Communication: A new class of biodegradable polyampholytes, poly[(aspartic acid)-*co*-lysine], were synthesized by thermal polycondensation of aspartic acid and lysine under reduced pressure and subsequent hydrolysis. Polymerization conditions were optimized to yield maximal water-soluble poly(succinimide-*co*-lysine) with high molecular weight (160 °C/3.5 h). The succinimide/lysine ratio in the polyampholytes could be adjusted by their feed ratio. Characterization of the poly(succinimide-*co*lysine) by ¹H NMR revealed that ω -amine and carboxylic groups in lysine participated in the polymerization, leaving *a*-amino groups as pendant cationic moieties.



Synthetic route for the production of amphoteric poly[(aspartic acid)-*co*-lysine].

Synthesis of Biodegradable Amphoteric Poly[(aspartic acid)-*co*-lysine] by Thermal Polycondensation

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Introduction

Poly(amino acid)s, such as poly(aspartic acid), poly(glutamic acid) and polylysine, have always been synthesized by polymerization of *N*-carboxyanhydrides of *a*-amino acids, i.e., the NCA method. However, the NCA method has a cost disadvantage and a production problem because the pendant reactive groups carried by the amino acids should be protected prior to polymerization, then deprotected under harsh conditions to give poly(amino acids). In addition, phosgene, diphosgene or triphosgene is needed for the synthesis of *N*-carboxyanhydrides.^[1]

There is considerable research into the production of poly(succinimide) by the thermal polycondensation of aspartic acid.^[2-4] Compared with the NCA method, thermal polycondensation has the advantages of simple operation and low cost. In addition, other amino acids, such as glysine, ornithine and ω -amino acid can also be incorporated to the polymer main chain by simple copolycondensation.^[5-8] However, to our knowledge, there

are few reports on the co-polycondensation of aspartic acid and lysine to give biodegradable amphoteric poly-[(aspartic acid)-*co*-lysine] [P(ASP-*co*-LYS)] (Scheme 1). Such biodegradable polyampholytes are promising for the preparation of absorbable polyelectrolyte complexes with high pH-sensitivity for controlled protein release by complexation with polyanions.^[9–10] In this work, the synthesis and characterization of [P(ASP-*co*-LYS)] are reported.

Experimental Part

Materials and Measurements

D,L-Aspartic acid (ASP) and L-lysine (LYS) were purchased from Weikang Amino Acid Corp (Shanghai). ¹H NMR spectra were obtained with a Bruker DMX500 NMR spectrometer operating at 500 MHz using D₂O as a solvent and tetramethylsilane (TMS) as the internal standard. Infrared (IR) spectra were recorded on a Bruker Vector 22 Spectrometer.



Scheme 1. Synthetic route for the production of amphoteric poly[(aspartic acid)-*co*-lysine].

Samples were either film cast in chloroform onto NaCl plates or pressed into KBr pellets. Molecular weights of the polymers were estimated using gel permeation chromatography (GPC, Waters GPC Model 208) on a bank of Waters columns (Ultrahydrogel 2000, 500 and 120). The eluant was water containing 0.1 M sodium nitrate and 5 mmol sodium azide; the flow rate was 0.8 ml \cdot min⁻¹ and the temperature was 45 °C.

Polycondensation

A typical procedure for the co-polycondensation of ASP with LYS is as follows: ASP (0.25 g, 1.88 mmol), LYS (0.75 g, 5.14 mmol) and 85% o-phosphoric acid (0.4 g, 3.51 mmol) were mixed for 15 min at room temperature manually. The mixture was heated at 160°C for 3.5 h under reduced pressure (ca. 5 mmHg) using a rotary evaporator. The reaction system changed from syrupy heterogeneous liquid to a glassy solid as the reaction proceeded. After completion of the reaction, the pale brown glassy product was dissolved at room temperature by adding 5 ml of distilled water. The solution was filtered and adjusted to pH10.0 with diluted NaOH, then poured dropwise into methanol (50 ml) to yield a viscous precipitate. The purifying step described above was repeated twice and the precipitate was dried at room temperature under reduced pressure to give a yellowish lump of poly(succinimide-co-lysine) (P(SI-co-LYS)).

Alkali Hydrolysis

The hydrolysis of P(SI-*co*-LYS) was carried out as follows: the pH of the aqueous polymer solution was adjusted to 10.8 with 0.1 \times NaOH solution. The solution was stirred for 1 h, then acidified to ca. pH8.0 by adding 35% aqueous HCl and finally poured into methanol. The precipitate was filtered and dried at room temperature under reduced pressure to yield a yellowish powder of P(ASP-*co*-LYS).

Results and Discussion

Synthesis and Characterization of P(SI-co-LYS)

It was observed that significant water-insoluble products were generated after the thermal co-polycondensation of aspartic acid and lysine at 180°C for 3.5 h, which could not dissolve in common organic solvents, such as, N,Ndimethylformamide, dimethyl sulfoxide and trifluroacetic acid. From their insoluble characteristic, it may be concluded that the water-insoluble species are the crosslinking products which were generated by the nucleophilic reaction of the pendant amino groups presented in lysine units with succinimides in the polymer main chain during polymerization.^[8] The water-soluble parts were precipitated into methanol to give a yellowish viscous product. It should be noted that the two monomers could dissolve in methanol/water mixed solution (90:10, v/v), ensuring the purity of the precipitated products. After drying under vacuum, a hard lump was obtained. ¹H NMR characterization of the polymers confirms their chemical structures as P(SI-co-LYS) (Scheme 1), which is described in the following section.

