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Effect of iron (III) hydroxide sol as a support for oligomerization of L-phenylalanine in aqueous solution

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Abstract

An oligomerization of L-phenylalanine in aqueous solution was studied in the presence of iron (III) hydroxide sols. To an aqueous solution of the iron hydroxide sols prepared from iron (III) chloride,L-phenylalanine with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride was added. After stirring for 48 h at 0 °C, 3 M HCl solution was added to the reaction mixture to decompose the colloidal iron hydroxide. Oligo (L-phenylalanine) was isolated by centrifugation and washed with acidic water. The maximum yield of 15% was obtained at around pH 6.3. This value was 2.5 times higher than that obtained without the iron hydroxide sols at the same pH. Adsorption study of L-phenylalanine indicated that assemblies of L-phenylalanine onto the iron hydroxide sols were predominant factor to improve the yield of the oligomerization product. The presence of nitrate anions changes the pattern of the yield of oligo (L-phenylalanine) against pH values. The maximum yield of 20% was obtained at around pH 3. This value was four times higher than that obtained without the iron hydroxide sols at the same pH. Effect of NaCl for the oligomerization of L-phenylalanine in an aqueous solution was also studied.

Keywords: Amino acid; Iron hydroxide sol; Polycondensation

1. Introduction

Polycondensation of amino acids in water phase is growing importance in both scientific interest and industrial requirement for environmental aspect. Peptide chemistry offers activating agents such as carbodiimides to condense amino acids in organic solvents. Water-soluble condensation agents can be used in water [1]. In a homogeneous aqueous solution, however, deactivation via hydrolysis remained the main pathway. To solve this problem, molecular assemblies of amino acids would be required. By using molecular assemblies for chemical reactions, increase in the reaction rate and the conversion by concentration or orientation effects are expected. Polycondensation of long-chain esters of α-amino acids proceeded in the monolayers at the air/water interface and in the LB films [2]. The active β-alanine and glycine esters of *N*-hydroxy-2,3-dihexadecylsuccinimide form multilamellar assemblies in water [3]. Molecular recognition by templates for amino acids in polycondensation was also reported. Successive addition of *N*-carboxyanhydride (NCA) of L-triptophan to liposomes showed that multiple feeding permits the formation of much longer oligo(L-triptophan) compared to the reference system with no liposomes [4]. Orgel et al. reached polymerization degrees up to 55 using glutamic acid by successive 'feedings' with the monomers, in which clay minerals served as supports for the polymerization [5]. Orgel et al. were also able to induce the polymerization of negatively charged NCA-amino acids using positively charged micelles in an aqueous solution [6]. In the case of the clays and the micelles, electrostatic interactions were a prerequisite for efficient oligomerization. These kinds of studies are not only relevant to the

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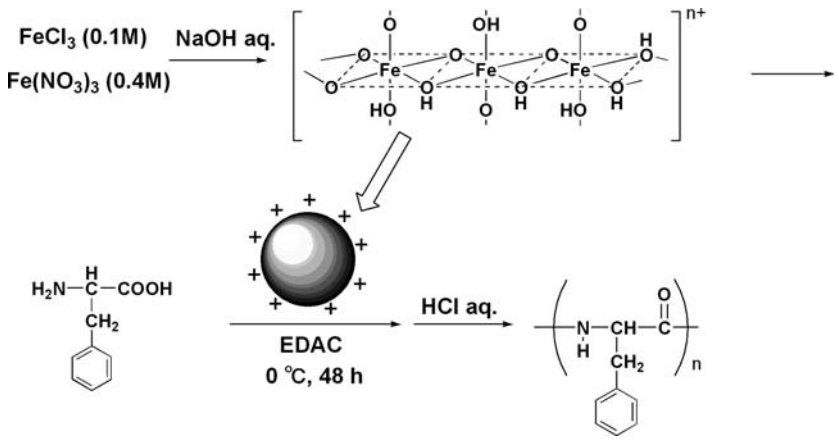

Scheme 1.

macromolecular chemistry of amino acids and to general research on amino acids and peptide interactions with nanosized matrix, but also to the field of prebiotic chemistry [7,8].

