Peptide Polyelectrolyte Complexes

Self-Assembling Peptide Polyelectrolyte β-Sheet Complexes Form Nematic Hydrogels**

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An emergent activity is exploration of the prospect for exploiting proteinlike self-assembly as a route to novel soft solidlike materials, with the objective of being able to incorporate proteinlike responsivity into their nanostructure.[1–5] Our approach has been to exploit the intrinsic one-dimensional self-assembling propensity of peptides to form cross-β-structures.[2–4] A hierarchy of structures: helical tapes (single-molecule thick), twisted ribbons (double tapes), fibrils (twisted stacks of ribbons), and fibers (entwined fibrils) are formed successively with increasing concentration in water.[2,3] The fibrils are semirigid and can form nematic fluids at concentrations of about 0.001 v/v, whilst fiber formation occurs at higher concentrations and gives rise to networks comprised of fibrils linked at fiberlike junctions that create nematic hydrogels.[3] This behavior has been shown to stem from the chirality of the peptide molecule, which in turn originates from the intrinsic chirality of the constituent amino acids (L in the case of naturally occurring ones). Another ubiquitous property of peptides is their ability to become electrostatically charged in response to changes in pH values.

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Here we demonstrate how the Coulombic attraction between oppositely charged monomeric peptides, in appropriate pH windows, leads to the spontaneous self-assembly of fibrillar networks and associated nematic hydrogels simply by mixing the component solutions. This behavior may be likened to the polyelectrolyte complexes (PECs) formed on mixing oppositely charged polyelectrolytes.[6]

To illustrate this principle, we make use of two peptides A and C, whose phase behavior[4] in 6.3 mM aqueous solutions is depicted as a function of pH in Figure 1 a. (In previous work,[4] A and C were termed P_{11}-4 and P_{11}-5, respectively.) Both peptides are in their monomeric states in the pH window $7.2 < pH > 7.4$. The three Glu ($-CH_2CH_2COOH$) residues at positions 5, 7, and 9 in A are in their deprotonated states which gives the peptide a net two units of negative charge (Figure 1 b). The resultant Coulombic repulsion keeps the peptides apart and suppresses self-assembly. Similarly, for peptide C, the three Orn ($-CH_2CH_2CH_2NH_2$) residues in positions 3, 5, and 7 are positively charged and give the peptide three units of net positive charge (Figure 1 b).

