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Ultrasound-Induced Gelation of Organic Fluids with Metalated Peptides**

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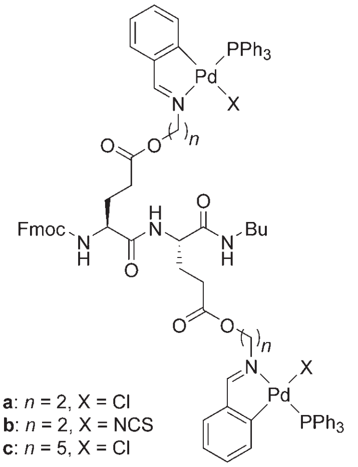
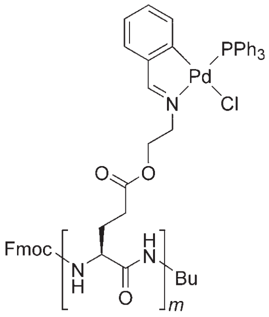
Stimuli-responsive supramolecular assembly has been studied extensively as a forward-looking technology for the precise control of the physical properties and functions of aggregates.[1–3] Recently, it was found that a blast of ultrasound can act as a trigger for the instant gelation of stable organic fluids when a small amount of a clothespin-shaped dinuclear palladium complex is used as a type of switchable gelator.[4,5] The ultrasound waves were thought to cleave the intramolecular π-stacking interactions of the complexes, inducing rapid and spontaneous aggregation through interpenetrating stacking interactions. Now this self-lock/interlock switching has been carried out with hydrogen-bonded aggregates, using the newly designed metalated dipeptide **1a** (Fmoc = 9-fluorenylmethyloxycarbonyl). This work is expected to provide a new methodology for the creation of stimuli-responsive H-bonded supramolecular assemblies,[1b–f,2b–d] especially those containing peptide nanoarchitectures.[1c–f,2c,6] This paper describes the sound-induced gelation of palladium-bound peptides, and the precise control of their switchable aggregation by the tuning of sound factors.



1a: $n = 2$, X = Cl
1b: $n = 2$, X = NCS
1c: $n = 5$, X = Cl



2: $m = 1$
3: $m = 3$
4: $m = 4$

Brief ultrasound irradiation (0.45 W cm^{-2}, 40.0 kHz, 60 s) of a homogeneous 1.50×10^{-2} M solution of dipeptide **1a** ($n = 2$, X = Cl) in EtOAc turned the stable pale-yellow solution into a stable opaque gel (Figure 1). Gelation was observed exclusively when ultrasound was used as an external stimulus, and spontaneous aggregation or formation of pregels did not occur without sonication. When nonsonicated samples were cooled or left to stand for a long time, ordinary precipitation of a small amount of amorphous solids or crystals resulted. Gels formed by sonication were stable but were readily converted to the original stable solutions upon heating and subsequent cooling to room temperature. This switchable sol–gel transition occurred exclusively when esters or chlorobenzene were used as solvents, while other solvents such as benzene, toluene, acetone, and acetonitrile did not provide gels regardless of concentration and sonication conditions. The chloro ligand and the short methylene spacer have proven to be indispensable for this switchable gelation. Typically, the dipeptidyl NCS complex **1b** and the Cl complex with longer spacer **1c** did not cause the gelation of any organic solvents. Also, solutions of amino acid **2**, tripeptide **3**, and tetrapeptide **4** in various organic solvents were stable under similar sonication conditions.

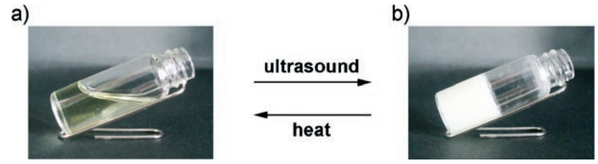


Figure 1. Reversible gelation of a 1.50×10^{-2} M solution of **1a** in EtOAc at 25 °C. Solution a) before and b) just after sonication (0.45 W cm^{-2}, 40.0 kHz, 60 s).

Kinetic studies on the gelation of a 7.00×10^{-3} M solution of **1a** in [D$_8$]EtOAc at 25 °C after sonication (0.45 W cm^{-2}, 43.5 kHz) were carried out by means of ^{1}H NMR (500 MHz) analysis, in which mobile, unaggregated **1a** in sol and gel states could be observed as distinct detectable species similar to conventional gelators.[7] The time dependence of the concentration of **1a** during the entire gelation process indicated that gelation began immediately after sonication, and proceeded until **1a** was almost completely consumed (Figure 2). The rate of consumption of **1a** clearly exhibited

