-sheet structures, respectively.[11] ^{13}C═O substitution causes the frequency to shift down by ∼35 cm^{-1} due to a mass effect. In addition, the TDC between adjacent ^{13}C═O oscillators, if present, would lower the frequency further; its magnitude is a function of the separation and relative orientation between two ^{13}C═O oscillators. In fact, it is expected that the band would appear at ∼1586 and ∼1595 cm^{-1} for parallel and antiparallel β-sheets, respectively, without slipping of the labeled position.[12] For the latter, it appears around ∼1602 and ∼1608 cm^{-1} for one- or two- and three-residue-slipped structures, respectively.

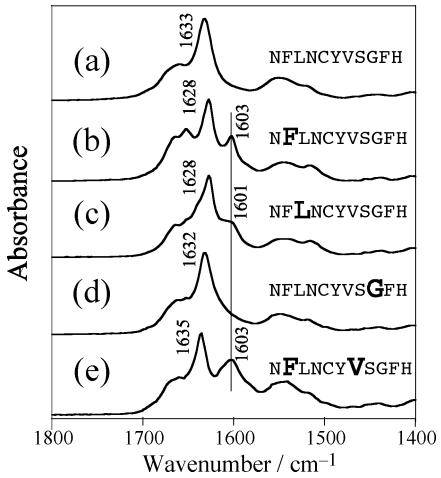

Figure 1. IR absorption spectrum of the fibril of (a) the #21−31 fragment and (b−e) the isotope-labeled species; the ^{12}C═O of peptide main chain is substituted to ^{13}C═O at (b) F22, (c) L23, (d) G29, and (e) F22 and V27, respectively (substituted residue is depicted in the figure by bold face).

In Figure 1b,c, the ^{13}C═O band appeared at 1603 and 1601 cm^{-1}, consistent with substitutions at F22 and L23, respectively. Their peak positions indicate that F22 (and L23) adopts a β-sheet, and that two ^{13}C═O oscillators in the adjacent molecules are slipped by two or three residues.[12] In contrast, the spectrum in Figure 1d is similar to that in Figure 1a, indicating that the shifted ^{13}C═O band is buried in the envelope of the ∼1630 cm^{-1} band in the ^{12}C═O β-sheet. This means that G29 adopts a random structure.

The IR absorption spectrum of the #21−31 fibril (Figure 1a) indicates the presence of a two-residue non-α/non-β structure, which we tentatively assigned as a "β-bulge",[8] although a β-turn is also a plausible candidate. To determine which structure is correct, both F22 and V27 were labeled with ^{13}C. If a β-turn were present, it would be at N24 and C25 because the β-sheet is five residues long in the molecule that would contain F22 and L23. In this case, F22 and V27 would come in close proximity and, due to the stronger TDC between the ^{13}C═O oscillators, should give rise to an additional peak at a lower position than the F22 monosubstituted species. Figure 1e shows that the additional peak is observed at 1603 cm^{-1} with an intensity nearly twice that of the monosubstituted species (Figure 1b,c). This clearly demonstrates that the TDC between F22 and V27 is small enough (i.e., their distance is sufficiently long) for there to be no turn structure in the #21−31 fibril because of the long intramolecular distance. Rather, TDC that causes the shift to 1603 cm^{-1} arises from the disposition of extended units.[13] Also, Figure 1e shows that both F22 and V27 are in a β-sheet because they yield a band at around 1603 cm^{-1}.

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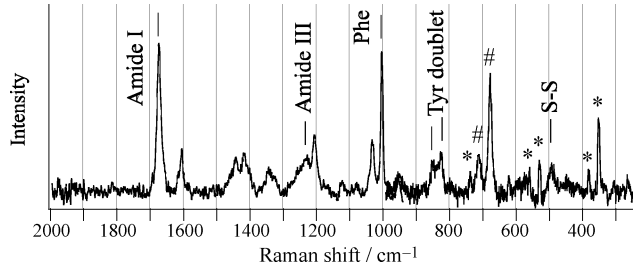

Figure 2. Raman spectrum of the #21−31 fibril: (∗) spontaneous emission lines of Ar^+ laser; (#) solvent.

