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Self-Assembly and Hydrogelation of Peptide Amphiphiles

Irwansyah[*)], Riwandi Sihombing, and Wahyudi Priyono Suwarso

Department of Chemistry, Faculty of Mathematics and Natural Sciences,
Universitas Indonesia, Depok 16424, Indonesia

[*)]*E-mail: irwansyahchem@yahoo.com*

Abstract

Seven peptide amphiphiles were successfully synthesized using solid phase peptide synthesis method. Peptide amphiphiles were characterized using Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS). Atomic force microscopy (AFM) study showed that peptide amphiphiles having glycine, valine, or proline as linker, self-assembled into 100-200 nm nanofibers structure. According to our research, both peptide amphiphile with positive and negative charges bear similar self-assembly properties. Peptide amphiphile also showed its capability as low molecular weight gelator (LMWG). Peptide amphiphiles bearing C-16 and C-12 as alkyl showed better hydrogelation properties than C-8 alkyl. Five out of seven peptide amphiphiles have minimum gelation concentration (MGC) lower than 1% (w/v).

Abstrak

Self-Assembly dan Hidrogelasi Peptida Ampifil. Tujuh peptida ampifil berhasil disintesis dengan metode sintesis peptida fasa padat. Karakterisasi peptida ampifil dilakukan dengan *matrix assisted laser desorption/ionization* Time-of-Flight Mass Spectrometer (MALDI-TOF MS). Studi dengan *atomic force microscopy* (AFM) menunjukkan bahwa peptida ampifil dengan *linker* glisin, valin dan prolin melakukan *self-assembly* dalam pelarut air membentuk struktur nanofiber berukuran 100-200 nm. Berdasarkan penelitian, peptida ampifil yang bermuatan positif atau negatif memiliki kemampuan *self-assembly* yang sama. Uji pembentukan hidrogel memperlihatkan peptida ampifil memiliki kemampuan sebagai material *low molecular weight gelator* (LMWG). Peptida ampifil yang memiliki alkil C-12 dan C-16 memiliki kemampuan hidrogelasi yang lebih baik dibandingkan C-8. Lima dari tujuh peptida ampifil memiliki nilai *minimum gelation concentration* (MGC) kurang dari 1% (w/v).

Keywords: low molecular weight gelator, MALDI, peptide amphiphile, self-assembly, solid phase peptide synthesis

1. Introduction

Molecular self-assembly is a very useful approach in the fabrication of supramolecular architectures [1]. Although mediated by weak non-covalent bonds such as hydrogen bonding, ionic bonds (electrostatic interactions), hidrophobic interaction, or van der Waals interactions, self-assembly structures are able to build a very stable conformation and affect its interaction with other molecules. The challenge in molecular self-assembly is to design a building block that is able to experience spontaneous organization to form well-ordered and stable macroscopic structure [2].

One molecule of potential builders in the self-assembly is amino acid. Amino acids are the building blocks in peptides and proteins. With the advances in peptide synthesis process, scientists can easily synthesize a wide range of peptides that have differences in amino acid composition and sequence to mimic the natural process. With the availability of the twenty naturally occurring amino acids, chemists can easily design a wide variety of molecules and study the nature of its self-assembly. Self-assembly with biomolecules also have a number of potential applications such as tissue repair, especially with the ability of amphiphilic molecules to form hydrogels, drug delivery, and surface modification by biological molecules [3-7].

The capability of self-assembly of a molecule can be used as a gelling technique (hydrogel or organogel). Gels resulting from the self-assembly of low molar-mass building molecules are known as physical gels or supramolecular gels. In contrast to the resulting gels of

macromolecules or chemical gels (chemically-crosslinked gel), supramolecular gels are thermoreversible, which changes from a gel into solution can be controlled by changing the temperature [8]. The gel can be converted into a solution with increasing temperature, whereas the solution can be converted into a gel by lowering the temperature.

In this study, the designed building blocks were peptide amphiphiles composed of amino and fatty acids. A number of amphiphilic molecular designs are proven to perform self-assembly to form nanotube and nanovesicle structures [3-4,9]. Peptide synthesis was done by using fmoc solid phase peptide synthesis, a peptide synthesis method that is effective, fast and simple [10]. The capability of peptide amphiphile molecules to perform self assembly in water was then explored as low molecular weight gelators (hydrogelator).

