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Effects of Gadolinium Chelate on the Evolution of the Nanoscale Structure in Peptide Hydrogels

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Abstract

Small-angle X-ray scattering (SAXS) was used to monitor peptide hydrogelation with and without the MRI contrast agent gadolinium chelate (Gd(III)-chelate). The gelators are a pair of decapeptides that are self-repulsive but mutually attractive. Gd(III)-chelate was either free or covalently bound to one of the decapeptides. Free and conjugated Gd(III)-chelate have the opposite effects on peptide gelation: free Gd(III)-chelate slows down gelation while having little effect on the cross-sectional area of peptide fibers; covalently conjugated Gd(III)-chelate speeds up gelation and results in peptide fibers with significantly greater cross-sectional area. After 24 hrs of gelation, the cross-sectional areas of hydrogels with no Gd(III)-chelate, with free Gd(III)-chelate and with conjugated Gd(III) chelate are 3700 Å², 3800 Å² and 6300 Å², respectively. Free Gd(III)-chelate is not incorporated into peptide fibers and remains in solution with little effect on MRI intensity upon gelation. In contrast, covalently conjugated Gd(III)-chelate is not only incorporated into peptide fibers, but further aggregates toward the center of the peptide fibers. In conclusion, to visualize hydrogelation using ¹H MRI, it is necessary to conjugate Gd(III)-chelate to the material covalently and use T₂-weighted imaging.

Keywords

Hydrogels; peptides; gadolinium chelate; small-angle X-ray scattering (SAXS); biomaterials; magnetic resonance imaging (MRI)

Introduction

Design of new biocompatible and biodegradable materials used for tissue engineering and drug delivery requires detailed knowledge of the structural characteristics of potential

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Supporting Information Available. MS and HPLC data for the peptides and Gd(III)-chelates as well as SAXS data and parameters are available free of charge via <http://pubs.acs.org>

biomaterials at the nanoscale level. It is often the nanoarchitecture that defines the properties of bulk materials relevant for their biomedical applications.^{1,2} Peptide-based hydrogels are among the most widely explored sources of such biomaterials because, in addition to their biocompatibility and biodegradability, their mechanical properties can be tuned to match those of various human tissues.³

Recently, significant attention has been focused on the importance of the structural organization of biomaterials because such organization is believed to define the biological response of the material.^{4,5} Of special interest are factors that give bioengineers the means to influence the structures and properties of biomaterials. The efficiency of such factors is likely to depend upon the kinetics and mechanisms of material assembly. Therefore, it is useful to know the details of structural changes that occur during material assembly.

We have designed mutually-complementary but self-repulsive oligopeptide pairs that form shear-responsive hydrogels upon mixing.^{6,7} Each oligopeptide pair comprises a positively charged module and a negatively charged module. In a preceding study, we conjugated a gadolinium chelate (Gd(III)-chelate) to the positive module to explore the possibility of using magnetic resonance imaging (MRI) to visualize hydrogelation.⁸ Gd(III)-chelates are MRI contrast agents.⁹ Hydrogelation of the oligo-peptide pair shortened T_2 to a much greater extent than T_1 and produced dark spots in both T_1 - and T_2 -weighted images. This result is indicative of local enrichment of Gd(III)-chelate. Using small angle X-ray scattering (SAXS) techniques, we showed that there are two contributing factors to this phenomenon: the aggregation of oligopeptides into nanofibers and the clustering of Gd(III)-chelates within the peptide nanofibers.

In this work, we monitored the incorporation of Gd(III)-chelate into the peptide fibers at different time points using SAXS. A control sample, in which Gd(III)-chelate is simply mixed with, rather than conjugated to, the positive peptide module, is also investigated. In addition, gelation, w/o Gd(III)-chelate, is monitored by T_1 - and T_2 -weighted MRI. By following the time course of Gd(III)-chelate incorporation, we hope to better understand the assembly process.

