ORIGINAL

Solution Properties of Fibrous Chains Constructed of Amphiphilic Molecules

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Gel-like solutions of *N*-acyl-L-aspartic acids (C_nAsp) and *N*-dodecanoyl-β-alanine (C_{12}Ala) were investigated by infrared absorption spectroscopy and optical microscopy. The structures of fibrous assemblies and their construction mechanisms were discussed. Examination was also carried out on the addition of 2-ethylhexylamine (2-EHA) as a stabilizer, and its effects on fibrous assemblies were examined. C_{18}Asp fibers were stabilized over long periods by the addition of 2-EHA. Amide bonds in amino acid surfactants played an important part in the formation of high-order structures such as fibrous assembries, globular particles, and crystals. Globular particles produced at high temperature were transformed into fibrous assemblies at room temperature. Two models for this mechanism were proposed.

1 Introduction

It has been reported that aqueous and organic solutions of some amphiphilic molecules present gel-like character, and fibrous molecular assemblies are formed in the gel-like solutions[1),3)~12)]. Fibrous chains are constructed by the non-covalent self-organization of amphiphilic molecules, and fibers constituted by chiral molecules exhibit helical sense. Helical structures were investigated by optical and electron microscopy[1),3)~12)], atomic force microscopy (AFM)[12)], infrared spectroscopy[11)], circular dichroism[9)], differential scanning calorimetry[7)], nuclear magnetic resonance[8)], X-ray diffraction[2)], small-angle X-ray scattering (SAXS)[10),12)], and small-angle neutron scattering (SANS)[10),12)]. Many investigations of fibrous assemblies were concerned in structures of fibers, and some workers assumed the molecular arrangement in fibrous chains in association with the construction mechanism of fibers.

It was confirmed from phase diagrams that gel-like solutions of chiral *N*-acyl-L-aspartic acids (C_nAsp, n=12, 14, 16, 18) were obtained at medium pH and lower temperature[11)]. The cryo-tramsmission electron microscopic (TEM) observation revealed that helical, fibrous assemblies were formed in the gel-like solutions. SANS measurement proposed more information about molecular arrangement in fibers[12)]. Fibrous assemblies of C_nAsp were not stable, and the solutions precipitated with time. The existence of cylindrical fibers in gel-like solutions of *N*-dodecanoyl-β-alanine (C_{12}Ala) without asymmetric carbon was displayed by cryo-TEM[11)]. Similar textures were observed even on AFM[12)]. Small-angyle X-ray scattering profile presented some Bragg peaks. The distances calculated from the peak positions were related to the molecular arrangement in cylindrical fibers.

In this work, gel-like solutions of C_nAsp and C_{12}Ala are investigated by infrared spectroscopy and optical microscopy to confirm fine structures of fibers and their construction mechanisms. Examination is also carried out in addition of 2-ethylhexylamine (2-EHA) as a stabilizer, and its effect for fibrous assemblies is discussed.

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2 Experimental Section

C_nAsp, C_{12}Ala, and *N*-dodecanoyl-L-glutamic acid (C_{12}Glu) which were supplied by Mitsubishi Petrochemical Co. Ltd., Japan, were same samples previously used[11]. 2-EHA and standardized HCl and NaOH solutions were commercial products. Water was redistilled from alkaline $KMnO_4$ and degassed by the ultrasonication under the reduced pressure.

The pH measurement for aqueous C_{18}Asp solutions with 2-EHA was carried out at 25 ℃ on an Iwaki Glass pH/ion meter A-225 under nitrogen atmosphere. Solutions were prepared as described before[11].

Phase diagrams were drawn as follows : C_{18}Asp in water was mixed with adequate amounts of 2-EHA and standarized NaOH solution, and the mixtures were dissolved at 85℃. After the solutions were maintained for 2 h at the desired temperature in a water bath, the aspect of solutions such as transparent, opalescent, solid, and gel-like was inspected visually and by using an Olympus BH optical microscope. Then the temperature was lowered and the same procedure was repeated.

The specimens for infrared absorption spectrum measurement were prepared on a KRS-5 window : the solution prepared as described above was mounted on the window and solvent was quickly evapolated in air. Infrared spectra were recorded at room temperature by a JASCO JRA-2 diffraction grating infrared spectrophotometer. The measurement was performed for surfactant solutions with and without 2-EHA.