2. Experiment

The study consisted of two main stages, peptide amphiphile synthesis and self-assembly study. Ampifil peptide synthesis was performed by solid phase peptide synthesis method. Characterization of the synthesized products was done by using Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS), while the self-assembly was observed with Atomic Force Microscopy (AFM).

Chemicals. Chemicals used in peptide amphiphile synthesis was α-N-Fmoc-glycine (Fmoc-Gly-OH), N-α-Fmoc-L-valine (Fmoc-Val-OH), N-α-Fmoc-L-proline (Fmoc-Pro-OH), N-α-Fmoc-L-phenylalanine (Fmoc-Phe-OH), N-α-Fmoc-L-α-asparat acid t-butyl ester (Fmoc-Asp-OtBu), Fmoc-Asp (OtBu)-Wang resin (resin loading 0.7 mmol/g), Fmoc-Lys (Boc)-OH, Fmoc-Lys (Boc)-Wang resin (resin loading 0.7 mmol/g), N, N-Diisopropiletilamin (DIPEA), O-Benzotriazol-N,N,N',N'-tetrametil-uronium-heksafloro-phosphate (HBTU), piperidine, trifloroacetic acid (TFA), Triisopropilsilane (TIS), N-Methyl-2-pyrrolidone (NMP), Dichloromethane (DCM), octanoic acid, dodecanoic acid, palmitic acid, Si wafers and NaOH. All amino acids were dissolved in NMP with a concentration of 0.2 molar. HBTU activator solution was made in 0.4 molar concentration in NMP.

Synthesis of peptide amphiphile. Peptide amphiphile synthesis was performed in a 200 μmol scale. There were 7 synthesized peptide amphiphiles, namely C16-GGGGDD-COOH (PA1), C12-GGGGDD-COOH (PA2), C8-GGGGDD-COOH (PA3), C16-VVVVDD-COOH (PA4), C16-FFFFDD-COOH (PA5), C16-PPPPDD-COOH (PA6) and C16-GGGGK-COOH (PA7). Peptide amphiphile structures are shown in Figure 1.

Peptide amphiphiles 1 to 6 were synthesized by using Fmoc-Asp (OtBu)-Wang resin as starting materials,

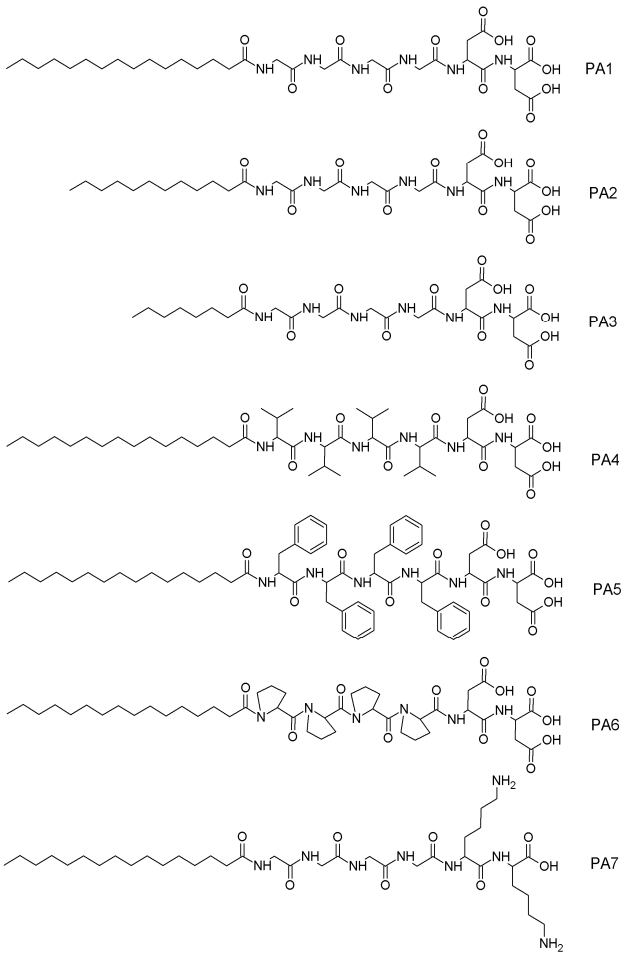

Figure 1. Structures of Peptide Amphiphile 1 (PA1) to Peptide Amphiphile 7 (PA7)

while peptide amphiphile 7 using Fmoc-Lys (Boc)-Wang resin. In general, peptide amphiphile synthesis procedure involves several stages, which were resin preparation, Fmoc group deprotection, coupling of amino acids, capping with fatty acid chain, and the termination reaction of the resin (cleavage). Figure 2 shows schematically the peptide amphiphile 1 synthesis.