Materials and Methods

Peptide design, synthesis and purification

The peptide sequences and Gd(III)-chelate structure are given in Table 1. A total of four compounds were utilized in the experiments: **1** is the negative peptide module, **2** is the positive peptide module, **3'** is the free Gd(III)-chelate, and **3** is the covalent conjugate of **2** and **3'**. Gelation is initiated through *co*-assembly of the oppositely charged peptide modules upon mixing, and such design allows gelation to take place at precisely specified pH, ionic strength and temperature. The benefit of mutually-complementary peptides is that it offers an *additional* gelation mode: mixing. Gelation is easily achieved in phosphate-buffered saline at ambient temperature and neutral pH with a pair of mutually-complementary peptides. In contrast, mixing-induced gelation is not possible with self-complementary peptides and gelation has to be accompanied by changes in pH, ionic strength, temperature, etc. Compounds **2** and **3** contain ornithine (designated as 'O') instead of lysine to increase aqueous solubility (ornithine has one less $-CH_2-$ group than lysine). Also, due to the solubility concerns, the *N*-termini of the peptide modules were formylated instead of acetylated.¹⁰ The Gd(III)-chelate contains a tri-glycine segment (GGG) which serves as spacer between the chelator and the peptide. Without such a spacer, **3** cannot form a hydrogel with **1** (unpublished experimental observation).

Oligopeptide modules **1** and **2** were synthesized using Fmoc solid-phase peptide synthesis¹¹ following the procedure described in detail in a previous publication.⁸ The mass value for each oligopeptide was verified using MALDI-MS. The purity of the peptides was assessed by analytical HPLC. (See MALDI-MS spectra and HPLC chromatograms in a previous publication⁸ and in the Supporting Information.)

The [(do3a)]GGG-*amide* was synthesized by adding a 4-fold excess of Fmoc-GGG (the synthesis of Fmoc-GGG is described in details in a previous publication⁸) to rink amide MBHA resin followed by a 2.5-fold excess of [(do3a)]-*tris*-(*t*-butyl ester) (from Macrocyclics, Texas). After cleavage from the resin with 90% trifluoroacetic acid and 2.5% each of ethylene dithiol, tri-isopropyl silane, water and dichloromethane, the products were isolated by rotary evaporation, washed with ether, dissolved in water/acetonitrile and lyophilized. The resulting solids were each purified using cation-exchange and reversed-phase HPLC to obtain [(do3a)]GGG-*amide*.

To obtain compound **3'**, [(do3a)]GGG-*amide* (30 mg, 0.05 mmole) was dissolved in 1 M NH₄OAc buffer at pH 7. To this, GdCl₃ (26 mg, 0.10 mmole) was added and the pH of the resulting solution was adjusted to 7. After stirring at room temperature for 1 hr, the pH of the solution was increased to 9 in order to precipitate excess Gd(III) ions. The pH was adjusted back to 7 after filtration. The resulting compound **3'** was purified by reversed-phase HPLC at pH 7. A similar procedure was used to obtain **3** from [(do3a)]GGGOFOAOAOAOW-*amide*, and this synthesis is described in details in a previous publication.⁸

Peptides were dissolved in 100 mM NaCl and 50 mM sodium phosphate buffer (phosphate buffered saline, PBS) prepared from the deionized water (18.2 MΩ) and deuterated water (Cambridge Isotope Laboratories, 99.99% of D content): for SAXS experiments the mixture of 5% H₂O and 95% D₂O was used; MRI samples were prepared using 70% H₂O and 30% D₂O mixtures. In both cases, pH was adjusted to 7 without taking the D₂O effect into account. Concentrations of the peptide solutions were determined on the basis of the molar absorptivity of tryptophan at 280 nm ($\epsilon_{280} = 5690 \text{ M}^{-1} \text{ cm}^{-1}$)¹² and ICP-OES (Perkin-Elmer, Optima 3100XL).

The gelation process for three samples, **1+2** (no Gd(III)), **1+2+3'** (free Gd(III)-chelate), **1+3** (covalently conjugated Gd(III)), was monitored using SAXS for 24 hrs. Samples **1+2+3'** (gel) and **3'** (sol) were also subjected to MRI studies.

