



Title	Self-Assembly and Liquid-Liquid Phase Separation of Lysine Derivatives in Solution
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Abstract of Thesis

Name (Nikesh Narang)

Title

Self-Assembly and Liquid-Liquid Phase Separation of Lysine Derivatives in Solution (溶液中におけるリジン誘導体の自己組織化と液-液相分離)

Abstract of Thesis

[Introduction]

Amino acids and their derivatives are important building blocks in supramolecular chemistry. The hydrogen bonding, electrostatic attraction, and hydrophobic interaction of amino acids and the derivatives induce higher-order self-assembly structures. So far, self-assemblies of many amino acids and their derivatives have been investigated mainly by electron and optical microscopy on dry samples prepared from solutions.

However, the self-assembling process of amino acids and their derivatives in solution has been little studied. When their self-assemblies are utilized in various fields of nanotechnology, the tuning and refinement of the self-assembly structures are an important task, and the investigation of the self-assembling process can help with this task.

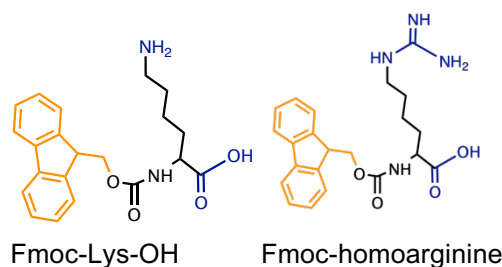
In this thesis, 9-fluorenylmethyloxy-carbonyl (Fmoc)-lysine derivatives (cf. Scheme 1) have been used for understanding the self-assembling process in solutions. The self-assembly of the derivatives was induced by addition of water to their dimethylsulfoxide (DMSO) solution, and the self-assembling process was investigated by time-resolved static and dynamic light scattering (SLS and DLS), small angle X-ray scattering (SAXS), as well as optical and scanning electron microscopy (OM and SEM).

[Experimental Section]

Commercially available Fmoc-lysine and Fmoc-homoarginine samples were dissolved in DMSO, and water was added to the DMSO solutions stepwise to obtain the ternary phase diagram from

SLS at the scattering angle of 90° . The water content of the DMSO solutions were also abruptly changed by adding water at once, and SLS, DLS, SAXS, and OM

measurements were carried out as functions of time t after water addition. The composition of the test solution was expressed in terms of the volume fraction of water ϕ_H° in the DMSO-water mixture, and the mass concentration c of Fmoc-lysine derivative. SEM observations were made on a Fmoc-lysine solution with $\phi_H^\circ = 0.8$ after the evaporation of the solvent.



Scheme 1. Chemical structures of Fmoc-lysine and Fmoc-homoarginine.

[Results & Discussion]

Figure 1 shows the ternary phase diagram for Fmoc-lysine in DMSO-water mixtures. It was found that the phase separation takes place at about $\phi_H^\circ \approx 0.4$ almost independent of the Fmoc-lysine concentration c . When the water content is abruptly increased from 0 to 0.8, light scattering intensity from the solution slightly increases or stays constant with t at lower concentration $c = 0.0165 \text{ g/cm}^3$, but starts fluctuating after an incubation time at higher concentration $c = 0.026 \text{ g/cm}^3$, as shown in Figure 2.

The growth of concentrated phase droplets in the solutions is very slow, and at the higher concentration particles of a new phase appear after the incubation time. The SAXS measurement indicates that the Fmoc-lysine concentration in the concentrated phase droplet is as high as 0.6 g/cm^3 and the size distribution of the droplets is very broad ranging from 10 nm to 500 nm.

The Fmoc-lysine solution with $\phi_H^\circ = 0.8$ and

$c = 0.026 \text{ g/cm}^3$, investigated by SLS and DLS, was observed by optical microscopy (OM). In an early stage ($t = 6 \text{ min}$), no particles were observed, but at $t = 186 \text{ min}$ sub-micron size particles were observed by OM. This time of the particle appearance almost coincides with the time when the light scattering intensity starts fluctuating

(cf. Figure 2). These optical micrographs indicate that the light scattering intensity fluctuation comes from the sub-micron size particles coming into and going out of the scattering volume of the SLS measurement, where the laser beam width used was less than 100 micron.