Preparation of resin. A 0.285 g Fmoc-Asp(OtBu)-Wang resin was introduced into the synthesis reactor (Disposable PP reaction vessels (syringes with a frit)). A number of 3 mL of NMP was added in the reactor and then shaken for 2 hours. All peptide amphiphile synthesis used Fmoc-Asp(OtBu)-Wang resin except for peptide amphiphile 7 utilized Fmoc-Lys(Boc)-Wang resin.

Deprotection of fmoc group. A total of 4 mL of deprotection solution was added to the synthesis reactor and shaken for 5 minutes. All solution is then removed from the reactor and the resin was washed with 2 x 3 ml of NMP.

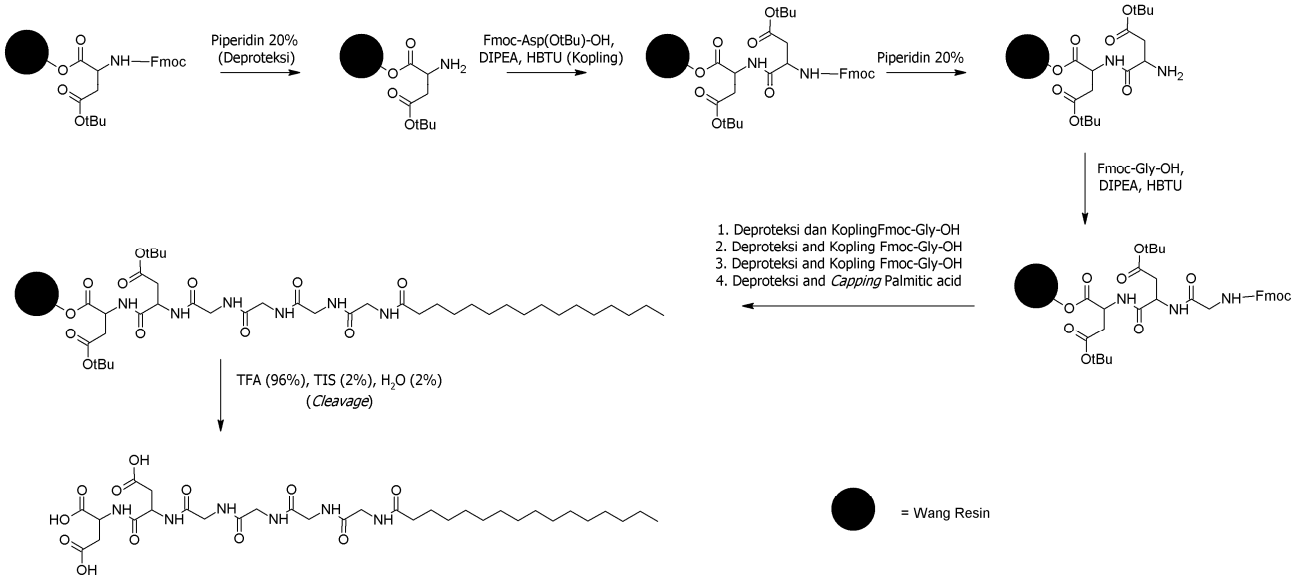

Figure 2. Synthetic Scheme of Peptide Amphiphile 1 (PA1)

Coupling reactions. 4 ml of Fmoc-amino acid 0.2 M, 2ml of DIPEA 1.6 M and 2 ml of HBTU 0.4 M were introduced into the synthesis reactor and shaken for 30 minutes. After the reaction complete, the solution was removed leaving only resin. The resin was then washed with 2 x 3 ml of NMP. The reaction was repeated by adding 4 mL of Fmoc-amino acid 0.2 M, 2 ml of 1.6 M DIPEA and 2 ml of 0.4 M HBTU into the reactor followed by shaking for 30 minutes and washing the resin with 2 x 3 ml of NMP. The reaction was continued with deprotection of fmoc group and coupling of the adjacent amino acid until the intended amino acid sequences were achieved.

