

An Amphiphilic Folding Molecule That Undergoes an Irreversible Conformational Change

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An emerging field of chemistry involves the design, synthesis, and characterization of large synthetic molecules with abiotic higher order structure.¹ Large folding molecules promise the opportunity to explore increased molecular size and well-defined complexity, while obviating the synthetic difficulties associated with constraining conformations by networks of covalent connections. We have been studying the folding properties and chemistry of aedamers; relatively large synthetic molecules whose conformations are driven by the stacking interactions of alternating electron-rich (1,5-dialkoxynaphthalene) and electron-poor (1,4,5,8-naphthalenetetracarboxylic acid diimide) aromatic units.^{1a} *Aedamers* are named for these *aromatic electron donor–acceptor* interactions. The presumed pleated structures of folded aedamers have a stacked aromatic core with derivatizable, flexible linkers on the periphery.

By alternating the linker amino acids between leucine and aspartic acid (Figure 1), an amphiphilic design, conceptually similar to the leucine zipper motif, was created. The hydrophobic leucine residues are located on one side and the negatively charged aspartate residues are located on the other side of a folded aedamer (1).²

The amphiphilic aedamer, **1**, was synthesized by using standard FMOC-based peptide chemistry.³ Following reverse-phase chromatography, compound **1** gave satisfactory high-resolution mass spectral analysis (calcd 2746.042; found 2746.037). However, compound **1** produced unresolved ¹H NMR spectra in D₂O, indicating extensive aggregation. Dynamic light scattering experiments determined an apparent molecular weight of ~400 000 Da at 2.0 mM for aqueous solutions of **1**, consistent with the aggregate interpretation of the ¹H NMR spectra.⁴

The aromatic units that make up the stacked core of aedamers are valuable reporters of folded structure. Hypochromism and charge-transfer absorbances can result when aromatic units are held in a face-centered stacked geometry, as predicted for the folded **1**.^{1a,6} Aedamer **1** exhibited 56% hypochromism at 382 nm, the absorbance band of the diimide moiety.⁵ In addition, a

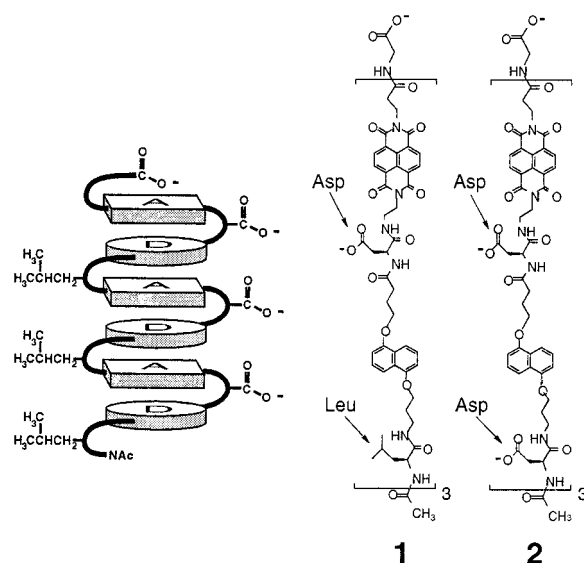


Figure 1. Chemical formulas of the aedamer **1** and the original aedamer **2**. Also shown is a cartoon of the folded conformation of the amphiphilic aedamer **1**.

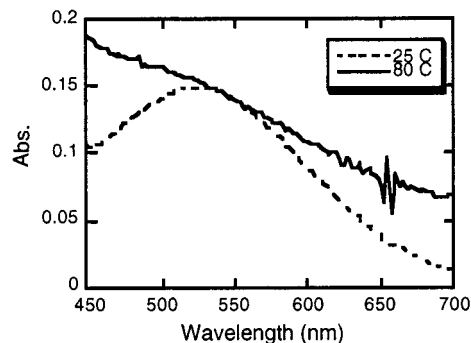


Figure 2. The visible region spectrum of aedamer **1** before (dashed line) and after (solid line) heating at 80 °C. The dramatically increased baseline observed in the heated sample indicates increased light scattering caused by the tangled aggregate.

strong charge-transfer absorbance was observed in the visible spectrum of **1**, with λ_{max} at 526 nm ($\epsilon_{526} = 1500$). This absorbance gives solutions of **1** a red wine color reminiscent of a fine Merlot.