Video enhanced microscopy (VEM) for a gel-like C_{12}Ala solution was observed on Nicon differential interference contrast microscope NTF 2 equipped with a C 2400 CCD video camera and a digital image processing system ARGAS-10 (Hamamatsu Photonics Co. Ltd.)

3 Results and Discussion

3・1 pH Titration Curves and Phase Diagrams of Aqueous C_{18}Asp Solutions with and without 2-Ethylhexylamine

Fig.−1 illustrates the preparation scheme of gel-like solutions. C_nAsp solids were dissolved at 85℃. If transparent solutions are cooled down to lower temperature (room temperature), the solutions precipitate. However, when the solutions are aged at high temperature for more than 2∼3 h, the opalescent solutions are obtained. Those so-

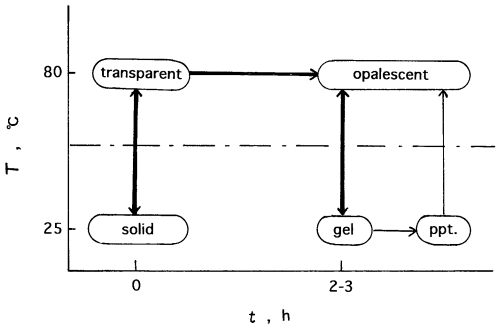
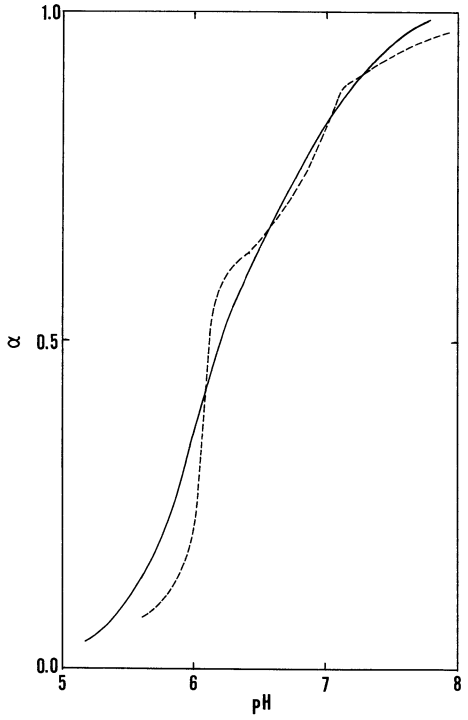


Fig.−1 Schematic representation for the preparation scheme of gel-like C_nAsp solutions.



——, with 2-EHA (mole ratio=1.0) ;
---, without 2-EHA (Ref. 11).

Fig.−2 The degree of ionization α of surfactant as a function of pH for aqueous C_{18}Asp solutions of 0.1 % with and without 2-EHA at 25℃.

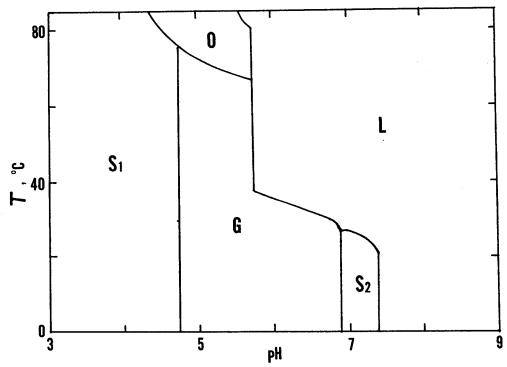
lutions become gel-like at room temperature. Gel-like solutions transform into gels or occasionally precipitate after a long period at room temperature. Gels and precipitates become reversibly opalescent by changing the temperature. The preparation scheme is same even if 2-EHA is added.