Capping reaction. 4 ml of palmitic acid 0.2 M, 2 ml of DIPEA 1.6 M, and 2 ml of HBTU 0.4 M were added to the reactor and shaken for 30 minutes. After the reaction complete, the solution was removed. The resin was washed with 2 x 3 ml of NMP. The reaction was repeated once more. After the resin was washed with NMP, the resin was washed with DCM and dried in a freeze-drier.

Cleavage reaction from the resin. The reacted resin was transferred into centrifuge tube. A total of 5 ml solution of TFA:water:TIS = 96:2:2 (v/v) was added to the centrifuge tube and shaken for 2 hours. The mixture was filtered and the filtrate was collected and added with 10 ml of cold ether. The filtrate is then centrifuged for 5 minutes at 2000 rpm. The precipitate was separated and as much as 15 ml of water was added thereto and stored at -20 °C. The crystal obtained was lyophilized for 2 days. The peptide amphiphile sample were then characterized by MALDI-TOFMS.

Self-assembly of peptide amphiphiles. Peptide amphiphile stock solution was prepared by dissolving 1 mg of peptide amphiphile in 500 µl of water, addition of a few µl of 0.1 M NaOH was performed until the peptide solubilized perfectly. An aliquot of 50 µl was diluted with water until the volume reached 1 ml. A total of 1 µl of this solution was dripped on the surface of the Si wafer (1 cm x 1 cm) that had previously cleaned with plasma and allowed to dry. Topography of Si wafer was then observed by atomic force microscopy (AFM).

Determination of minimum gelation concentration (MGC). A certain amount (mg) of peptide amphiphile was dispersed in 200 µL of water in 2 ml vial resulting in a concentration of 0.2 : 0.4 : 0.6 and 0.8% (w (mg)/v (ml)). The mixture was heated with a heater gun and then cooled to room temperature. Gel formation was done by reversing the vial. Gel resulted in the vial was then added with 50 µl of water followed by heating and cooling processes. Addition of 50 µl of water was continued until it produced a viscous liquid.

3. Results and Discussion

Figure 3 shows a MALDI-TOF MS spectrum of peptide amphiphile 1 with m/z value of 714.80. Signals at 741.31, 763.67, and 786.05, respectively, correspond to $[M+Na]^+$, $[M-H+2Na]^+$ and $[M-2H+3Na]^+$. Peptide amphiphiles have carboxylic groups easily ionize and subsequently react with Na^+ ion present in solution to form salt. Replacement of one H^+ ion will result in an anion with charge value minus one (-1) and react with one Na^+ ion to produce a neutral species. This neutral

species, then undergoes protonation by protons from the matrix producing positively charged ion. Thus, the signal $[M+Na]^+$ is actually derived from the $[M-H+Na+H]^+$. A similar process occurs when there is replacement of two protons by two Na^+ ions and subsequent protonation by proton from the matrix so that the signal $[M-H+2Na]^+$ is actually derived from the $[M-2H+Na+H]^+$. Because the peptide amphiphile 1 has three carboxylic groups, then, there are three protons that can be replaced by Na^+ ion. Signal $[M-2H+3Na]^+$ is actually derived from the $[M-3H+3H+Na]^+$. The three are not a single signal but experienced splitting forming a multiplet signal as a result of the isotope distribution. In the structure of the peptide, atoms are in the isotope form. For example, the 741.31 signal is a multiplet of signals 742.33 and 743.28. Peptide amphiphile 2 to 6 have three carboxylic groups that can undergo ionization and replaced by Na^+ ion, so that in general it will have MALDI-TOF MS spectrum similar to peptide amphiphile 1. Table 1 summarizes the signals of MALDI-TOF MS of peptide amphiphile 1 to 7.

Figure 4 shows a height profile AFM of peptide amphiphiles 1 to 3. It appears that all three peptides self-assemble to form nanofiber. Differences in the alkyl chain length resulted in a very noticeable difference. Peptide amphiphiles 1 and 2 produce unaggregated fibers, while peptide 3 produces aggregated fibers. These three self-assembly structures produce nanofiber that does not have rigid structure or have high flexibility. The three peptide amphiphiles produced a curved fiber due to glycine residue that provides a high flexibility. In the absence of side chains on glycine, no steric obstacles were encountered as in other amino acids. Figure 5 is an height profile AFM of peptide 4 to 7. Unlike peptide 1, peptide 4 produced a more rigid nanofiber. Fiber character changes that occured in peptide amphiphile 4 was due to valine residue replacing glycine.

