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Aggregation of the Amphipathic Peptides $(AAKA)_n$ into Antiparallel β-Sheets

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The self-assembly of biomolecules such as peptides and proteins has become an important issue in biomedical, biotechnological, and material research. The aggregation of peptides can be considered as a noncovalent form of a polymerization process. Its biomedical relevance results from the fact that different disorders such as spongiform encephalopathies, Alzheimer's, Parkinson's, and Huntington's disease are due to the aggregation and subsequent fibril formation of naturally unfolded or misfolded peptides and proteins.[1−4] The self-assembly of biomolecules generally yields a paracrystalline fibrillar structural arrangement of β-sheets.[5] With respect to biotechnology, the self-assembly process has very positive aspects. It allows the generation of material with incorporated biofunctionality like biocompatibility and ligand and metal recognition.[6] Hydrogels, that is, a self-assembled mixture of, for example, peptides and water, are used for tissue engineering and drug delivery.[7,8] Finally, self-assembled molecules offer the possibility of creating new supramolecular architectures such as ribbons, nanotubes, and monolayers exhibiting a nanoscale order.[9,10]

Self-aggregating peptides share some common features. They are often hydrophobic with charged residues added so that they dissolve at low concentrations (e.g., Ac-$KYA_{13}K$-NH_2 [11,12] or $D_2A_{10}K_2$ [13]). Peptides with alternating hydrophilic and hydrophobic residues (e.g., VKVKVK...[14] or KFEFK...[15]) are also used, which are amphipathic with respect to a β-strand or β-sheet structure. Other types of peptides with antibacterial and hemolytic activity have a mixture of hydrophilic and hydrophobic groups and prefer helical structures in membrane environments.[16,17] The helical wheel projections of these peptides show a clustering of equally charged groups and hydrophobic groups, respectively.

Alanine-based peptides doped with some charged residues generally form short α-helices in solution if the number of residues exceeds a certain threshold value.[18] In principle, this was what we expected for Ac-$(AAKA)_{3,4}$-NH_2. However, as described in the present study, for which we utilized IR, Raman, vibrational circular, and electronic circular dichroism spectroscopy, both peptides are capable of forming very stable hydrogels. A Materials section is included in the Supporting Information, which describes the sample preparation.

Figure 1 depicts the IR, isotropic and anisotropic Raman, and VCD spectra of the amide I′ region of the Ac-$(AAKA)_4$-NH_2 hydrogel. The IR-spectrum exhibits a strong, asymmetric band at 1616 cm^{-1} with its high wavenumber wing extending over a very broad spectral region. A much smaller peak is depicted at ∼1684 cm^{-1}. The VCD spectrum displays two weak negative peaks, one at 1620 cm^{-1}, and an even less-pronounced peak at 1685 cm^{-1}. Both the IR and the rather weak VCD spectra are expected of an antiparallel β-sheet conformation.[19] A very strong interstrand excitonic coupling gives rise to the observed band splitting. The "continuum" between the two IR bands stems from the distribution of excitonic states typical for a not perfectly regular β-strand.[20] The large splitting suggests the absence of significant twisting.

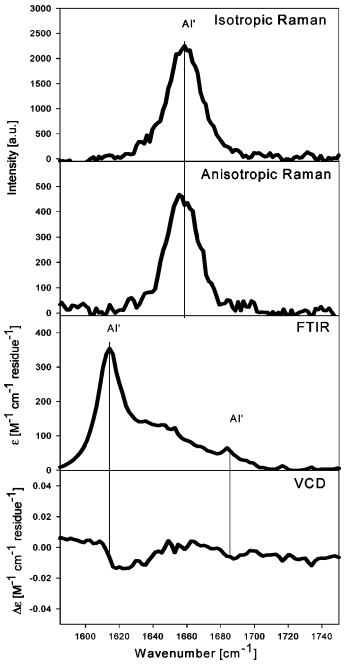

Figure 1. Isotropic and anisotropic Raman, FTIR, and VCD spectra of the amide I′ band of Ac-$(AAKA)_4$-NH_2 measured at room temperature.

The Raman spectra in Figure 1 exhibit a broad band at approximately 1655 cm^{-1}. A fit of a Voigtian profile to the isotropic and anisotropic Raman band revealed a slight noncoincidence (∼2.0 cm^{-1}) between the respective peak positions with the isotropic band at higher wavenumbers. This reflects a distribution of excitonic states rather than the single band expected for an ideal β-sheet.[21] We performed a preliminary simulation of the IR and Raman spectra of a β-hairpin with a type I′-β turn, on the basis of our excitonic coupling model,[22] which nearly exactly yielded the observed noncoincidence.