Fig.−2 shows a pH titration curve at 25℃, where the degree of ionization α of surfactant is illustrated as a function of pH for aqueous C_{18}Asp solutions at a surfactant concentration of 0.1 % (10^{-3} g·cm^{-3}) with 2-EHA (mole ratio=[2-EHA]/[surfactant] =1.0). The degree of ionization was calculated by

$$\alpha = \{3-(C_{H^+,\,total}-C_{H^+,\,free})/C\}/2 \quad (1)$$

where $C_{H^+,\,total}$ and $C_{H^+,\,free}$ are the total and free molar amounts of hydrogen ions, respectively. C is the molar amount of surfactant and equal to that of 2-EHA. It was supposed that the degree of ionization of 2-EHA was unity at pH below 8. The degree of ionization of surfactant changes sharply at pH=5.5∼7 as well as that for solutions without 2-EHA which was already reported[11] and included in **Fig.**−2. While the titration curve of solutions without 2-EHA present two inflections, that of solutions with 2-EHA changes rather smooth. It is suggested that the phase transitions resulting from the hydrolysis of carboxylates are less distiguishable for a C_{18}Asp system with 2-EHA than for that without 2-EHA.

Fig.−3 illustrates the temperature-pH phase diagram for aqueous C_{18}Asp solutions with 2-EHA (mole ratio=0.67) at a total concentration of 1 %. Five regions are distinguished. At high temperature, solutions are transparent at alkaline pH and opalescent at acid pH. In opalescent solutions, spherical particles are observed on an optical microscope. At lower temperature, there is a viscous gel-like region between two solid regions at acid and alkaline pH. It is apparent in comparison with the pH titration curve that the gel-like solutions are obtained at the transition region between carboxyl and carboxylate species. Similar temperature-pH phase diagram was already obtained for aqueous C_{18}Asp solutions without 2-EHA[11]. By adding 2-EHA, the gel-like and solid regions shift to lower pH, and the opalescent region moves to higher temperature.

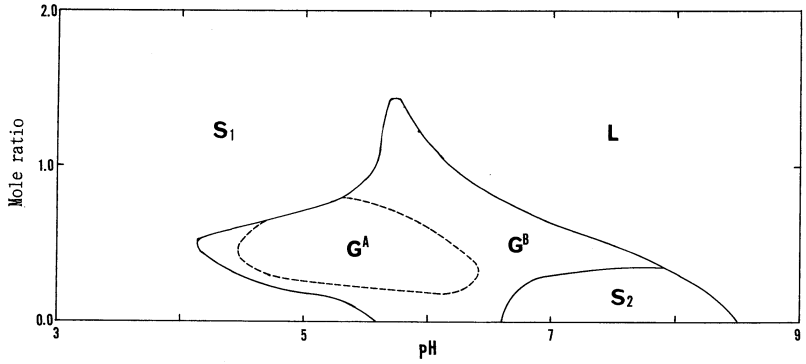


Total concentration of C_{18}Asp and 2-EHA is 1 %. Notation : L, transparent ; O, opalescent ; S_1, S_2, solid ; G, gel-like.

Fig.−3 The temperature-pH phase diagram for aqueous C_{18}Asp solutions with 2-EHA (mole ratio=0.67).

The mole ratio-pH phase diagram for aqueous C_{18}Asp solutions of 1 % total concentration with 2-EHA at 25℃ is shown in **Fig.**−4. The solid region at alkaline pH disappears at higher mole ratio than 0.35. The pH region of gel-like solutions increases with mole ratio up to 0.5, but the region disappears above mole ratio 1.45. At higher mole ratio, there is the solid region at acid pH and the transparent region at alkaline pH. The gel-like region can be separated into two regions G^A and G^B. The G^A region at mole ratio 0.2∼0.8 and pH 4.5∼6.4 is more stable than the G^B region. Gel-like solutions in the G^A region do not precipitate for more than several months. This means that 2-EHA acts to stabilize the gel-like state of C_nAsp solutions.

Fibrous assemblies are formed in gel-like C_nAsp solutions[11),12)], where carboxyl and carboxylate groups coexist, as suggested from pH titration. Then the hydrogen bonding between COO- and COOH occurs between fibers, and fibers partly make lateral arrangement, resulting in the formation of their network aggregation. C_nAsp molecules in gel-like assemblies rearrange to form thermodynamically stable crystals, and pre-



Notation : L, transparent ; S_1, S_2, solid ; G^A, G^B, gel-like. G^A region is more stable than G^B region.