Small-Angle X-ray Scattering (SAXS)

Sample Preparation—A 10 mM stock solution of each peptide was prepared in PBS buffer (5% H₂O, 95% D₂O, pH 7) and equilibrated on ice. In each experiment, solutions of individual peptides were gravimetrically aliquoted and mixed in equal amounts so the concentration of each peptide in the mixture was 5 mM. The mixture was vortexed in a 1.5 mL centrifuge tube kept on ice to slow down gelation initiation. To monitor the gelation of the **1+2** pair in the presence of free **3'**, **2** and **3'** were first mixed to form a solution with the concentration of each component being 10 mM. This 10 mM solution of **2+3'** was then mixed with 10 mM solution of **1** in equal proportions to achieve the final 5 mM concentration for each individual component. Immediately after mixing the sample was loaded into a capillary for SAXS measurements as described earlier.⁸ The gelation kinetics was monitored at 12 °C, and the capillary was sealed to exclude water evaporation during overnight data collection.

SAXS Data Measurements and Analysis—SAXS data were acquired at 12°C in 15 min intervals during the first hour of gelation, then in 1 hr intervals during next the 4–7 hrs

of gelation. The last set of data was collected 24 hrs after mixing of the peptide pair. In our previous SAXS studies¹³ of temperature-dependent gelation for similar co-assembled decapeptide hydrogels, we have observed noticeable temperature effect on the gelation kinetics (and on the dimensional parameters of the resulting fiber). It has been shown that based on SAXS intensity profiles, the gelation at 25°C proceeds much faster than at 5°C, and is virtually completed within 5 hrs after mixing.¹³ At 5°C, gelation is significantly slower, and the scattering intensity continues to grow even 24 hrs after mixing.¹³ Therefore, in the present study, the gelation was monitored at 12°C. At this temperature, gelation was slow enough to allow fiber morphology to be traced by SAXS at various time points and fast enough to complete within 24 hrs (see, also the Supporting Information). Data were collected and reduced to $I(q)$ versus q using the small-angle X-ray scattering instrument and procedures described previously.¹⁴ The variable q is the amplitude of the scattering vector (measured q -range in our experiments was 0.006–0.32 Å⁻¹), and is equal to $4\pi(\sin\theta)/\lambda$, where 2θ is the scattering angle and λ is the wavelength of the incident X-ray ($\lambda = 1.54$ Å). The fibrils that form the hydrogel network have one dimension much longer than the other two and this longest dimension is beyond the resolution limit (q_{min}) of the scattering data (in our case $q_{min} \sim 0.006$ Å⁻¹ which means the maximum dimension we can reliably characterize is ~ 600 Å). We therefore analyzed the scattering data in terms of the cross-sectional dimensions of the scattering particles by multiplying $I(q)$ by q which effectively removes information about the longest dimension of the scattering particle.¹⁵ Guinier analysis for the cross-section parameters of the scattering particle involves plotting $qI(q)$ versus q^2 to yield a linear dependence with a slope proportional to the average radius of gyration of cross section, R_c , which is the contrast weighted mean distance of all area elements from the centre of scattering density. For elongated particles of finite length, this Guinier plot will have a distinctive roll-over when $L \geq 2\sqrt{2}d_{max}$, where L is the length of the particle and d_{max} is the maximum dimension of the cross-section.¹⁶