The UV–visible spectral features observed for **1** are similar to the 54% hypochromism at 386 nm and $\epsilon_{540} = 1400$ observed for compound **2**. These data suggest aedamer **1** is folded to a similar extent as the entirely hydrophilic **2** in aqueous solution, presumably in a similar conformation. Like aedamer **2**, aedamer **1** was unfolded by the addition of 2% cetyltrimethylammonium bromide (CTAB), thereby eliminating the hypochromism and charge-transfer band.⁷

Upon heating at or above 80 °C, 1.5 mM solutions of aedamer **1** undergo a thermally irreversible transition to a solution that is considerably more viscous, essentially a gel, accompanied by almost complete loss of the 526 nm absorbance (Figure 2). Upon cooling then standing at room temperature for extended periods, for example 3 weeks, the properties of the pale, highly viscous sample remained constant, not reverting back to those of the original solution.

(7) Because of aggregation, it is impossible to rule out rigorously that the hypochromism and charge-transfer bands are derived in part from intermolecular stacking interactions. However, the similarity in spectral results for **1** and the monomeric **2** can be used to argue against intermolecular stacking effects playing a major role in the spectra observed for **1**.

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(4) Protein Solutions Dyna Pro-801 Molecular Sizing Instrument (2 mM solution of aedamer **1** in 20 mM phosphate buffer, filtered through a 0.02 mm 13 mm Whatman Anodisc 13).

(5) A 56% hypochromism corresponds to an absorbance of the folded aedamer **1** in aqueous buffer that is 44% of that observed for the same concentration of unfolded aedamer in 2% CTAB.

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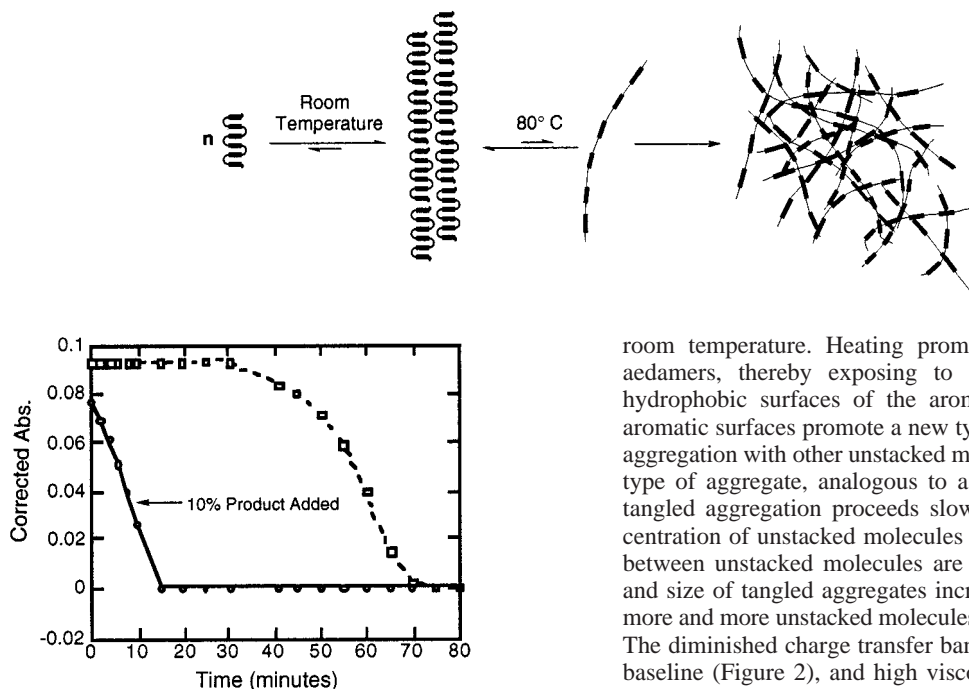
Scheme 1. Proposed Scheme for the Conversion of Amphiphilic Aedamer **1** to the Tangled Aggregate State upon Heating

Figure 3. Kinetics of the conformational transition of aedamer **1** upon heating at 80 °C, as monitored at 526 nm. The upper trace shows the amphiphilic aedamer **1** at 1.5 mM (1.0 mM phosphate pH 7.5, 100 mM NaCl). The lower trace is the same as before, except 0.15 mM material that had already been converted to the product tangled aggregate was added prior to heating. Absorbance was corrected for baseline changes due to increasing aggregation.