Here we report the effect of iron (III) hydroxide sols as supports for the oligomerization of amino acid in aqueous solution (Scheme 1). Although the forms of the iron (III) hydroxide sols and the mechanisms of their transformations in aqueous solutions are still not fully clear, polynuclear hydrolysis products are formed through progressing olation and oxolation reactions [9]. The polynuclear products of iron (III) hydrolysis readily depolymerize upon acidification of solutions to homogeneous solution. When hydrophobic amino acids are polymerized on the iron (III) hydroxide sols, the resulting products would be simply isolated by filtration after acidification. To our knowledge, this is the first example for oligomerization of amino acids with the iron hydroxide sols.

2. Experimental part

2.1. Materials

L-Phenylalanine, iron (III) chloride and iron (III) nitrate nonahydrate were purchased from WAKO Pure Chemistries Ltd. 1-Fluoro-2,4-dinitrobenzene was obtained from nacalai tesque Inc. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) and 8-anilino-1-naphthalensulfonic acid ammonium salt (ANS) were obtained from Tokyo Kasei Inc. All materials were used without further purification.

2.2. Measurements

^{1}H NMR spectra were recorded on a 270 MHz JEOL JNM-EX270 spectrometer. IR spectra were obtained on a Perkin Elmer 2000 spectrometer. Gel permeation chromatographic analysis was carried out on a TOSOH α-3000 column by using DMF as an eluent at 40 °C after calibration with polystyrene standards. UV–vis absorption spectra were obtained on a JASCO V-530 spectrophotometer. Fluorescence spectra were obtained on a Perkin Elmer LS50B luminescence spectrometer.

2.3. Polycondensation of L-phenylalanine

A typical procedure is described as follows. To an aqueous solution (2.0 ml) of 0.1 M iron (III) chloride, 0.1 M NaOH aqueous solution (4.0 ml) was added to hydrolyzed iron hydroxide (adjusted pH 3.0). To an aqueous solution of the resulting iron hydroxide sols, 132 mg (0.8 mmol) of L-phenylalanine with 767 mg (4.0 mmol) of EDAC was added. The solution's pH was adjusted by 0.5 M HCl. After the reaction mixture was stirred for 48 h at 0 °C, 3 M HCl was added to dissolve colloidal iron (III) hydroxide and the resulting precipitate was obtained by centrifugation, followed by reprecipitation into acidic water to give a white powder. IR (KBr) 1520, 1652 cm^{-1}. ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.68, 2.84 (2H, CH_2), δ 4.40–4.60 (bs, 1H, CH), δ 7.00–7.23 (bs, 5H, C_6H_5) δ 7.90 (s, 1H, NH).

In the control experiments, 6.0 ml of distilled water was added instead of iron (III) chloride. Iron (III) hydroxide sols were also prepared from 0.4 M iron (III) nitrate instead of 0.1 M iron (III) chloride.

2.4. Molecular weight determination

The dinitrophenyl method was carried out to determine the number of the amino end group in L-phenylalanine oligomer. *N*-dinitrophenyl terminated L-phenylalanine oligomer was prepared according to the reported method [10]. After sufficiently washed with water and ether, the polymer was dissolved in DMF. Absorption of the solution was determined at 360 nm. The number of the amino end group and the degree of polycondensation was determined by the calibration curve of *N*-2,4-dinitrophenyl-L-phenylalanine and by ^{1}H NMR (DMSO-d_6) of the polymer.

2.5. Adsorption experiment

The amount of L-phenylalanine adsorbed on the iron (III) hydroxide was determined by UV–vis measurement. To an aqueous solution (2.0 ml) of 0.1 M iron (III) chloride, 0.1 M NaOH aqueous solution (4.0 ml) was added to hydrolyzed iron hydroxide (adjusted pH 3.0). To an aqueous solution of the resulting iron hydroxide sols, 132 mg (0.8 mmol) of L-phenylalanine was added and the solution's pH was adjusted by 0.5 M HCl or 0.5 M NaOH. After stirring for 24 h at room temperature, the mixture was filtered through ultra-filtration membrane (ADVANTEC USY-5, M.W. Cut-off 50000) to remove the iron (III) hydroxide sols and the adsorbed L-phenylalanine. Absorption of the solution was determined at 257 nm and the content of L-phenylalanine in the filtrate was calculated. The adsorption ratio was determined by the equation

$$\text{Adsorption ratio } (\%) = (W_0 - W_f)/W_0 \times 100$$

where W_0 and W_f are weight of L-phenylalanine at the initial and in the filtrate, respectively.