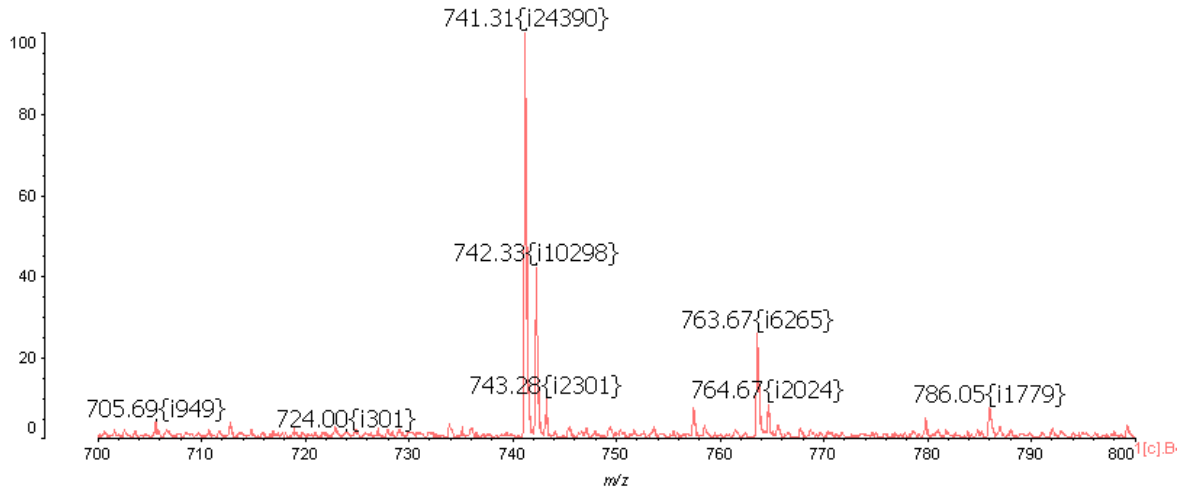

Figure 3. MALDI-TOF MS Spectrum of Peptide Amphiphile 1 (PA1)

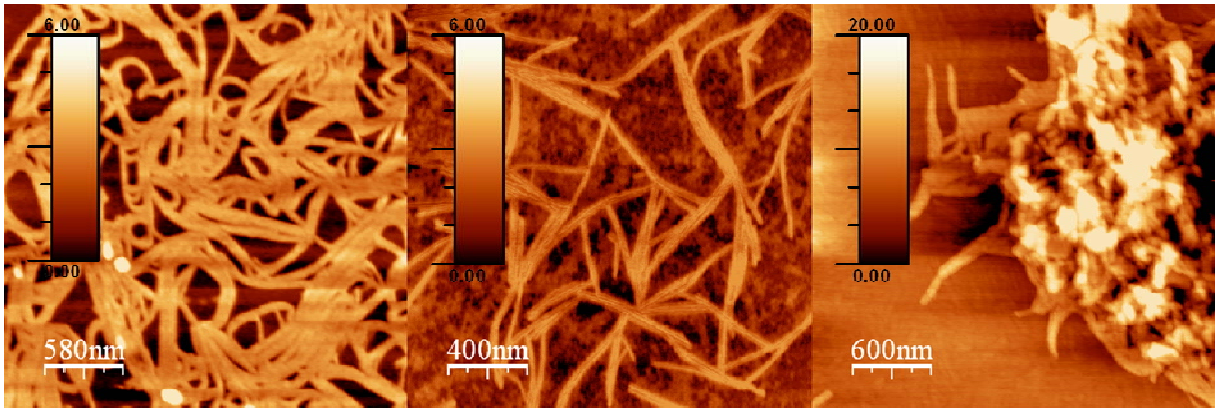

Figure 4. AFM Height Profile of Peptide Amphiphile 1 (Left), Peptide Amphiphile 2 (Middle) and Peptide Amphiphile 3 (Right)