Similar to the approach used for 3D dummy atom shape restoration,¹⁷ we used a simulated annealing algorithm, implemented in a purpose-written program that is described elsewhere,¹⁸ to model the cross-sectional shape of the peptide fibers. In all these calculations, dummy atoms were arranged on a flat grid of 50×50 close packed dummy atoms. The size of each atom was 3 Å in diameter which is stipulated by the resolution limit of the SAXS technique¹⁵ (to achieve the best model fit, the size of dummy atom should be $< \pi/(2q_{max})$ which is 4.9 Å for our $q_{max} \sim 0.32$ Å⁻¹). The program calculates the pair distance or vector length distribution function, $P_c(r)$, for the model cross-section composed of the dummy atoms. $P_c(r)$ is the distribution of distances between area elements in the cross section, weighted by the scattering density at each radial value, r , and Fourier transformation of $P_c(r)$ yields the model scattering profile $qI_{mod}(q)$. In the optimization procedure, the program minimizes the discrepancy between the model scattering data $I_{mod}(q)$ and the experimental scattering data $I_{exp}(q)$.¹⁸ After optimization, values for $I_c(0)$, the area of the cross-section S_c , d_{max} , and R_c , are determined from $P_c(r)$; d_{max} is the r value at which $P_c(r)$ goes to 0. The zeroth and the second moments of $P_c(r)$ yield $I_c(0)$ and R_c values, respectively. The program also outputs the model cross-sections as atomic coordinate files in the Protein Data Bank format. Corrections for instrument slit geometry, scaling and incoherent background are applied to the model scattering profile so it can be compared directly with experimental scattering data.¹⁸

Electron density contrast values $\Delta\rho_g$ for the three gels, **1+2**, **1+2+3'** and **1+3**, were calculated as the difference between the electron density, ρ_g , of each peptide pair and the electron density of the solvent, ρ_s , which is 0.3345 e/Å³ for diluted aqueous buffer solutions (for the PBS buffer containing 95% D₂O + 5% H₂O).¹⁹ The electron densities for these gels were determined using $\rho_g = Z/V_m$ where Z and V_m denote the number of electrons in and the volume of each molecular scattering unit: for the **1+2** and **1+3** gels, the scattering units

are (**1** + **2**) and (**1** + **3**), respectively. V_m for each peptide was calculated based on the amino acid sequences and with standard molecular volumes of the functional groups for peptides and Gd(III)-chelate calculated using Voronoi approximation.²⁰ Values of Z and V_m for each individual peptide are listed in Table 1. The resulting contrast values for the **1**+**2** and **1**+**3** gels are $\Delta \rho_{1+2} = 0.115 \text{ e}/\text{\AA}^3$ and $\Delta \rho_{1+3} = 0.151 \text{ e}/\text{\AA}^3$, respectively. For the **1**+**2**+**3'** gel, based on the structural similarity of the cross-section of the fibers the **1**+**2** and **1**+**2**+**3'** gels (Figure 2), we treated this gel under the assumption that **3'** is not incorporated into the fibers formed by **1** and **2**. However, accounting for 5 mM of Gd(III)-chelate as part of the solvent results in $\rho_s = 0.336 \text{ e}/\text{\AA}^3$, and therefore in this instance $\Delta \rho_{1+2+3'} = 0.114 \text{ e}/\text{\AA}^3$ (see also Table S1), which is not significantly different to the corresponding value for the **1**+**2** gel.

Mass-fractal analysis is used to analyze the characteristics of a repetitive mass unit which might be present in the hydrogels under study. In the higher q region (0.01 – $0.04 \text{ \AA}^{-1}$), the scattering profile could be fitted to power-law decay which obeys the following equation²¹

$$I(q) = B_f q^{-d_f} \quad (1)$$

where d_f is the fractal dimension of the unit and B_f is the power-law scaling prefactor. $d_f = 1$ in the case of stiff rod-like repetitive units and for these particles B_f is proportional to the length and the fourth power of the radius of the rod; $d_f = 2$ for the Gaussian-coil-shaped structures, and $d_f = 1.5$ for the swollen Gaussian-coils in a good solvent. In the case of Gaussian-coils, $d_f = 2$ and $d_f = 1.5$, B_f is proportional to the average end-to-end distance of Gaussian coils.