3. Results and discussion

3.1. Polymerization of L-phenylalanine in water

Oligomerization of L-phenylalanine was tested in the absence of the iron hydroxide sols as a control experiment. To an aqueous solution of L-phenylalanine, a water-soluble carbodiimide (EDAC) was added. Solution's pH was adjusted to 2.1–7.8 by 0.5 M HCl. Concentration of L-phenylalanine was 0.13 M. After stirring for 48 h at 0 °C, an insoluble matter was filtered and washed with water. The yields of the precipitates were around 6% within the experimental solution's pH. According to the ^{1}H NMR and the IR analysis, the obtained precipitates were condensation products of L-phenylalanine. The molecular weights of the products were estimated by GPC after calibration with standard polystyrenes. The molecular weights of the all obtained products were independent within the experimental solution's pH. The number–average molecular weight and the weight–average molecular weight were 550 and 1080, respectively. The dinitrophenyl method [9] was carried out to determine the absolute degree of polymerization and found the number–average degree of polymerization (DP) as 11.6 ($M_n = 1500$).

The aggregation behavior of L-phenylalanine in an aqueous solution might promote the oligmerization. The fluorescence probe method was used to estimate the aggregate formation of L-phenylalanine in an aqueous solution at different pH. ANS in the absence of L-phenylalanine showed an emission maximum of 515 nm. When ANS was added to an aqueous solution of 0.13 M L-phenylalanine with the solution's pH of 5.2–2.3, a new emission maximum at 475 nm was observed in addition to the peak at 515 nm. Fig. 1 shows the fluorescence intensity of ANS at 475 nm against the concentration of Lphenylalanine in an aqueous solution at pH 4.0. A critical point is observed at 0.11 M of L-phenylalanine. Linear relationship between the intensity and the concentration of L-phenylalanine was observed above and below this critical point and the slope above 0.11 M was larger than that below the critical concentration. These results indicate that stronger interaction between L-phenylalanine and ANS existed above the critical point. Although the intensity at 475 nm in the presence of L-phenylalanine above the critical point was not drastically increased as in the usual micelle cases [11], these results indicate the aggregate formation of L-phenylalanine under the above polymerization condition.

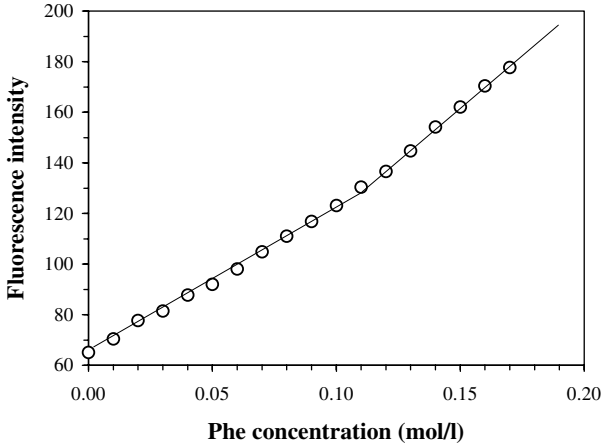

Fig. 1. Fluorescence intensity of ANS at 475 nm against the concentration of L-phenylalanine in aqueous solution at pH 4.0. $[ANS] = 2.0 \times 10^{-5}$ M. Intensity was plotted at 475 nm of emission spectra (Ex = 360 nm).

3.2. Polymerization of L-phenylalanine with iron hydroxide sols

The iron (III) hydroxide sols were prepared from iron (III) chloride. To an aqueous solution of 0.1 M iron (III) chloride, 0.1 M NaOH aqueous solution was added to hydrolyze the iron hydroxide. To an aqueous solution of the iron hydroxide sols, L-phenylalanine and EDAC were added. Solution's pH was varied from 2 to 9 by an aqueous NaOH solution. After stirring for 48 h at 0 °C, 3 M HCl solution was added to the reaction mixture to decompose the colloidal iron hydroxide. The remaining white solid was isolated by centrifugation and washed with acidic water. According to the ^{1}H NMR and the IR analysis, the obtained white precipitates were condensation products of L-phenylalanine.