Material heated at 80 °C to completion of the transformation (no further decrease in the 526 nm absorbance) was loaded onto a C18 reverse phase column. Only a single component eluted from the column, with the identical retention time and UV–visible spectrum as the unheated material, including recovery of the 526 nm absorbance.⁸ In addition, the mass spectrum of material recovered from the heated sample was identical with that of unheated material. These data indicate that a conformational change, rather than a covalent change, has taken place upon heating.

The kinetics of the thermally induced conformational transition were investigated by examining the decrease in the 526 nm absorbance upon heating (Figure 3). An entirely reproducible slow phase of the conversion is followed by a significantly more rapid phase, consistent with a mechanism in which the product of the conformational transformation promotes the process. Promotion by product was confirmed as a plausible mechanism by adding 10 vol % of material that had already been thermally “converted” to the transformed product prior to heating at 80 °C. In this case, there was no slow phase, but rather only the rapid conversion to the same product state (lower line in Figure 3). In other words, the added product caused the immediate conversion of material at 80 °C.

A plausible mechanism for the thermal conversion of aedamer **1** to the more viscous state is shown in Scheme 1. Molecules of **1** are initially associated into an aggregate of folded aedamers at

room temperature. Heating promotes the unfolding of some aedamers, thereby exposing to solvent the relatively large hydrophobic surfaces of the aromatic moieties. The exposed aromatic surfaces promote a new type of hydrophobically driven aggregation with other unstacked molecules, leading to a “tangle” type of aggregate, analogous to a tangled clump of hair. This tangled aggregation proceeds slowly at first, because the concentration of unstacked molecules is initially low and collisions between unstacked molecules are rare. With time, the number and size of tangled aggregates increase, enabling the capture of more and more unstacked molecules, and the reaction accelerates. The diminished charge transfer band, the dramatically increased baseline (Figure 2), and high viscosity observed for the heated product are all consistent with a tangled aggregate being formed. A tangled three-dimensional aggregate product would also explain the lack of reversibility upon cooling, since the molecules would be trapped by noncovalent interactions with so many other molecules that “untangling” is kinetically impossible. An important aspect of the proposed scheme is that a highly folded initial state is assumed, as any substantial population of significantly unfolded molecules would lead to immediate aggregation at room temperature.

The general features of the kinetically irreversible behavior seen with **1** are not unique. Collagen denatures from a triple helix structure to form gelatin, a tangled aggregate, when heated. Concentrated solutions of other proteins behave similarly. In these cases, the hydrophobic interior residues of the initially folded proteins are exposed to solvent by heat denaturation, allowing for tangled aggregation between unfolded molecules. It should be pointed out that the component protein molecules of collagen are generally 120 000 Da,⁹ considerably larger than the 2 750 Da of aedamer **1**.

In conclusion, we propose that the viscosity and spectroscopic changes observed upon heating the amphiphilic aedamer **1** are due to the kinetically irreversible conversion from a folded state to a tangled aggregate. The unique loss of color associated with this transformation may be useful as a temperature sensor, which could irreversibly indicate whether a sample had ever reached a threshold temperature. Whatever ultimate applications may be possible, we have shown that abiotic molecules can be produced that exhibit interesting and novel property transitions, likely the result of folding. We are continuing to examine new aedamer structures with the intent of understanding and ultimately controlling conformation-dependent behavior.

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(8) On the basis of the integration of the HPLC peaks, the material recovered after heating during different runs amounts to an average of 40 ± 10% of that expected. The remainder of the tangled aggregate material was likely irreversibly adsorbed onto the HPLC precolumn.