The density of the cross-linking networks in a hydrogel has been estimated using the Debye-Bueche model²² which is implemented using the *Irena SAS* macros (ver. 2.35)²³ for *IGOR Pro* 6.0 in the following form

$$I(q) \propto \frac{l_c^3}{(1 + q^2 l_c^2)^2} \quad (2)$$

where l_c is the correlation length. The correlation length of a gel is a measure of the spatial dimensions of the cross-linking regions and is equivalent to the mesh size of the network. The greater is the correlation length, the greater is the mesh size.²⁴

In order to attain greater scattering intensity in small angle X-ray scattering experiments and to minimize the divergence of the incoming beam so it is not interfering with comparatively less intensive scattering data, our instrument used slit collimation system.¹⁴ As this leads to the broadening of the SAXS profiles, wherever possible, we smear our model data to compare directly with experimental data, but for some analyses (specifically the mass fractal analysis) we must correct the experimental data for the effects of slit-smearing prior to the analysis. Based on Lake's algorithm,²⁵ slit-smearing corrections of the scattering profiles (desmearing) were performed using the *Irena SAS* macros (ver. 2.35)²³ for *IGOR Pro* 6.0 for the case of two-dimensional slit with trapezoidal beam-shape kindly developed at our request by Dr. J. Ilavsky (Argonne National Lab). Desmeared scattering data were then used in mass-fractal analysis and correlation length calculations.

Magnetic Resonance Imaging

Sample Preparation—Each compound under study —**1**, **2** and **3'**—was dissolved in deuterated PBS buffer (70% H₂O + 30% D₂O, pH 7, no correction for D₂O). MRI images

were collected for gel **1+2+3'** (5mM+5mM+5mM) and sol **3'** (5mM). MRI images of gel **1+2** from the previous study are used as control.⁸

Magnetic Resonance Imaging— T_1 - and T_2 -weighted MRI images were obtained using a Siemens Trio 3 Tesla clinical MRI system (Siemens Medical Solutions, Erlangen, Germany) with Avanto gradients (45 mT/m strength and 150 T/m/s slew rate). The transmit/receive wrist coil was used for the transmission of the RF wave and signal detection to and from the sample. Net magnetization amplitude for each sample was determined from the appropriate region of interest (ROI) using *MRIcro* software.²⁶

T_1 -weighted images were obtained using the inversion-recovery spin echo pulse sequence,²⁷ and for T_2 -weighted imaging, a turbo-spin echo (TSE)²⁸ was employed. In both experiments, all MRI parameters such as the imaging matrix, field of view, in-plane resolutions as well as the array of the variable inversion recovery times (TI) and echo times (TE) were the same as in a previous publication.⁸

Results and Discussions

Co-assembly of mutually complementary oligopeptide modules **1** and **2** into a hydrogel was investigated by SAXS with the Gd(III)-chelate either covalently conjugated to or simply mixed with the positive module **2**. For each of the three combinations, **1+2**, **1+2+3'** and **1+3**, the measured X-ray scattering intensity $I(q)$ vs. q (Supporting Information, Figure S3, top row) increased with time, consistent with the formation of cross-linked fiber networks. Guinier plots of $\ln q I(q)$ vs q^2 for all three hydrogels show a distinctive roll-over at lower q ($q^2 < 2 \times 10^{-4} \text{ \AA}^{-2}$) indicating fibril formation as such a roll-over feature is characteristic of particles that have one dimension much larger than the other two. In fact, fibrils form as early as 15 minutes after mixing (Supporting Information Figure S3, bottom row). The measured scattering intensity is directly proportional to the total concentration (in mg/mL) of the scattering particles and to the square of their volume. Comparison of the zero-angle scattering intensity $I(0)$ which characterizes the total mass of the scattering particles (Figure 1) indicates that among the three gels under study, the kinetics of gelation is fastest for the **1+3** pair. This is confirmed by its fastest rate of increase such that within the first 15 min a sharp jump in the $I(0)$ value is observed followed by much slower changes over the remainder of the 24 hrs of measurement. In contrast, the other two pairs (**1+2** and **1+2+3'**) show slow growth in the 15 min to 1 hr period and also continue the growth during ensuing 24 hrs.