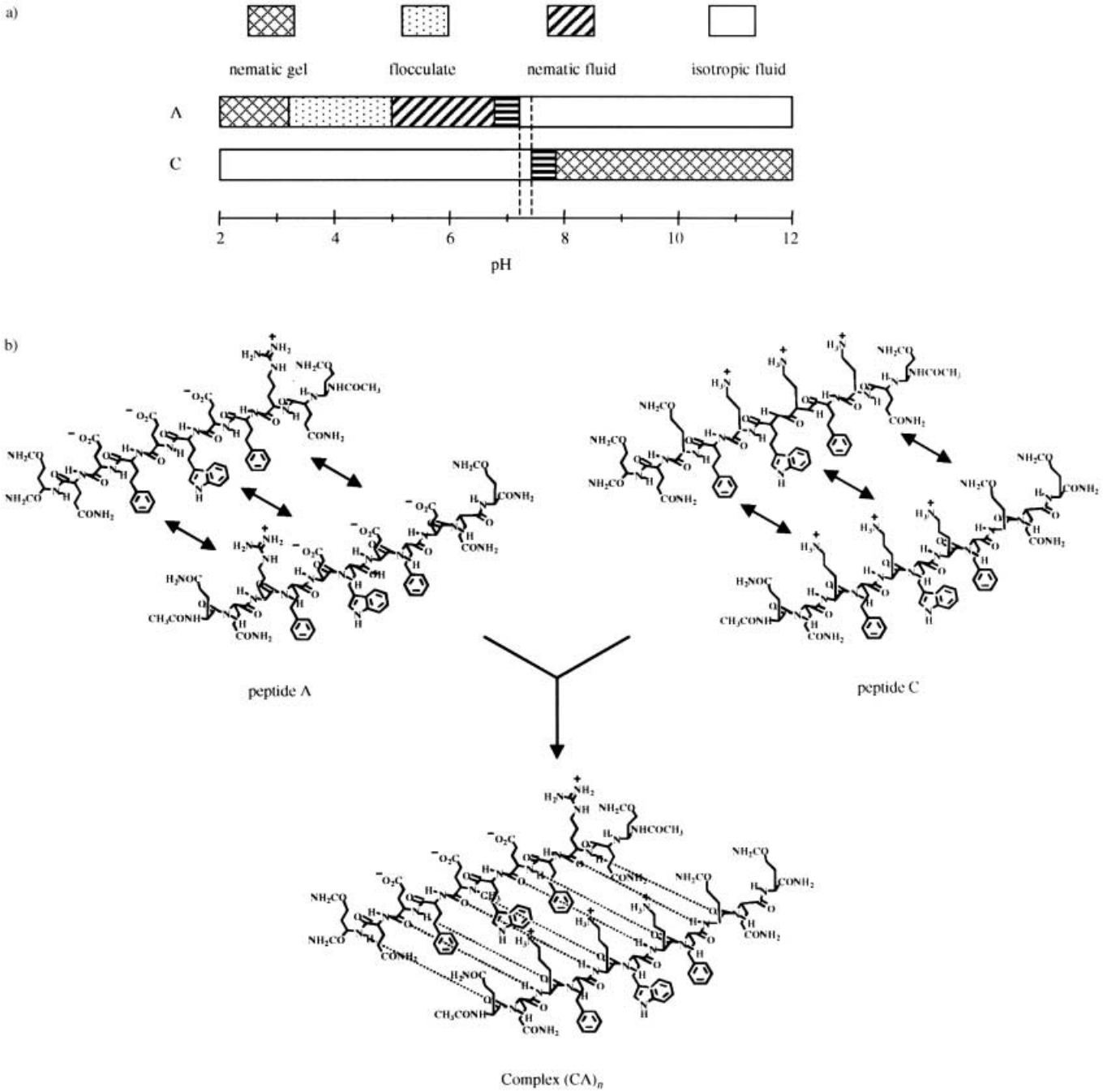

Figure 1. a) Phase behavior of 6.3 mM aqueous solutions of peptides A and C as a function of pH. The nematic-to-isotropic biphasic region of A extends from pH 6.8 to 7.2 and for C from 7.4 to 7.8, which defines the pH interval 7.2 to 7.4 for the mixing of A and C. The horizontal lines spanning the biphasic intervals denote pH values of co-existing nematic and isotropic phases. b) Molecular structures of A, C, and the complex CA showing the electrostatic charge distributions at pH 7.3.

When equal quantities of these two solutions at pH 7.3 were mixed a self-supporting, turbid gel was obtained instantaneously. The gel is birefringent when viewed between crossed polarizers, and the optical micrograph (Figure 2a) is characteristic of a nematic gel.[7] The FTIR (Figure 3a) and

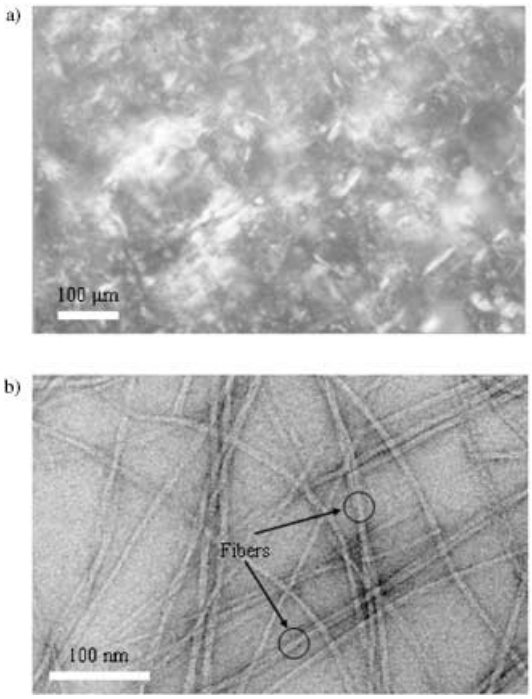

Figure 2. a) Polarizing optical micrograph of the gel ($c = 6.3$ mM) formed after mixing aqueous solutions of monomeric peptides A and C at pH 7.3 showing a typical nematic gel texture.[7] b) Transmission electron micrograph showing mainly fibrils and a few fibers (having twice the diameter of the fibrils) in the nematic gel ($c = 6.3$ mM).