When the Fmoc-lysine solution with $\phi_H^\circ = 0.8$ and $c = 0.026 \text{ g/cm}^3$ is kept standing for 3 weeks, the sub-micron size particles grow to form particles with branched structure as shown in the left panel of Figure 3. The particles were optically anisotropic (by checking under the cross Nicol condition), indicating a crystal-like orientation of Fmoc-lysine molecules inside the particles. After the evaporation of the solvent from the Fmoc-lysine solution, Fmoc-lysine forms the self-assembly with fringed morphology, as shown in the right panel of Figure 3.

Similar measurements were carried out also on Fmoc-homoarginine dissolved in DMSO-water mixtures. As shown in Figure 4a, Fmoc-homoarginine does not dissolved in DMSO, but dissolved at an intermediate ϕ_H° , and the phase separation takes place again at higher ϕ_H° . Figure 4b shows OM

Figure 1. Ternary phase diagram of Fmoc-lysine + DMSO + water.

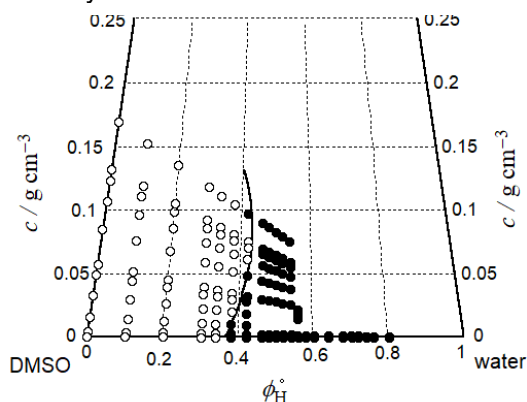


Figure 2. Time evolution of light scattering intensity from Fmoc-lysine solutions after water addition.

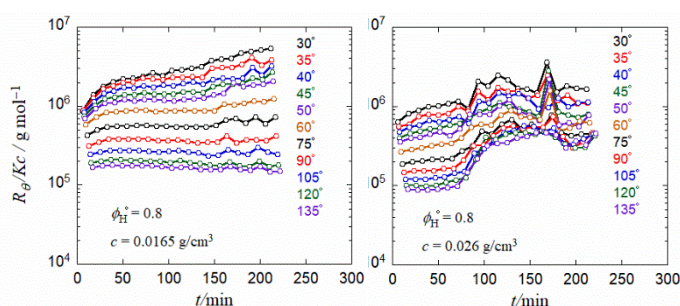
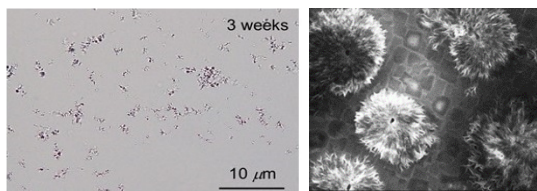


Figure 3. (Left) OM image of Fmoc-lysine in DMSO-water mixture with $\phi_H^\circ = 0.8$; (right) SEM image of Fmoc-lysine after the evaporation of the solvent from the



images of two Fmoc-homoarginine solutions developing fibrous self-assembly and spherulites.

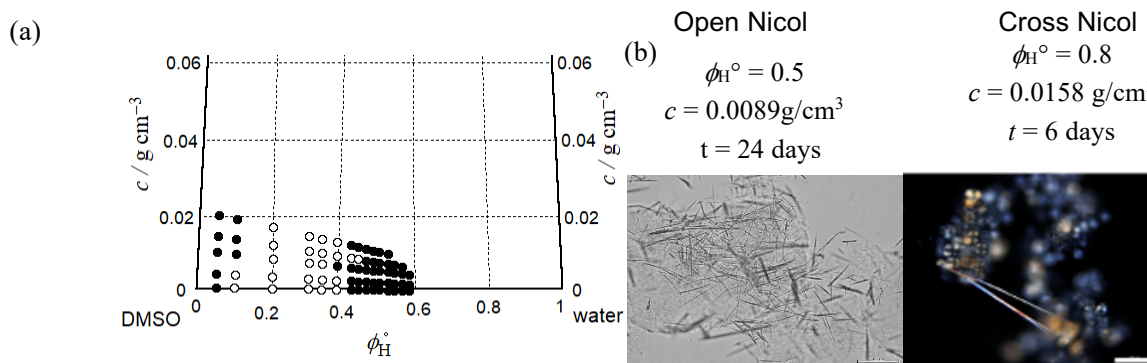


Figure 4. Ternary phase diagram and OM images of Fmoc-homoarginine solutions.

Similar observations were made from lisinopril, a lysine based drug from ACE-inhibitor class. The lisinopril shows β -sheet signature in CD in aged aqueous solution and the shape factor shows the presence of spheres. The aged sample at higher concentrations shows the long fibrillar structures in the microscope.