Fig. 2 shows the yields of the oligo(Lphenylalanine)s against the pH value in the presence and the absence of the colloidal iron hydroxide. The maximum yield of 15% was obtained at around pH 6.3 in the presence of the colloidal iron hydroxide. This value was 2.5 times higher than that obtained without the iron hydroxide sols at the same pH. According to the GPC analysis, the number–average molecular weight and the weight–average molecular weight of the product were 550 and 1100, respectively. The molec-

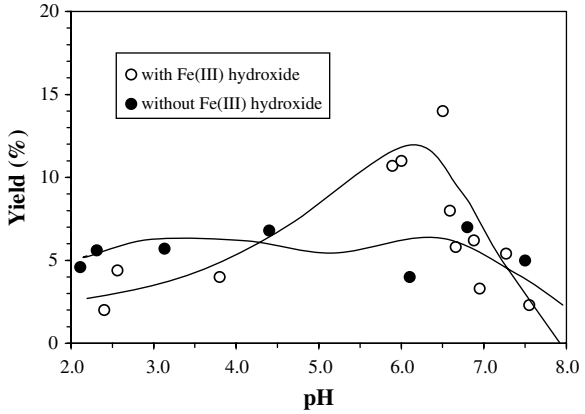

Fig. 2. Yields of oligo(L-phenylalanine) against the pH value (○) in the presence and (●) the absence of the colloidal iron hydroxide from iron (III) chloride.

ular weight of oligo(L-phenylalanine) was same as that in the absence of the iron (III) hydroxide sols as described above.

Adsorption behavior of L-phenylalanine on the iron hydroxide sols was measured to clear the mechanism of the oligomerization. An aqueous solution of the iron hydroxide sols and L-phenylalanine was adjusted to a desired pH by adding aqueous NaOH solution. After this mixture was incubated for 24 h at room temperature, the remaining concentration of L-phenylalanine after dialyzation through a membrane (M.W. Cut-off 20000) was determined by UV spectroscopy. The amount of adsorbed L-phenylalanine was plotted as a function of the solution's pH. The curve shape is similar to that of Fig. 2. Fig. 3 indicates that the maximum amount (8.8%) of L-phenylalanine was adsorbed at pH 6.4. The sum of the adsorption amount and the yields of the precipitates of 6% in the absence of the iron hydroxide sols are good agreement of the maximum yield of 15% which was obtained at around pH 6.3 in the presence of the iron (III) hydroxide sols. These results indicate that the assemblies of L-phenylalanine onto the iron hydroxide sols were predominant factor to improve the yield of the oligomerization product.

According to the literature [12,13], the point of zero charge of the iron hydroxide sols and L-phenylalanine are 7.6 and 5.48, respectively. Therefore, above 5.48, cationic character of L-phenylalanine might nonspecifically adsorb on the positively charged iron hydroxide sols. The maximum adsorbed mount of L-phenylalanine and the yield of oligo(L-phenylalanine) were resulted at pH around 6. Electrostatic interactions were a prerequisite for efficient oligomerization.

3.3. Effect of anion

The presence of other anions changes the pattern of iron (III) hydrolysis [9]. Iron (III) hydroxide sols were prepared from iron (III) nitrate instead of iron (III) chloride. Solubility of iron (III) nitrate is higher than that of iron (III) chloride in an aqueous solution. Thus, 0.4 M iron (III) nitrate was used to form iron hydroxide sols. To an aqueous solution of the iron (III) hydroxide sols from iron (III) nitrate, an aqueous solution of L-phenylalanine with EDAC was added as described above. Fig. 4 shows the yields of oligo(L-phenylalanine) against the pH value in the presence and the absence of the colloidal iron hydroxide prepared from iron (III) nitrate. Although the yields of the oligo(L-phenylalanine) were almost same between pH 6 and 8, the maximum yield of 20% was obtained at around pH 3. This value was 4 times higher than that obtained without the iron hydroxide sols at the same pH. According to the GPC analysis, the number–average molecular weight and the weight–average molecular weight of the oligomer obtained at around pH 3 were 720 and 1160, respectively. The molecular weights of the all obtained oligomers were independent within the experimental solution's pH.