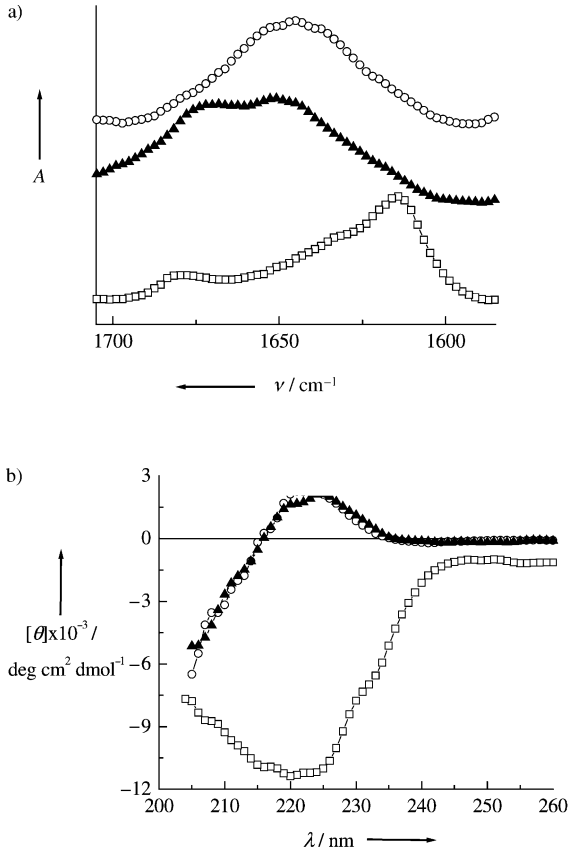

Figure 3. a) FTIR spectra showing amide I′ bands centered at 1645 cm^{-1}, a signature of the initial random coil state of peptide A (○) and peptide C (▲) in 6.3 mM aqueous solutions at pH 7.3 prior to mixing, and the β-sheet conformation of the polyelectrolyte complex after mixing (□). b) Far-UV CD spectra showing the initial random coil states of peptide A (○) and peptide C (▲) in aqueous solutions ($c = 3.1$ mM) prior to mixing, and the β-sheet complex formed after mixing (□).

CD (Figure 3b) spectra of both A (○) and C (▲) prior to mixing are indicative of monomeric (random coil) peptide.[4] After mixing, the spectra (□) are characteristic of an antiparallel cross-β-sheet structure within fibrils.[8] The ^{1}H NMR spectra also reveal distinctive changes. The high-resolution spectra obtained from monomeric solutions of A or C (Figure 4a and b) are indicative of peptide molecules undergoing fast, random, reorientational motion. In contrast, a solidlike, structureless, broad-banded spectrum (Figure 4c) is obtained from the nematic gel state of A ($c = 3.13$ mM, pH 2), which is indicative of peptide molecules locked into a relatively static fibrillar network. On mixing equal volumes of the solutions of A and C, as used to obtain the spectra in Figure 4a and b, respectively, the spectrum in Figure 4d was obtained, which is indicative of the formation of a gel with a small excess (5%) of monomeric C arising from the slight inequality of the molar ratios of A and C in the mixture. Addition of a greater excess of C led to the spectrum in Figure 4e (ca. 66% monomeric C). In contrast, the corresponding addition of an excess of A led to the spectrum in Figure 4f (ca. 66% monomeric A). These spectroscopic results are consistent with the fibrils being a 1:1 complex of A and C which we anticipate must be arranged alternately in an antiparallel cross-β-structure within the tapelike fibrillar substructure (Figure 1b).

The bulk viscosity and ^{1}H NMR experiments reveal that the peptide PECs and their nematic hydrogels appear to be stable over a wider pH interval (ca. 1–12) than the access window (ca. 7.2–7.4). The peptide PEC was converted into a gel of C and a monomeric solution of the anionic peptide A over a period of days at pH values higher than about 12 (see Supporting Information). The stability of the peptide PEC to pH appears to arise from the effects of the electrostatic attractive forces between neighboring Glu^- and Orn^+ residues in the complex (Figure 1b). This would shift the effective pK values of the Glu and Orn units to lower and higher values, respectively, than those of the free amino acids (Glu 4.1, Orn 10.8). Typically, the gels were stable up to approximately 90 °C.