In another lysine derivative, Fmoc-Lys(biotinyl)-OH, that undergoes gelation in $\phi_H^o = 0.9$. The time dependent optical microscopy shows the presence of spheres in fresh solutions that later turns into fibrillary branched structures.

Thus, the thesis demonstrates liquid liquid phase separation in lysine derivatives undergoing fibrilization.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨 アミノ酸やオリゴペプチド、およびそれらの誘導体は超分子化学における重要な化合物である。それらの中には、溶液中で繊維状の自己集合体を形成するものが多く、ヒドロゲルや有機ゲルのゲル化剤として利用されている。また、それらが生体適合性に優れている点に着目して、細胞培養の足場や、バイオセンサー、ドラッグデリバリーシステムなどの生体医療材料・組織工学材料としての利用が期待されている。最近では、様々な病気の原因となることが知られているタンパク質のアミロイド線維と類似の自己集合体を、アミノ酸やオリゴペプチドも水溶液中で形成することが見出され、その病原性も報告されて、注目を集めている。 これらのアミノ酸・オリゴペプチド、およびそれらの誘導体の自己集合体の研究は、主として光学・電子顕微鏡観察によって行われてきたが、この研究手法では、自己集合の途中のプロセスを調べるのが困難で、その形成動力学については、未だに不明な点が多い。そこで本論文では、アミノ酸の一種であるリシンの誘導体が希薄溶液中で形成する自己集合体形成の動力学を、時間分解光散乱・小角 X 線散乱を顕微鏡観察と組み合わせて調べた。研究対象としたアミノ酸誘導体は、9-fluorenylmethyloxycarbonyl L-lysine (Fmoc-Lys-OH; Chapter II) および 9-fluorenylmethyloxycarbonyl homoarginine (Fmoc-homoarginine; Chapter III) である。 Fmoc-Lys-OH は、ジメチルスルホキシド (DMSO) に可溶で、その DMSO 溶液に水を添加すると不溶化し、自己組織化する。まず、静的光散乱 (SLS) 測定を利用して Fmoc-Lys-OH—DMSO—水の 3 成分系相図を求めた。さらに、2 相領域である水の含量が 80 vol% の溶液 (Fmoc-Lys-OH の濃度は 0.0165~0.026 g/cm³) に対して SLS・動的光散乱 (DLS)・小角 X 線散乱 (SAXS) の時間分解測定を行い、この溶液中では Fmoc-Lys-OH の濃度が約 0.6 g/cm³ の濃厚相が相分離して、粒子サイズが 10~500 nm の範囲の球状コロイド粒子として存在していることを見出した。溶液中の濃厚相コロイド粒子の成長は非常に遅いが、Fmoc-Lys-OH 濃度 0.026 g/cm³ の溶液では、水を添加してから 100 min を経過したところから SLS の散乱光強度が時間とともにゆらぎだし、非常に数の少ない巨大散乱粒子が生じていることが示された。同溶液を光学顕微鏡観察したところ、散乱光強度がゆらぎだす時間帯で、ミクロンサイズの微粒子が観察され、水添加より 3 週間ほど経過すると分岐構造を有する光学的異方性粒子が観察された。さらに同溶液を乾燥し走査型電子顕微鏡で観察したところ、他のアミノ酸誘導体で報告されているような樹状あるいは房状の構造体が観察された。以上より、Fmoc-Lys-OH は、DMSO-水混合物中でまず液-液相分離が起こり、生じた濃厚相から高次の秩序性を有する自己集合体が形成されることが示された。また、この自己集合挙動は、同 3 成分系に対する格子理論により再現されることが実証された。 Fmoc-Lys-OH の一つのアミノ基をグアニジノ基で置換された Fmoc-homoarginine についても同様の実験を行った。Fmoc-Lys-OH と同様、DMSO 溶液に水を 40 vol% 以上添加すると、初期には液-液相分離を起こし、長時間後にはミクロンサイズの繊維状自己集合体および球晶の形成を確認した。 以上のように、本論文はアミノ酸誘導体が DMSO-水混合物中で形成する自己集合体の形成過程を、時間分解 SLS・DLS・SAXS により詳細に調べ、液-液相分離後に濃厚相から高次の秩序性を有する自己集合体が形成されることを明らかにした。この論文の内容は新規かつ学術的に重要で、博士 (理学) の学位論文として十分価値あるものと認める。		