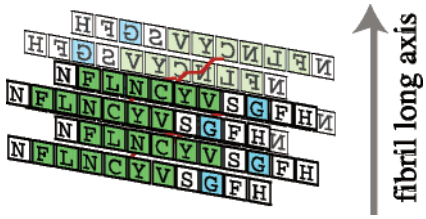

Figure 3. Plausible model of #21−31 fibril. Parallel β-sheet is shown. Color depicts the secondary structures: β-sheet (green) and random (blue). Red line indicates a disulfide bridge.

To examine the structure further, the Raman spectrum was measured for a dispersed fibril in an aqueous buffer under nonresonant conditions[14] (Figure 2). Amide I was observed at ~1670 cm^{-1} as an intense band, consistent with a β-sheet. Also, the S−S stretching band, ν(S−S), is clearly seen at 494 cm^{-1}, and the amide III (1229 cm^{-1}), Tyr doublet (826 and 852 cm^{-1}), and Phe (1004 cm^{-1}) bands are also clearly visible. At the same time, there was no trace of a band around 2560 cm^{-1} in the IR spectrum that could be assigned to a S−H stretching band (data not shown).

Our finding of a ν(S−S) at 494 cm^{-1} strongly suggests that the disulfide bridge is constrained,[15,16] which means that the C25 residue is in a regular structure. Given the steric hindrance of the CCSSCC linkage, this bridge should connect molecules in different β-sheets. It is highly likely that the N24 and Y26 residues also adopt a regular structure because they would be held in place by the regular L23, C25, and V27 residues. Therefore, a β-sheet is very possible for this region. Furthermore, the presence of the ν(S−S) band agrees with the findings of Hasegawa,[9] which suggested the predominance of the dimeric species in the fibril.

A plausible structural model based on the present results is shown in Figure 3. Two β-sheets are formed with hydrogen bonds between molecules and are linked by a disulfide bridge. This agrees well with our previous model[8] and also with the conformation of this fragment in the native protein.[10] The secondary structure preference in the native protein is retained (if not complete) in the fibril.[17] In other words, the side chain interactions in the native protein would probably be correctly reproduced in the fibril if it is formed at neutral pH. This is in agreement with the fact that the Chou−Fasman method[1] predicts a high propensity for β-sheet formation by the #22−28 fragment.

Therefore, the side chain interactions in the peptide fibril seem to be similar to those in the protein, and, at least for the short peptide fibril, are an important determinant of the secondary structure.[18] This view contradicts the idea that sequence effects are much less significant than main chain interactions for the fibril core structure.[19] Indeed, this previous idea cannot explain the fact that (i) the β-sheet core does not spread to the whole molecule, and (ii) the location of the β-strand in the unit agrees with that of the sequence that prefers β-sheet. A β-helix[20,21] can be a real core structure only when its physicochemical properties are consistent with (i) and (ii).

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(13) This illustration shows an intramolecular TDC magnitude between F22 and V27 as large as 0.0008 mdyn $Å^{-1}$ amu^{-1} (antiparallel two-transition dipole moments are assumed to be 3.70 D $Å^{-1}$ $amu^{-1/2}$, 12 Å apart (3 Å/residue), and with coincident normal vectors). This value is not as significant as the off-diagonal force constant arising from the TDC; the IR spectrum can be reproduced successfully even if such a small value is disregarded. See: Torii, H.; Tasumi, M. *J. Chem. Phys.* **1992**, *96*, 3379−3387.
(14) The fibril was sonicated to avoid intense scattering of the excitation light (488 nm from an Ar^+ laser). It was then placed back in elongation conditions in case the peptide was converted to its constituent monomers by sonication. AFM imaging suggested that the fibril was shortened by sonication (data not shown).
(15) The ν(S−S) band usually appears around 510−540 cm^{-1}. A good correlation has been established between the frequency and the conformation of the CCSSCC linkage, which is essentially unchanged as long as the dihedral angle of CSSC [χ(CS−SC)] is ~90°. See: Sugeta, H.; Go, A.; Miyazawa, T. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3407−3411.
(16) For example, ν(S−S) decreases to as low as 495 cm^{-1} when χ(CS−SC) is ~30°. See: Van Wart, H. E.; Scheraga, H. A. *J. Phys. Chem.* **1976**, *80*, 1823−1832.
(17) Note that the side chain interactions in this fibril are not exactly the same as those in the native protein because the disulfide bridge binds strands B of two molecules in the present fibril, while it binds strands B and F in the native structure.
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