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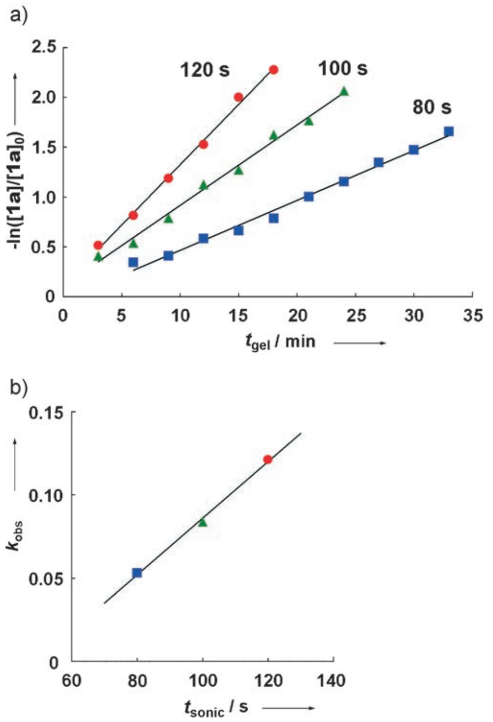

Figure 2. a) Time dependence of $-\ln([\mathbf{1a}]_{\text{unaggregated}}/[\mathbf{1a}]_0)$ for the gelation of a 7.00×10^{-3} M solution of **1a** in [D$_8$]EtOAc at 25 °C after sonication (0.45 W cm^{-2}, 43.5 kHz) for 80–120 s. b) Dependence of k_{obs} on sonication time.

first-order dependence ($R^2 = 0.990$–0.996) on the concentration of **1a**. At 25 °C, k_{obs} depended linearly ($R^2 = 0.994$) upon sonication time (t_{sonic}) ($5.00\times10^{-2}\,\text{s}^{-1}$ for $t_{\text{sonic}} = 80$ s, $8.09\times10^{-2}\,\text{s}^{-1}$ for $t_{\text{sonic}} = 100$ s, and $1.21\times10^{-1}\,\text{s}^{-1}$ for $t_{\text{sonic}} = 120$ s) as shown in Figure 2. The clear first-order kinetics and the linearity of k_{obs} vs. t_{sonic} strongly suggests that gelation consists of a sonication-induced initiation step and a subsequent spontaneous propagation step.

Most importantly, the heat resistance of the aggregates could be precisely controlled by adjusting the sonication time. Figure 3 shows typical differential scanning calorimetry (DSC) traces obtained by heating an amorphous solid and xerogels of **1a** at 5.0 K min^{-1}. Xerogel samples were prepared from a 1.50×10^{-2} M solution of **1a** in EtOAc by varying t_{sonic} from 15 to 60 s at 25 °C. These thermograms showed endothermic peaks at 80.0 °C (12.5 kJ mol^{-1}, amorphous solid), 106.0 °C (29.6 kJ mol^{-1}, $t_{\text{sonic}} = 15$ s), 110.0 °C (29.9 kJ mol^{-1}, $t_{\text{sonic}} = 30$ s), and 116.0 °C (22.5 kJ mol^{-1}, $t_{\text{sonic}} = 60$ s), respectively. Thus, xerogels prepared using longer sonication times tended to exhibit endothermic peaks at higher temperatures than those of nonirradiated or briefly sonicated samples. This unprecedented level of control could be ascribed to the sound-induced formation of the initial domains for subsequent aggregation, which determined the higher-order structure of the gel fibers.

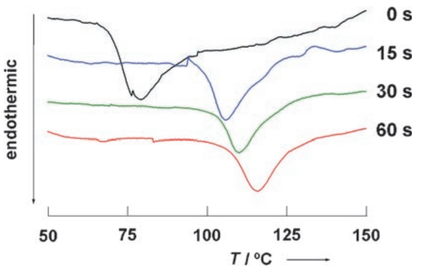

Figure 3. DSC profiles of **1a**; amorphous solid (black trace), xerogels (colored traces) after sonication (0.45 W cm^{-2}, 40.0 kHz) for 15–60 s.

SEM images of the Pt-coated xerogel revealed that complex **1a** self-assembled into beltlike structures with a breadth of roughly 200 nm and a maximum length of 7.5 µm, forming an extended network. Thin layers with a thickness of approximately 4 nm were observed as the smallest unit of the laminated structures (Figure 4). AFM observations showed the same order of length and thin units with a thickness of 3.5 nm. These thickness values were in good accord with the maximum molecular length (3.3 nm) of **1a** in β sheets, as estimated by computer-aided molecular modeling. Lamellar aggregation of β-sheet monolayers of **1a** in the gel phase were clearly identified by their XRD patterns, which showed features at 4.3 and 32.2 Å ($2\theta = 20.1$ and 2.67°, respectively; see the Supporting Information), the former of which is a typical value for the interstrand distance of β sheets,[1d,e] while the latter coincides with the above-mentioned values for the thickness of the β sheets.

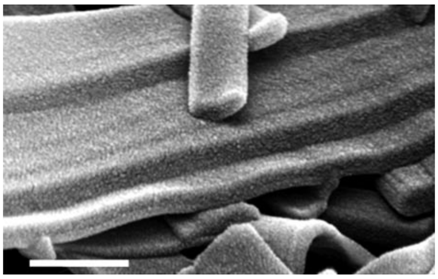

Figure 4. SEM image of xerogel **1a** showing lamellar aggregates of β-sheet monolayers (scale bar: 200 nm).