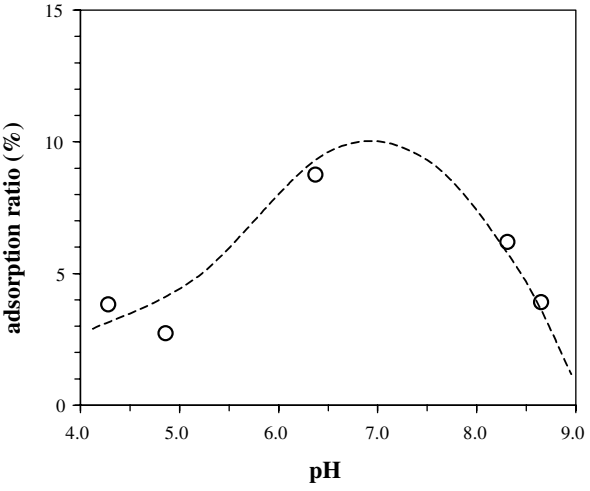

Fig. 3. The amount of adsorbed L-phenylalanine onto the iron hydroxide sols from iron (III) chloride as a function of the solution's pH.

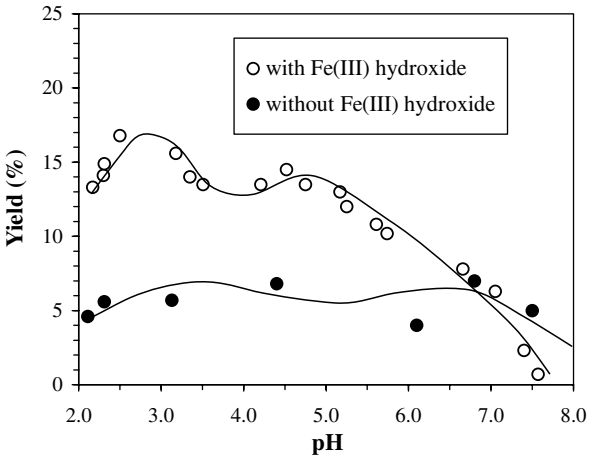

Fig. 4. Yields of oligo(L-phenylalanine) against the pH value (○) in the presence and (●) the absence of the colloidal iron hydroxide from iron (III) nitrate.

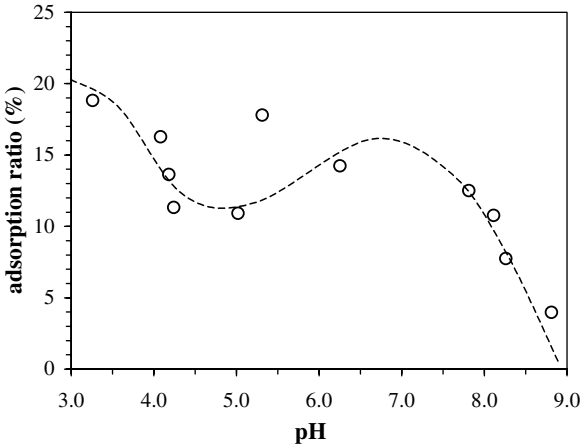

Fig. 5. The amount of adsorbed L-phenylalanine onto the iron hydroxide sols from iron (III) nitrate as a function of the solution's pH.

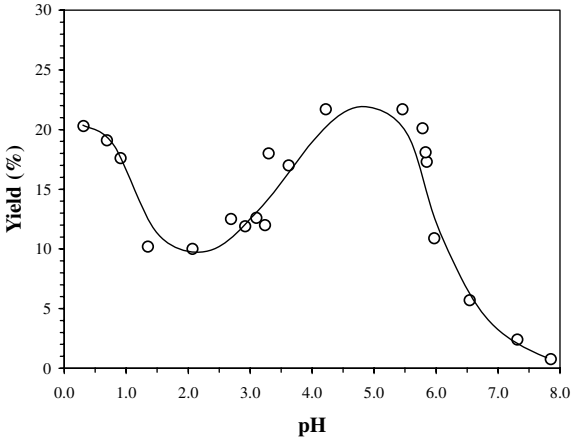

Fig. 6. Yields of oligo(L-phenylalanine) against the pH value in the presence of the colloidal iron hydroxide from iron (III) chloride and NaCl.