Table 1. Summary of MALDI-TOF MS Signals of Peptide Amphiphile

Peptide amphiphiles	m/z MALDI-Tof	Identity
PA1, m/z=714.80	741.31	$[M+Na]^+$
	763.67	$[M+2Na-H]^+$
	786.05	$[M+3Na-2H]^+$
PA2, m/z=658.70	658.00	$[M+H]^+$
	684.45	$[M+Na]^+$
	706.75	$[M+2Na-H]^+$
	722.95	$[M+3Na-2H]^+$
PA3, m/z=602.59	600.33	$[M+H]^+$
	627.63	$[M+Na]^+$
	649.90	$[M+2Na-H]^+$
	672.19	$[M+3Na-2H]^+$
PA4, m/z=883.12	905.19	$[M+Na]^+$
	927.20	$[M+2Na-H]^+$
	949.25	$[M+3Na-2H]^+$
PA5, m/z=1075.29	1098.79	$[M+Na]^+$
	1121.53	$[M+2Na-H]^+$
	1144.17	$[M+3Na-2H]^+$
PA6, m/z=875.05	897.18	$[M+Na]^+$
	919.18	$[M+2Na-H]^+$
	941.22	$[M+3Na-2H]^+$
PA7, m/z=740.97	746.67	$[M+H]^+$
	767.97	$[M+Na]^+$
	782.21	$[M+K]^+$

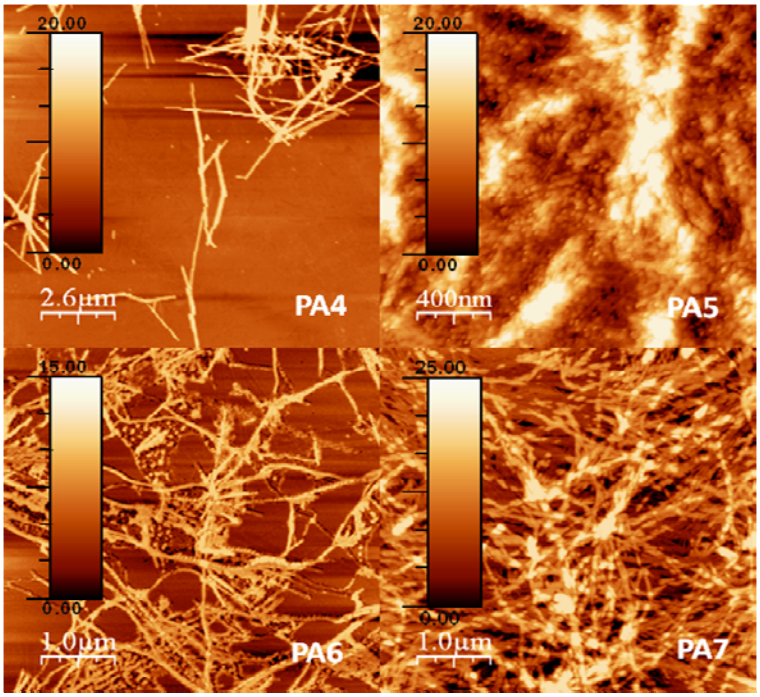

Figure 5. AFM Height Profile of Peptide Amphiphile 4 to 7

Valine residue has a more steric side group than glycine so as the formed structure does not have high flexibility. AFM images of peptide amphiphile 5 shows that there is no formation of fiber structure, which indicates that the peptide failed to perform self assembly. Peptide 5 contained phenylalanine with aromatic ring side-group. The presence of the aromatic ring is thought to inhibit the formation of hydrogen bonds between peptide molecules, so as a consequence the hydrophobic part, in such a way, will distance themselves from the polar solvent. Same as peptide 1 to 4, peptide amphiphiles 6 also form self-assembly nanofiber. Nanofiber produced by self-assembly of peptide 7 have commonality with fiber produced by peptide 1, they have a high flexibility. Although it has a total of positive charge on the hydrophilic part, peptide amphiphile 7 still capable of performing self-assembly. This indicates that the peptide self-assembly can be performed on peptide with positively or negatively charged. This study sought to study the potential of peptides as a low molecular weight gelator (LMWG). To be regarded as an effective LMWG, gelator must have a minimum gelation concentration (MGC) or low concentrations of molecules that can produce a gel in the range 0.2-0.7% (w/v) [11]. Gel derived from LMWG was prepared by heating the gelator in a particular solvent followed by cooling to give a solution supersaturated at room temperature. To find out whether or not the formation of gel in solution took place, physical observations were made by reversing the vial. If the gel formed, then the material will not fall, while if it only produces a viscous fluid, then, when the vial is inverted the liquid will fall soon.