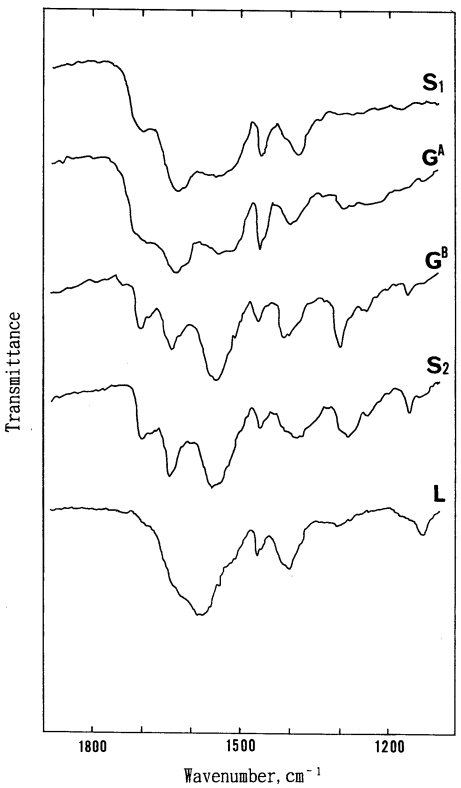
Fig.-4 The mole ratio-pH phase diagram for aqueous C_{18}Asp solutions of 1 % total concentration with 2-EHA at 25℃.

cipitates occur with time. 2-EHA ionized at pH below 8 makes ion-pair with carboxylate ions. Such ion-pairing disturbs the hydrogen bonding between fibers and the crystal arrangement of C_nAsp molecules. Then the fibers are stabilized. Bulky 2-EHA can be bound on assembly surfaces but not intercalated into assemblies. Such molecular character is valuable as a stabilizer of fibers.

3·2 Infrared Absorption Spectra of Aqueous Surfactant Solutions with and without 2-Ethylhexylamine

Infrared spectra for the dried specimens prepared from C_{18}Asp solutions of 1 % total concentration wth 2-EHA in different regions at 25℃ are given in **Fig.-5**. The wavelengths of main absorption bands and their assignments are listed in **Table-1**. The 3,265~3,300 cm^{-1} band which is observed at all regions is assigned to amide A (hydrogen bonded NH str.). The strong amide I (1,626~1,644 cm^{-1}) and II (1,534~1,554 cm^{-1}) bands are observed at the regions except the transparent region, where a strong COO- antisym. str. band overlaps on the amide I and II bands, since pH of the solution is 7.99. It is suggested that the amide groups are hydrogen-bonded and take some ordered arrangement in C_nAsp assemblies.

1,692~1,706, 1,560~1,583, and ~1,415 cm^{-1} bands are assignable to C=O (COOH) str., COO- antisym. str., and COO- sym. str., respectively. While the COO- antisym. and sym. str. bands are observed at higher pH,



Notations in figure have same meanings as those in **Fig.-4**.

Fig.-5 Infrared absorption spectra for aqueous C_{18}Asp solutions of 1 % total concentration with 2-EHA.

the C=O str. band is dominant at lower pH. This suggests that the content of carboxyl and carboxylate species depends on the pH of C_{18}Asp solutions. It should be noticed

Table-1 Infrared absorption bands[a] and their assignments for aqueous surfactant solutions of 1 % total concentration at 25℃.