Fig. 5 shows the adsorbed amount of L-phenylalanine as a function of pH. The maximum amount (17%) of the adsorbed L-phenylalanine was observed at around pH 3. The absorption curve was close to the yield curve as shown in Fig. 4. These results also support that the assemblies of L-phenylalanine onto the iron hydroxide sols were predominant factor to improve the yield of the oligomerization product. The higher yield of oligo(L-phenylalanine) and the adsorbed amount of L-phenylalanine in the presence of the iron hydroxide sols prepared from iron (III) nitrate compared with those in the presence of the iron hydroxide sols from iron (III) chloride might be due to the higher concentration of the iron hydroxide sols from iron (III) nitrate than that from iron (III) chloride, because the solubility of iron (III) nitrate is higher than that of iron (III) chloride in an aqueous solution.

The presence of different anions in the iron hydroxide sols changed the pattern of the absorption curves and the yield curves. By blocking one or more coordination sites of iron (III), the anions strongly affect the structure and the size of the polynuclear products. Nitrate anion is less coordinate power than chloride anion [8]. Although the forms of the iron (III) hydroxide sols and the mechanisms of their transformations in aqueous solutions are not fully understood, we speculate that the positively charged L-phenylalanine below its isoelectrical point might interact with the nitrate anions weakly adsorbed on the iron hydroxide sols. At around pH 3, electrostatic interactions between the nitrate anions and positively charged L-phenylalanine might increase the adsorbed amount of L-phenylalanine on the sols.

3.4. Effect of NaCl

Effect of NaCl in the oligomerization of L-phenylalanine in an aqueous solution was also studied. After L-phenylalanine and EDAC were added to an aqueous solution of the iron hydroxide sols, 3 g of NaCl was added to the reaction mixture. Fig. 6 shows the yields of oligo(L-phenylalanine) against the pH value in the presence of the colloidal iron hydroxide and NaCl. The maximum yield of 22% was obtained at around pH 5.0. This value was higher than that obtained in the presence of the iron hydroxide sols without NaCl. The maximum yield was shifted to lower pH compared with that without NaCl. Although the yields of the products were high, the number–average molecular weight and the weight–average molecular weight of the oligomerization products were lower than those in the presence of the iron hydroxide sols without NaCl. According to the GPC analysis, the number–average molecular weight and the weight–average molecular weight of the oligomer obtained at around pH 5 were 420 and 520, respectively. The high yield and the low molecular weight of the products might be due to a salting-out effect of L-phenylalanine from an aqueous phase to concentrate the monomer and to decrease the solubility of the product.

Fig. 6 also shows that the yields of oligo(L-phenylalanine) increase with decreasing the solution's pH below 2.0. To solve this observation, the fluorescence probe method was employed. When ANS was added to an aqueous solution of 0.13 M L-phenylalanine with the solution's pH at 0.8, the fluorescence intensity of 475 nm markedly increased compared with that with the solution's pH of 5.2–2.3. According to the literature [13], the isoelectric pH and the pK_a (–COOH) of L-phenylalanine are 5.48 and 1.83, respectively. Solubility of L-phenylalanine in aqueous phase might decrease when the carboxylate anion of L-phenylalanine is protonated at pH 0.8. The aggregate formation might be promoted at such low pH. These results suggest that polycondensation of L-phenylalanine might be promoted via molecular assemblies in water phase at lower pH.

4. Conclusion

The iron (III) hydroxide sols increased the conversion of oligomerization of L-phenylalanine. Molecular assembly of the amino acid onto the iron (III) hydroxide

matrix may play a predominant factor for this effect. Electrostatic interactions were a prerequisite for efficient oligomerization. The iron (III) hydroxide sols acted as a support for oligomerization of amino acid in an aqueous solution. It should be emphasized that the iron (III) hydroxide sols are readily depolymerized upon acidification of solutions. Thus, the oligomerization products of hydrophobic amino acids were easily isolated by simple filtration and washed with water. The presence of different anions or salts in the iron hydroxides changed the adsorption behavior of L-phenylalanine and the yield of oligo(L-phenylalanine). The present results indicate that increased adsorbed amounts of L-phenylalanine increase the yield of oligo(L-phenylalanine) and both the values are comparable.

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