To obtain insight into the mechanism of this gelation, we analyzed the dynamic behavior of a series of metalated amino acids and peptides, **1**–**4**, in solution by spectroscopic methods. ^{1}H NMR (920 MHz) analysis of **1a** in CDCl$_3$ showed that both C*H*=N doublet signals on two independent palladium moieties appear specifically at lower magnetic field ($\delta = 8.16$ and 8.17 ppm), while only two and three C*H*=N protons in tri- and tetrapeptides **3** and **4**, respectively, are shifted to $\delta = 8.17$ ppm, with one signal remaining at $\delta = 8.13$ ppm. Such downfield shifts of C*H*=N protons are not observed in complexes **1b** and **1c**. Detailed distance geometry analysis based on NOESY experiments carried out at 920 MHz revealed that these metalated peptides form specific helical structures in solutions with strong support from intramolecular H-bonding between chlorine atoms and hydrogens at adjacent nitrogens (see the Supporting Information). Thus, the two chloro ligands in dipeptide **1a** proved to be intramolecularly engaged with two adjacent amide hydrogen atoms.

The present gelation can be understood in terms of the mechanism of aggregation polymerization consisting of initiation and propagation steps.[4] Intramolecular H-bonding involving the chloro ligand of **1a** prevents this dipeptide from intermolecular self-assembly by H-bonding. Ultrasound irradiation releases this self-lock and induces the formation of semistable initial aggregates. After ultrasound irradiation, this initial domain undergoes spontaneous β-sheet aggregation with excess unaggregated **1a**. Increasing the duration of sonication increases the concentration of this active domain, leading to accelerated gelation rates (Figure 2) and formation of higher-order nanostructures with heat-resistant properties (Figure 3). The inertness of **3** and **4** to gelation is probably a result of their tight helical structures which are supported by specific multisite hydrogen bonds. Further studies on these molecules are now in progress.

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[1] Chemical stimuli: a) S. Kawano, N. Fujita, S. Shinkai, *J. Am. Chem. Soc.* **2004**, *126*, 8592; b) L. A. Estroff, A. D. Hamilton, *Angew. Chem.* **2000**, *112*, 3589; *Angew. Chem. Int. Ed.* **2000**, *39*, 3447; c) M. Kogiso, S. Ohnishi, K. Yase, M. Masuda, T. Shimizu, *Langmuir* **1998**, *14*, 4978; d) H. A. Lashuel, S. R. LaBrenz, L. Woo, L. C. Serpell, J. W. Kelly, *J. Am. Chem. Soc.* **2000**, *122*, 5262; e) A. Aggeli, M. Bell, N. Boden, J. N. Keen, P. F. Knowels, T. C. B. McLeish, M. Pitkeathly, S. E. Radford, *Nature* **1997**, *386*, 259; f) Y. Zimenkov, S. N. Dublin, R. Ni, R. S. Tu, V. Breedveld, R. P. Apkarian, V. P. Conticello, *J. Am. Chem. Soc.* **2006**, *128*, 6770.

[2] Light: a) K. Murata, M. Aoki, T. Suzuki, T. Harada, H. Kawabata, T. Komori, F. Ohseto, K. Ueda, S. Shinkai, *J. Am. Chem. Soc.* **1994**, *116*, 6664; b) L. Frkanec, M. Jokić, J. Makarević, K. Wolsperger, M. Žinić, *J. Am. Chem. Soc.* **2002**, *124*, 9716; c) J. H. Collier, B.-H. Hu, J. W. Ruberti, J. Zhang, P. Shum, D. H. Thompson, P. B. Messersmith, *J. Am. Chem. Soc.* **2001**, *123*, 9463; d) S. Yagai, T. Nakajima, K. Kishikawa, S. Kohmoto, T. Karatsu, A. Kitamura, *J. Am. Chem. Soc.* **2005**, *127*, 11134.

[3] Electrooxidation: K. Tsuchiya, Y. Orihara, Y. Kondo, N. Yoshino, T. Ohkubo, H. Sakai, M. Abe, *J. Am. Chem. Soc.* **2004**, *126*, 12282.

[4] T. Naota, H. Koori, *J. Am. Chem. Soc.* **2005**, *127*, 9324.

[5] J. M. J. Paulusse, R. P. Sijbesma, *Angew. Chem.* **2006**, *118*, 2392; *Angew. Chem. Int. Ed.* **2006**, *45*, 2334.

[6] a) S. Fernandez-Lopez, H.-S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxen, R. M. Ghadiri, *Nature* **2001**, *412*, 452; b) V. Percec, A. E. Dulcey, V. S. K. Balagurusamy, Y. Miura, J. Smidrkal, M. Peterca, S. Nummelin, U. Edlund, S. D. Hudson, P. A. Heiney, H. Duan, S. N. Magonov, S. A. Vinogradov, *Nature* **2004**, *430*, 764.

[7] J. Makarević, M. Jokić, B. Perić, V. Tomišić, B. Kojić-Prodić, M. Žinić, *Chem. Eur. J.* **2001**, *7*, 3328.

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