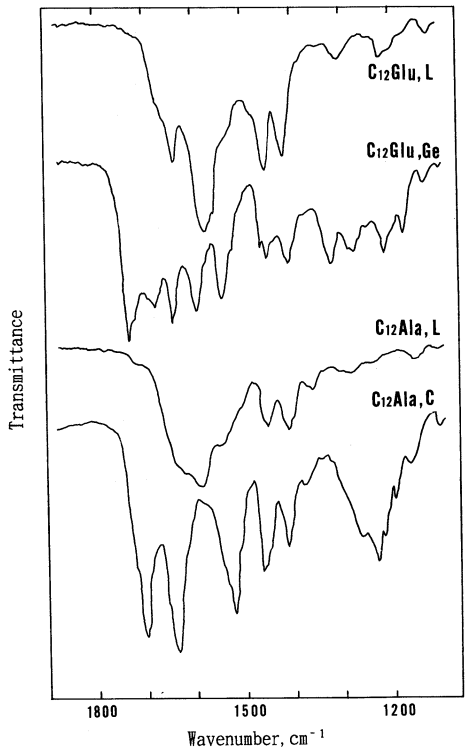
Surfactant	Region[b]	Mole ratio	pH	Amide A	Amide I	Amide II	C=Ostr.	COO- antisym. str.
C_{12}Glu	L		6.80	3,350 s	1,643 m	~1,530 sh	–	1,582 s
C_{12}Ala	L		6.86	3,300 m	1,626 sh	1,545 sh	–	1,592 s
C_{12}Asp	L		10.67	3,299 m	~1,640 sh	~1,520 sh	–	1,578 s
C_{18}Asp/2-EHA	L	0.50	7.99	3,280 m	1,639 sh	sh	–	1,583 s
C_{12}Asp	O		3.41	3,278 m	1,649 s	1,526 m	1,699 s	–
C_{12}Glu	Ge		4.77	3,316 m			1,738 m, 1,684 m	–
C_{12}Ala	C		6.16	3,317 s	1,644 s	1,531 s	1,707 s	–
C_{16}Asp[c]	G		5.82	3,308 s	1,645 s	1,530– 1,550 s	1,710– 1,700 m	~1,570 sh
C_{18}Asp	G		6.11	3,298 m	1,637 s	1,540 m	1,705 m	–
C_{18}Asp/2-EHA	G^A	0.50	5.16	3,265 m	1,635 s	1,534 m	1,692 m	–
C_{18}Asp/2-EHA	G^B	0.63	6.95	3,300 m	1,641 m	1,554 s	1,706 m	1,571 s
C_{18}Asp	S_1		3.70	3,278 m	1,652 s	1,528 m	1,693 s	–
C_{18}Asp/2-EHA	S_1	1.02	5.53		1,626 s	1,536 m	1,704 m	–
C_{18}Asp	S_2		8.42	3,285 s	1,638 s	–	1,695 m	~1,560 s
C_{18}Asp/2-EHA	S_2	0.10	7.73	3,300 m	1,644 m	1,542 s	1,703 m	1,562 s
2-EHA				3,300 m	–	–	–	–

a) s, strong ; m, medium ; sh, shoulder.
b) L, transparent ; O, opalescent ; Ge, gel ; C, fiber ; G^A, G^B, gel-like ; S_1, S_2, solid.
c) Ref. 11).

that the gel-like solutions are in the coexistent region of the carboxyl and carboxylate species. This is consistent with the result from the pH titration.

Infrared spectrum measurement was also carried out for C_{18}Asp, C_{12}Ala, and C_{12}Glu solutions (1 %) without 2-EHA in different regions at 25℃, as illustrated in **Fig.-6**. The wavelengths of main absorption bands and their assignments are listed in **Table-1**, where the corresponding data for aqueous C_{16}Asp solution[11] are also included. The phase diagrams and the characteristics for those solutions were previously reported[11]. At 25℃, a C_{12}Asp solution at pH 3.3~4.7 is opalescent, while a C_{16}Asp solution is gel-like at pH 4.8~5.8 and C_{12}Ala and C_{12}Glu solutions are gel-like at acid pH. Aqueous surfactant solutions are all transparent at alkaline pH.

Aqueous surfactant solutions with and without 2-EHA displayed similar spectra and their similar pH dependence, except for a C_{12}Glu gel solutions. Amide I and II bands are at 1,626~1,652 and 1,526~1,554



Notation : L, transparent ; Ge, gel ; C, fiber.
Fig.-6 Infrared absorption spectra for aqueous C_{12}Glu and C_{12}Ala solutions of 1 %.

cm^{-1}, respectively. It may be noted that the wavelengths of amide bands are between those of the characteristic absorption bands for α-helix and β-structure of polypeptide. Therefore, the ordered conformations in molecular assemblies of amino acid surfactants can not be specified from infrared spectra.