Figure 6 shows an optical image of gel formed by peptide amphiphile 1. Based on Figure 6, the gel is formed when the peptide concentration exceeds 0.2% (w/v). Table 2 summarizes the minimum gelation concentration (MGC) of peptide 1 to 7. Figure 7 illustrates the scheme of the formation of gel derived from LMWG. Based on Figure 7, when the hot supersaturated solution is cooled, the molecules begin to

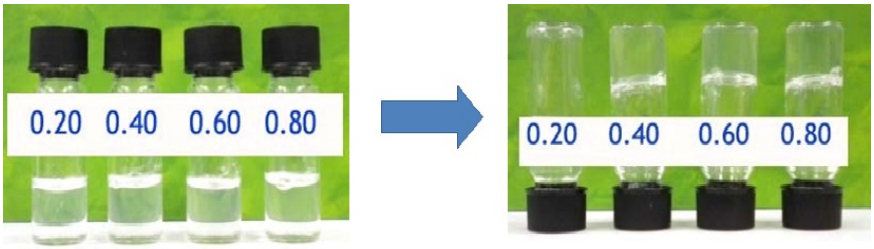

Figure 6. Optical Image of Peptide Amphiphile 1 Hydrogel

Table 2. MGC of Peptide Amphiphile

Peptide amphiphiles	MGC, %(w/v)	Forms
PA1	0.32	Gel
PA2	0.42	Gel
PA3	-	MGC>1%
PA4	0.28	Gel
PA5	-	Non Gelator
PA6	0.36	Gel
PA7	0.35	Gel

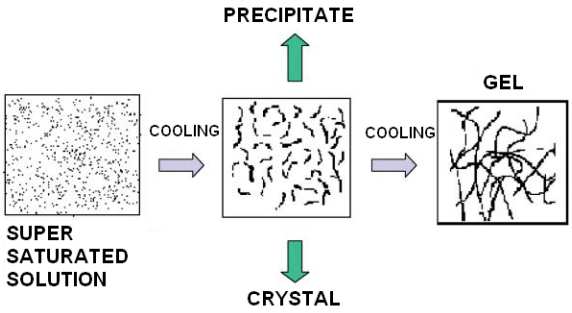

Figure 7. The Scheme of Gel Formation from LWMG [8]

condense and there are three possible situations, namely (1) aggregation with high regularity produces crystal, or better known as crystallization process, (2) random aggregation generates amorphous sediment, or (3) the process of intermediate aggregation to produce a gel. Gel formation process involves self-association of the gelator molecules forming long polymer-like fibrous aggregate intersecting each other during the aggregation process creating a matrix that traps solvent molecules through a process of surface tension. This process prevents the flow of solvent due to the gravitational force and mass that appears as a solid [8].

4. Conclusions

Peptide amphiphiles 1 to 7 were successfully synthesized by solid phase peptide synthesis method. Self-assembly structure and properties of hidrogelator were influenced by the structure of the peptide. Peptide amphiphiles formed nanofiber structure except peptide 5 which contained phenylalanine residue. Peptide amphiphile has potential as a low molecular weight gelator.

References

[1] S. Zhang, Nat. Biotech. 21 (2003) 1171.
[2] S. Zhang, D.M. Marini, W. Hwang, S. Santoso, Curr. Opin. Chem. Biol. 6 (2002) 865.
[3] Y. Yanlian, K. Ulung, W. Xiumei, A. Horii, H. Yokoi, Z. Shuguang, Nano Today 4 (2009) 193.
[4] X. Zhao, Curr. Opin. Colloid Interface Sci. 14 (2009) 340.
[5] L. Ruan, H. Zhang, H. Luo, J. Liu, F. Tang, Y. Shi, X. Zhao, Proceedings of the National Academy of Sciences 106 (2009) 5105.
[6] S. Koutsopoulos, L.D. Unsworth, Y. Nagai, S. Zhang, Proceedings of the National Academy of Sciences 106 (2009) 4623.
[7] Y. Nagai, L.D. Unsworth, S. Koutsopoulos, S. Zhang, J. Control. Release 115 (2006) 18.
[8] N.M. Sangeetha, U. Maitra, Chem. Soc. Rev. 34 (2005) 821.
[9] S. Santoso, W. Hwang, H. Hartman, S. Zhang, Nano Letters 2 (2002) 687.
[10] C.W.C. White, D. Peter, Fmoc Solid Phase Peptide Synthesis: A Practical Approach, W.C. Chan, P.D. White (Eds), New York, Oxford University Press, 2000.
[11] T. Kar, S. Debnath, D. Das, A. Shome, P. Das, Langmuir 25 (2009) 8639.