Using 2D dummy atom shape restoration we modeled cross-sectional shapes for the fibers underlying the hydrogel network that best fit the scattering data (Figure 2). The evolution of the cross-sections of all three hydrogels over time (Figure 2, Figure 3A) indicates that, as early as 15 minutes after mixing, the maximum cross-sectional dimension of the peptide fibers, d_{max} , are already 130–140 Å. During the ensuing 24 hrs, d_{max} continues to grow, reaching ~170 Å for all three pairs. While the d_{max} values for each fiber network measured are indistinguishable by SAXS, the **1+3** pair forms distinctly thicker fibers with one cross-sectional dimension significantly greater compared to the other two fibers (60 Å for **1+3**, 45 Å for **1+2** and **1+2+3'**, see Figure 2).

For the **1+2** and **1+2+3'** pairs, the estimated area of fiber cross-section, S_c , shows no systematic growth between 15 min and 24 hr (Figure 3C). In the same time period, the maximum dimension of the cross-section, d_{max} , and the radius of gyration of the cross-section, R_g , for these two pairs demonstrate clear systematic increases (Figures 3A and 3B). It therefore can be concluded that the cross-sectional (transverse) dimensions of the **1+2** and **1+2+3'** fibers are essentially maximized within the first 15 min of gelation. Subsequent

hydrogel maturation involved transverse reorganization (evidenced by increasing d_{max} and R_c) but no transverse growth (evidenced by constant S_c). Such transverse reorganization under constant S_c amounts to increasing d_{max} at the expense of the regularity of cross-sectional shape (see shape evolution in Figure 2). As $I(0)$ for these two gels keeps increasing during this time period while the cross-section area shows no systematic growth, the peptide fibers must be growing longitudinally. During this longitudinal growth, the average mass per unit length of the fibers, M_c , remains constant (Figure 3D).

In spite of the aforementioned similarities between the **1+2** and the **1+2+3'** gels, the presence of free Gd(III)-chelate (**3'**) does result in a deceleration of the gelation process, and it is evident from Figure 1 that the growth in $I(0)$ for **1+2+3'** gel is slower as compared to **1+2** gel. Further, R_c of the **1+2+3'** fibers is consistently smaller than those of the **1+2** fibers (Figure 3B). Thus, it appears that Gd(III)-chelate interferes with peptide assembly as gelation is slower and the resulting fibers are thinner in at least one dimension. One possible explanation for the slower growth of the **1+2+3'** gel is that, after mixing, **2** and **3'** associate to certain extent and dissociation is needed after **1** is added in order for **1** and **2** to form fibers.

The changes caused by free Gd(III)-chelate are minor in comparison to the changes caused by covalently conjugated Gd(III)-chelate (the **1+3** pair.) For the **1+3** pair, all four parameters of the fiber cross-section— d_{max} , R_c , S_c , and M_c —grow systematically during the 15 min – 24 hr time period (Figure 3). This result suggests that after the initial 15 min, the **1+3** fibers keep growing transversely and increasing amounts of peptide are being incorporated into the gel network. Of note, both the area of fiber cross-section, S_c , and the mass per unit length, M_c , show increases that sharply accelerate after 5 hr (Figures 3C and 3D). Interestingly, such rapid growth is preceded by a “plateau” region where the maximum dimension of the cross-section, d_{max} , and the radius of gyration of the cross section, R_c , remain essentially unchanged (~ 2–5 hr, Figure 3A and 3B). Notably, the **1+3** fibers have the lowest cross-sectional radius of gyration R_c even though they have the largest cross-sectional area S_c (Figure 3B vs. Figure 3C). As explained in our previous paper, this is caused by the clustering of Gd(III)-chelates within the peptide fibers.⁸ Based on the above observations, it would be reasonable to suggest that this “plateau” region of d_{max} and R_c time evolution reflects the transversal reorganization of the fiber. Meanwhile, the R_c value remains constant because Gd(III)-chelate, the strong scatterer, is clustering towards the center of the hydrogel fiber (R_c is the scattering density-weighted root-mean-square distance of area elements from the cross sectional center.) One might suggest that such movement will result in the decrease in R_c which will be counterbalanced by the transversal growth of the fiber (probably in one dimension), keeping the d_{max} and R_c constant. Understandably, once such seclusion and clustering of Gd(III)-chelate is completed, further transversal growth of all four parameters of the fiber cross-section is observed (Figure 3).