We also measured the IR spectra of Ac-$(AAKA)_4$-NH_2 at various temperatures between 5° and 65 °C (data not shown). The spectra display solely a slight upshift of the entire amide I′ band shape at higher temperatures, compared to those at lower temperatures. This rules out any significant melting of the aggregates and thus indicates a high thermal stability. Preliminary investigations of the gel by atomic force microscopy seems to indicate fibril formation (Measey, Yang, Schweitzer-Stenner; unpublished data).

The aggregation significantly influences the intensity of the amide I′ relative to that of the amide II′ band at ∼1450 cm^{-1}. The corresponding normal mode is a mixture of the CN stretching vibration and the antisymmetric CH_3 bending mode of the alanine side chain.[23] We found that for the unaggregated octamer, $(AAKA)_2$,[24] the relative integrated intensity ratio of amide I′ to amide II′ is ∼0.65; that is, the amide I′ band exhibits a significantly lower intensity in the *x*-polarized Raman spectra (Figure S2 of Supporting Information). The case is quite different, however, for the ag-

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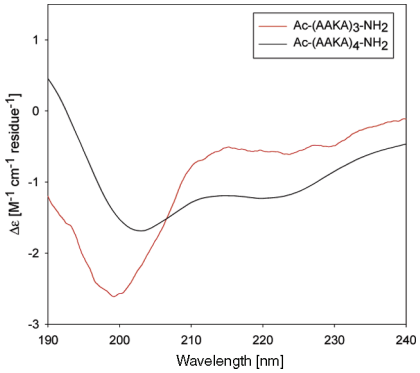

Figure 2. ECD spectra of Ac-$(AAKA)_3$-NH_2 and Ac-$(AAKA)_4$-NH_2 measured at room temperature.

gregated 16-mer Ac-$(AAKA)_4$-NH_2, for which we obtained an amide I′/amide II′ ratio of ∼1.4. The spectrum of Ac-$(AAKA)_3$-NH_2 displays a similar ratio (data not shown). This suggests a substantial intensity decrease of amide II′ owing to the hydrophobic interaction between alanine side chains in the β-sheet. Thus, this band may be used as a marker band for aggregation of alanine-based peptides.

Electronic circular dichroism (ECD) measurements were performed at room temperature and a lower peptide concentration (1−2 mg/mL) at which aggregation does not take place. This can be inferred from the room temperature ECD spectrum depicted in Figure 2. That of the 16-mer is clearly indicative of an α-helical conformation mixed with some PPII; hence, aggregation involves an $\alpha \rightarrow \beta$ transition at some still-to-be- determined concentration, which resembles the behavior of prion proteins.[25]

Some measurements were also performed on the gelated state of Ac-$(AAKA)_3$-NH_2. First results (IR and Raman spectra) are depicted in Figure S1 of the Supporting Information. They also reflect a β-sheet structure, but compared with the spectra of Ac-$(AAKA)_4$-NH_2 all bands are substantially broadened and the high wavenumber band in the IR spectrum is more intense. This suggests a more inhomogeneous solution and structural differences between the formed β-sheets. A significant fraction of nonaggregated peptides is revealed by the isotropic Raman intensity between 1660 and 1670 cm^{-1}. The respective ECD spectrum measured at room temperature (Figure 2) suggests a disordered peptide, that is, a mixture of PPII, β-strand, and α-helix-like conformations, as recently obtained for calcitonin.[26]

The formation of a β-sheet-containing gel is surprising for an amphipathic peptide with its charged groups clustering in the helical wheel, for which we would instead expect the formation of a helical structure with the tendency to layer on the aqueous surface. The positive charges on lysine exclude the possibility of regular multistrand sheet layers solely formed by interpeptide hydrogen bonds. The hydrophobic interaction between the alanine side chain could give rise to a β-hairpin like conformation with the lysine pointing outside and the inside occupied by alanines. However, a hairpin structure alone cannot explain the gel formation and the IR-splitting observed experimentally. Hence further stacking and in-plane aggregation is necessary, which might be caused by hydrogen bonding between lysine and peptide carbonyls of different hairpin structures. One reason for the peptide's sheet propensity can be inferred from our finding that AAKA exhibits more β-strand character than tetraalanine,[27,28] possibly because lysine prevents an efficient hydration of the protein backbone.

Taken together our results reveal that the amphipathic peptides Ac-$(AAKA)_3$-NH_2 and Ac-$(AAKA)_4$-NH_2 form hydrogels at particularly low concentrations. The formation apparently involves a PPII/$\beta \rightarrow \beta$-sheet transition for the former, and a $\alpha \rightarrow \beta$ sheet conversion for the latter.

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Supporting Information Available: Materials section, FTIR and Raman spectra of aggregated Ac-$(AAKA)_3$-NH_2, and a comparison of the amide II′ Raman profile of $(AAKA)_2$ and Ac-$(AAKA)_4$-NH_2. This material is available free of charge via the Internet at http://pubs.acs.org.

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