The fibrillar structure of the CA complex (Figure 2b) is similar to those of pure A and pure C: all have cross-sections of 8×4 nm and are comprised of four ribbons (eight tapes; see Supporting Information). This observation might appear surprising in view of the different cross-β-strand forces between peptides in the tapelike substructure of the CA

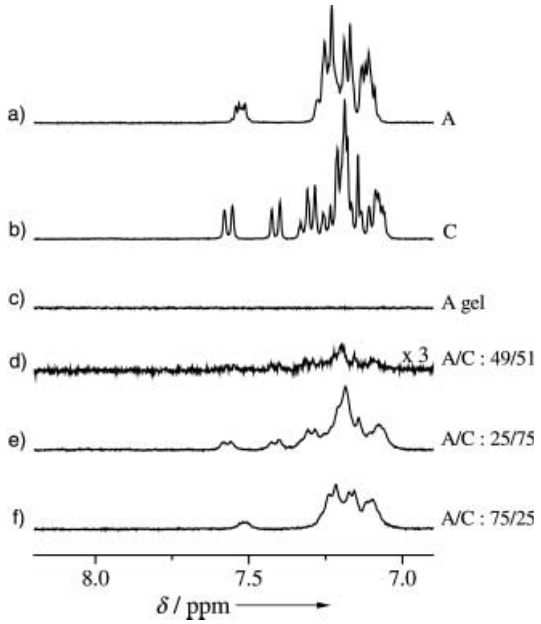

Figure 4. ^{1}H NMR (300 MHz) spectra of the aromatic side chains of residues 4, 6, and 8 in a) a solution of monomeric A at $c = 3.13$ mM, pH 7.46, b) a solution of monomeric C at $c = 3.28$ mM, pH 7.1, c) the nematic gel state of A at $c = 3.13$ mM, pH 2, d) the nematic gel state obtained by mixing equal volumes of solutions (a) and (b), e) as in (d) but with a 3:1 excess of peptide C, and f) as in (d) but with a 3:1 excess of peptide A (pH values are uncorrected meter readings).

complex as compared with the corresponding ones in those of pure A or C. This is because fibril structure is goverened primarily by the intrinsic twist of the ribbons and their mutual attraction.[3] Nevertheless, we might expect the apparently stronger mutual attraction between neighboring A and C peptides in the tapelike structure of the complex to be reflected in a significantly lower value of the onset concentration c^*_{tape} for self-assembly of tapes of CA compared to those of pure A and C.[3] From CD measurements we estimate $c^*_{tape} \approx (2 \pm 1)$ mM) for the CA complex, a value significantly greater than for either A ($c^*_{tape} \sim 20$ μM at pH 2) or C ($c^*_{tape} \sim 400$ μM at pH 10) at pH values appropriate for the formation of a gel (see Supporting Information). This paradoxical behavior would appear to have its origin in a high kinetic barrier, which leads to supersaturation of the solution beyond c^*_{tape}. To test this hypothesis a 6.3 mM gel at pH 7.5 was carefully diluted down to 0.05 mM. CD measurements revealed a transition in the peptide conformation from β-sheet to random coil at about 100 μM, which is a more realistic value for c^*_{tape}.

The stability of β-sheet fibrillar networks toward flocculation has previously[4] been linked to the electrical double layer forces resulting from the presence of a net positive or negative charge on each peptide monomer. One unit of net positive charge per CA pair of peptides appears to be sufficient in the case of the peptide polyelectrolyte complex CA. This charge comes from the single net positive charge located on the Arg^+ residue of A. We can expect this minimum net charge requirement to be raised with increasing ionic strength of the solution.

In conclusion, it has been demonstrated that polyelectrolyte β-sheet complexes (PECs) are formed on mixing aqueous solutions of cationic and anionic peptides and this results in the spontaneous self-assembly of fibrillar networks and the production of nematic hydrogels. These complexes have a 1:1 stoichiometry and their networks are quite robust to variations in pH values or peptide concentration. They may be likened to the PECs formed on mixing oppositely charged polymeric polyelectrolytes,[6] except that their supramolecular structures are quite different. In the case of peptide complexes, the fibrils have more definitive molecular and mesoscopic structures which makes it easier to specify the requisite molecular design. The two peptides C and A must have a propensity to form antiparallel β-sheets, appropriate complementarity in the disposition of their charged amino acid side chains, and at least one additional charged amino acid per peptide pair to stabilize the peptide PEC fibrillar network against flocculation. The presence of salt will attenuate the electrostatic forces and this must be accounted for in the peptide design. Potential applications envisaged for these biocompatible, biodegradable nematic hydrogels are, for example, encapsulation, immobilization, and separation of cells, proteins, antibodies, or enzymes.

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Keywords: β-sheets · gels · peptides · phase transitions · self-assembly

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