The impact of covalently conjugated Gd(III)-chelate on the morphology of the hydrogel also is evident from mass-fractal power-law analysis. Figure 4 shows the time evolution of the fractal dimension d_f and the scaling pre-factor B_f of the three gels. The d_f value of the **1+3** pair is lower than those of the **1+2** and **1+2+3'** pairs throughout the 15 min – 24 hr period, indicating that the **1+3** fibers are more swollen than the other two fibers, consistent with higher S_c value. B_f for the **1+3** pair is significantly larger than that for the **1+2** and **1+2+3'** pairs (Figure 4B) throughout the 15 min – 24 hr period. B_f for Gaussian coils is proportional to the end-to-end distance of the coils. Larger B_f is indicative of larger structure unit, consistent with higher scattering intensity $I(0)$ (Figure 1). The observed greater swelling for the **1+3** pair together with greater B_f value for this hydrogel are also consistent with the clustering of Gd(III)-chelates towards the center of the fibers.

Both the **1+2** and **1+2+3'** pairs show a steady decrease in the correlation length, l_c , over time (Figure 4C). In gel systems, l_c is a characteristic of the extension of the cross-linking network and is equivalent to the network mesh size. Therefore, the decrease in l_c amounts to the expansion of the cross-linking regions in the gel and the decrease in mesh size is consistent with the formation of more uniform fibril network. However, in the **1+3** gel, after a noticeable drop at the onset of gelation, the correlation length remains essentially unchanged, suggesting that the growth of this gel does not involve expansion of the cross-linking regions and the mesh size of the network remains unchanged. This observation supports the suggestion that the formation of the **1+3** gel after first 30 min of gelation occurs mainly through the structural reorganization and the transversal growth at the level of the individual fiber, increasing the mass of scattering particles, without the formation of more cross-links in the gel network.

Finally, the above mentioned similarity in the cross-sectional characteristics of the **1+2** and **1+2+3'** hydrogels suggests that free Gd(III)-chelate (**3'**) is not incorporated into the peptide fibers underlying the **1+2+3'** gel. This conclusion is corroborated by T_1 - and T_2 -weighted MR images (Figure 5A and 5B) as well as by the MRI intensity dependences on recovery time, TI, and echo time, TE (Figure 5C and 5D). Indeed, sol **3'** and gel **1+2+3'** demonstrate virtually identical MRI intensity profiles (Figure 5C and 5D), suggesting that in the **1+2+3'** hydrogel, water accessibility of the Gd(III)-chelate is hardly affected. This result is entirely consistent with SAXS result showing that **3'** is not incorporated into fibers formed by **1** and **2**. The results demonstrate that if ^1H MRI is used to monitor material assembly *in vivo*, it is necessary to covalently conjugate the contrast agents to the material.

Structural differences observed for all three hydrogels at the nanoscale have evident implications for T_1 - and T_2 -weighted MRI visualization of hydro-gelation. Still, to establish a clearer link between nanoscale structures and bulk properties, material characterizations, such as dynamic rheology, need to be performed.

Conclusions

Free and conjugated Gd(III)-chelate have the opposite effects on peptide gelation: free Gd(III)-chelate slows down gelation while having little effect on the cross-sectional area of peptide fibers; covalently conjugated Gd(III)-chelate speeds up gelation and results in peptide fibers with significantly greater cross-sectional area. Free Gd(III)-chelate is not incorporated into peptide fibers and remains in solution with little effect on MRI intensity upon gelation. In contrast, covalently conjugated Gd(III)-chelate is not only incorporated into peptide fibers, but further aggregates toward the center of the peptide fibers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Normalized $I(0)$ from Guinier analysis vs. time during the gelation of the **1+2** pair (red); the **1+2+3'** pair (blue), and the **1+3** pair (green). Observed $I_{obs}(0)$ values extracted from Guinier analysis were normalized to account for the difference in electron contrasts ($\Delta \rho_g^2$), $I_{obs}(0) \sim I(0) \times \Delta \rho_g^2$. $I(0)$ is proportional to the mass of the scattering particles.