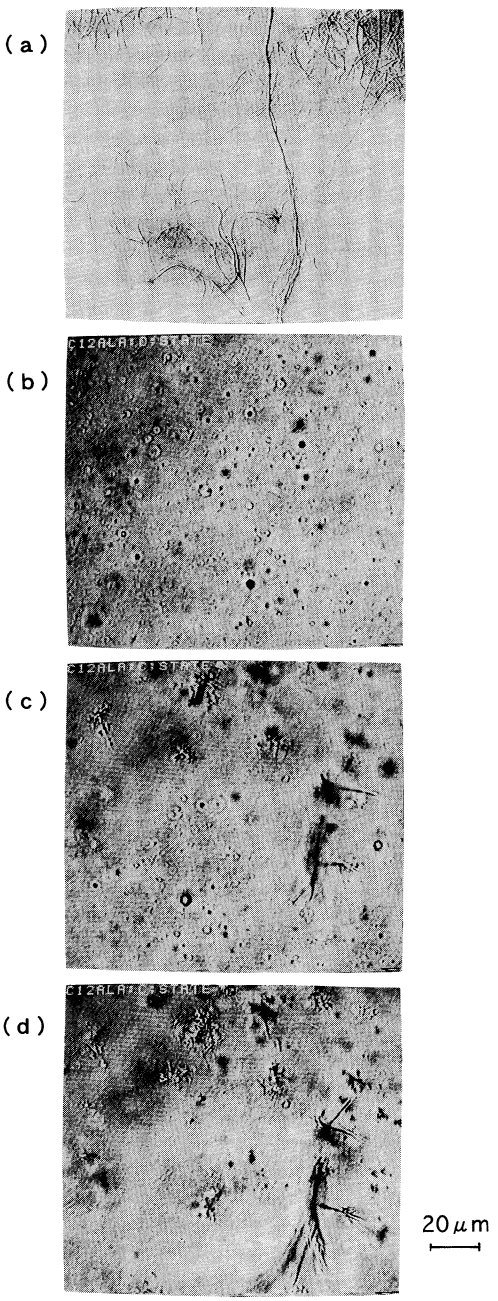
It has been confirmed that fibrous assemblies in gel-like C_nAsp and C_{12}Ala solutions and vesicular assemblies in opalescent surfactant solutions are formed with the structure of ordered molecular arrangement[11),12)]. Such ordered structure is kept even in solid state, even if it is rearranged from the original one. The amide bands in transparent solutions are usually not separated from the strong COO- antisym. str. band, but the amide I band (1,643 cm^{-1}) observed for the transparent C_{12}Glu solution supports the formation of the ordered structure, which may be assumed to be small vesicles or micelles.

The infrared absortion spectrum of a C_{12}Glu gel solution displays no characteristic amide bands, which are assigned for polypeptide, indicating that there is no ordered structure in the gel solution. Instead of it, many absorption bands are observed. Those bands can be assigned to vibrational modes for isolated amino acid surfactants. Molecules in gel aggregate into oil droplets in disordered arrangement[11)]. The infrared absorption result is consistent with such structure.

3·3 Video Enhanced Microscopic Observation of a Gel-like C_{12}Ala Solution

When C_{12}Ala molecules in aqueous acidic medium are incubated for more than 2 h at high temperature, the transparent solutions change to be opalescent[11)]. If the solutions are cooled down to room temperature, they transform into the gel-like solutions.

VEM observation was carried out for a gel-like C_{12}Ala solution of 1 % at pH 6.16. Bundles of fibrous assemblies are observed. When the VEM photograph is compared with that for 1/10 dilution of a 1 % solution, fibers are dispersed from bundles, as seen in **Fig.–7 (a)**. Similar texture of bundles and dispersed fibers was already observed by the electron microscopy[11)] and the atomic force microscopy[12)].



(a) at room temperature (before heating) ; (b) 90 s at room temperature after heated ; (c) 145 s ; (d) 180 s.

Fig.–7 VEM photographs of molecular assemblies in 1/10 dilution of a 1 % C_{12}Ala solution (pH=6.16).