Figure 2. Time evolution of the cross-sectional shapes of peptide fibers underlying the **1+2**, **1+2+3'** and **1+3** gels.

Figure 3.

(A) Maximum dimension of the cross-section, d_{max} , 1+2 pair, red (final value is $d_{max} = 170$ Å); 1+2+3' pair, blue (final value is $d_{max} = 167$ Å); 1+3 pair, green (final value is $d_{max} = 170$ Å). The final values of d_{max} for 1+2 and 1+3 pair are identical, therefore, they coincide. (B) Radius of gyration of the cross-section, R_g , 1+2 pair, red (final value is $R_g = 44.4$ Å); 1+2+3' pair, blue (final value is $R_g = 43.0$ Å); 1+3 pair, green (final value is $R_g = 41.7$ Å). (C) Cross-sectional area of individual fibers, S_c , for 1+2 (red); 1+2+3' (blue) and 1+3 (green). (D) Mass per unit length of individual fibers, M_c , for 1+2 (red); 1+2+3' (blue) and 1+3 (green). To obtain M_c , zero-angle scattering of the cross-section, $I_c(0)$, was normalized to account for the difference in electron contrast, i.e., $M_c = I_c(0)/\Delta \rho_g^2$. The lines represent a basic B-spline fit to the data.

Figure 4.

Time evolution of the mass-fractal parameters of the repetitive mass unit of the fibers of **1+2** (red), **1+2+3'** (blue) and **1+3** (green) gels: **(A)** fractal dimension d_f ; **(B)** power-law scaling pre-factor B_f . Fractal dimension d_f characterizes the structure of a repetitive mass unit of the hydrogels under study. $d_f = 2.0$ for Gaussian-coils, $d_f = 1.5$ defines a swollen Gaussian-coil in a good solvent. The deviation of d_f from 2.0 allows qualitatively estimate the degree of swollenness of the Gaussian-coils. Power-law scaling pre-factor B_f is proportional to the end-to-end distance of Gaussian-coils. **(C)** Time evolution of the correlation length l_c for **1+2** (red), **1+2+3'** (blue) and **1+3** (green) gels, respectively. The lines represent a basic B-spline fit to the data.

Figure 5. Comparison of the MRI images of hydrogels in the presence of free Gd(III)-chelate. (A) T₁-weighted images with different inversion recovery time TI; (B) T₂-weighted images with different echo time TE; (C) MRI intensity vs. TI; (D) MRI intensity vs. TE. The images were collected using a 3T clinical MR scanner.

Table 1

Sequences of oligopeptide modules and structures of Gd(III)-chelates.

Peptide	Sequences of Co-Assembling Modules	M_w (Da)	Z (e)	V_m (Å ³)
1	<i>formyl-EFEAEAEAEW-carboxyl</i>	1,235.7	654	1416.6
2	<i>formyl-OFOAOAOAOW-amide</i>	1,162.7	624	1426.6
3	[Gd(do3a)]GGGOF ⁺ OAOAOW-amide	1,848.0	948	1877.7
3'	[Gd(do3a)]GGG-amide	730.2	340	475.9

3

3'

Modular material assembly is achieved by pairing a positive module (**2** or **3**) with a negative module (**1**). The free Gd(III)-chelate, **3'**, carries zero net charge. Positively charged amino acids are in red, negatively charged amino acids are in blue, and neutral amino acids are in black. A, alanine; E, glutamic acid; F, phenylalanine; G, glycine; O, ornithine; W, tryptophan. Z is a number of electrons in each peptide; V_m is a molecular volume of each module. V_m is calculated by summing the standard volumes of various functional groups in peptides and Gd(III)-chelate using the Voronoi approximation.¹⁹