When the 1/10 dilution is heated at high temperature, fibers disappear and globular particles are formed in the opalescent solution. The heated solution was kept at room temperature and observed at VEM. While globular particles are maintained even at 90 s [**Fig.**–7 **(b)**], short fibers are partly formed after 140 s. Fibers grow longer [**Fig.**–7 **(c)**], globular particles disappear, and all changes finish at 180 s [**Fig.**–7 **(d)**]. These observation suggests that globular particles transform into rod shape. The suggestion of this process was also obtained from electron microscopic observation[11]. Tubular assemblies were formed in vesicular C_{12}Asp solutions, and vesicles contacted with tubes.

3·4 Formation Process of Fibrous Assemblies

It is apparent from VEM observation that globular C_{12}Ala particles are formed at high temperature and they transform into fibrous assemblies at low temperature. Two kinds of processes are possible for the transition from globular assemblies to tubes. One mechanism is the aggregation of globular particles (process Ⅱ). Globular particles linearly aggregate by the interparticle interaction and rearrange to fibers. Another kind of possibility is the transition of structure from globular assemblies to tubes (process Ⅰ). Schematic representation of possible mechanisms is illustrated in **Fig.**–8.

The necessary of preceding construction of vesicles was also recognized in the formation process of fibrous C_nAsp assemblies[11]. Vesicles are formed at high temperature, and fibers are transformed from vesicles when the temperature is lowered. The incubation time is needed for the formaion of fibers because of the rearrangement of ordered molecules. The assembly formation concerned with the incubation time was reported for helical fibers of dimyristoyl-5'-phosphatidyldeoxycitidine[9]. Phospholipid vesicles, which were prepared by ultrasonication treatment, converted into fibers after the dispersion was incubated for 10 h at 25 ℃. When globular C_{12}Ala particles are vesicles, the formation process of fibrous C_{12}Ala assemblies may be resemble to that of C_nAsp fibers.

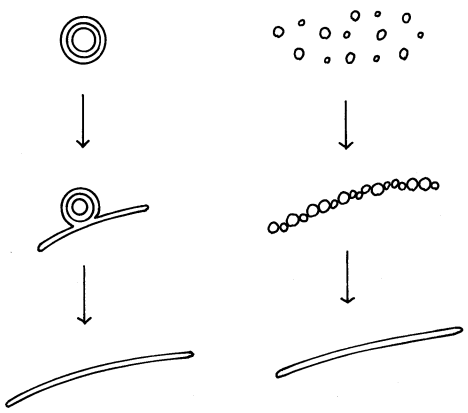


Fig.–8 Schematic models for the formation process of fibrous C_{12}Ala assemblies from vesicles.

Possible models of C_nAsp and C_{12}Ala fibers were estimated with the aid of the electron and atomic force microscopy and the small-angle neutron and X-ray scattering[12]. It was concluded that C_nAsp fibers can be the double strand or the super helical strand of helical bilayer strands. Such structure does not necessarily need vesicle as a precursor. However, the electron micrograph explained the existence of fibers contacted with vesicles[11]. Vesicles were far larger than the diameter of fibers. This may support the mechanism where fibers are formed by the transformation or the protuberance of vesicles. It was revealed that nonhelical C_{12}Ala fibers took cylindrical structure with uniform diameter which was consisted of multilamellar layers with common, constant width[12]. Such structure suggests the role of multilamellar vesicles as a precursor. The formation of fibrous assemblies from vesicular bilayers may be a model for the formation of projected organizations from native membranes.

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両親媒性分子によって構築された繊維鎖の溶液特性

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N-アシル-L-アスパラギン酸 (C_nAsp), *N*-ドデカノイル-*β*-アラニン (C_{12}Ala) のゲル状溶液が赤外吸収分光と光学顕微鏡によって研究された。繊維状集合体とその構築機構が議論された。安定化剤として 2-エチルヘキシルアミン (2-EHA) を添加した系も調べ，繊維状集合体に対するその効果を議論した。2-EHA を添加すると，C_{18}Asp 繊維は長期にわたって安定であった。アミノ酸界面活性剤中のアミド結合は繊維状集合体，球状粒子，結晶のような高次構造の形成に重要な役割をはたした。高温で構築された球状粒子は室温で繊維状集合体に転移する。この機構に対する 2 種のモデルが提出された。