In order to obtain maximal water-soluble polymers with relatively high molecular weight, the polymerization conditions were optimized. The effects of temperature on copolymerization of ASP and LYS (20/80 feed ratio in weight) are shown in Table 1. It can be seen that raising polymerization temperature results in an increase in the molecular weight (MW) of the water-soluble copolymers at the expense of their yields. Due to both the low fraction of water-insoluble species and the moderate MW of water-soluble copolymers at 160°C, the following polymerization was fixed under such conditions. Table 2 shows the effect of ASP/LYS feed ratio on the polymer vield, MW and SI/LYS ratio in the copolymers at 160°C. It can be seen that the copolymer \overline{M}_{w} varies from 15000 to 46000. In addition, the SI/LYS ratio is always lower than the ASP/LYS feed ratio indicating that LYS can be easily incorporated into the copolymers. In addition, the GPC traces show a single peak, indicating the absence of homopolymers of ASP and LYS (results not shown).

Table 1. The effect of polymerization temperature on percent of water-insoluble products, yield and MW of water-soluble poly(SI-*co*-LYS). ASP/LYS feed ratio (in weight) was fixed at 20/80, polymerization time was 3.5 h.

Temperature	Water-insoluble	Water-soluble copolymer		
C		Yield	$\overline{M}_{ m w}$	$\overline{M}_{ m n}$
	%	%		
140	0.6	32	13000	5600
160	2.2	79	34000	12000
180	38.1	58	38000	14000
200	78.2	16	44 000	19000

Table 2. The effect of ASP/LYS feed ratio on yield, MW of water-soluble poly(SI-*co*-LYS) and SI/LYS ratio in the copolymers. Polymerization was conducted at 160 °C for 3.5 h.

Polymer	$\frac{\text{Yield}^{a)}}{\%}$	$\overline{M}_{ m w}{}^{ m b)}$	$\overline{M}_{ m n}$	SI/LYS ratio ^{c)}
P(SI-co-LYS) 81:19d)	52	15000	4800	72:28
P(SI-co-LYS) 62:38	59	17000	5400	58:42
P(SI-co-LYS) 42:58	71	39000	16000	36:64
P(SI-co-LYS) 32:68	78	46000	21000	25:75
P(SI-co-LYS) 22:78	79	34000	12000	11:89

- ^{a)} Calculated by weight of water-soluble copolymer/weight of theoretic copolymer.
- ^{b)} Determined by GPC, distilled water as solvent and dextran as standard.
- ^{c)} In mole, calculated from ¹H NMR spectra.
- ^{d)} Feed ratio in mole corresponding to that in weight of 80:20, 60:40, 40:60, 30:70 and 20:80, respectively.



Figure 1. ¹H NMR spectra of poly(succinimide-co-lysine) with ASP/LYS feed ratio (wt.-%): (1) 20/80, (2) 40/60, (3) 60/40, (4) 80/20.

Figure 1 shows the ¹H NMR spectra of water-soluble copolymers obtained with different ASP/LYS feed ratios. It can be seen that with the increase in the ASP/LYS feed ratio the peak intensities at a and c increase, while the peaks at b, e, f and g gradually diminish, which indicates that the former two result from protons in SI units and the four latter can be attributed to those in LYS units. The peak at d shows no apparent variation in intensity with changes in the ASP/LYS feed ratio, suggesting it originated from protons in both SI and LYS units. The designation of the set of the set



Scheme 2. Chemical structure of poly(succinimide-*co*-lysine) with *a*-amino groups in lysine participating in the copolymerization.

nation of peaks obatined from the literature and the above results, is shown in Figure 1.^[6,7] At ca. 2.25 ppm in all the NMR spectra, no peaks of methylene protons presented as * in Scheme 2 are observable, a result which can be produced only when the *a*-amino groups in LYS participate in the copolymerization. However, the occurrence of peaks at b that can be designated as the methine protons reveals that it is the ω -amino groups in LYS taking part in the co-polycondensation.

Alkali Hydrolysis

P(SI-co-LYS) was further hydrolyzed in NaOH solution to give amphoteric poly[(aspartic acid)-co-lysine]. Figure 2 shows ¹H NMR spectrum of poly(ASP-co-LYS) 20:80. In comparison with the NMR spectrum of P(SIco-LYS) 20:80, new peaks can be observed at 4.40, 4.57 and 2.46-2.72 ppm. The two former can be designated as methine protons in ASP units and the latter as methylene protons in ASP units. In addition, the peak at 3.02-3.20 ppm resulting from methylene protons in SI units disappears, indicating the complete hydrolyzation of SI units. It was observed that precipitates were generated upon mixing poly(aspartic acid) (PASP) and P(SI-co-LYS) 40:60 within the pH range from 2.5–9.0, suggesting the formation of PASP/P(SI-co-LYS) complexes. In contrast, a mixture of PASP and LYS was homogeneous within the pH range 1-14, which further confirms the incorporation of cationic groups into the polymers. How-



Figure 2. ¹H NMR spectrum of poly[(aspartic acid)-co-lysine] 20:80. D₂O was the solvent and tetramethylsilane the internal standard.

ever, precipitates can only be produced within pH range 2.5–4.8 for the mixtures of PASP and P(ASP-*co*-LYS) 40:60, indicating the amphoteric nature of P(ASP-*co*-LYS). The entrapment of proteins into and protein release behavior from polyanion/P(ASP-*co*-LYS) complexes are under investigation.

Acknowledgement: Funding of this research by the Zhejiang Provincial Natural Science Foundation of China is gratefully acknowledged.

Received: January 21, 2001

Revised: May 25, 2001

- H. R. Kricheldorf, "a-Aminoacid-N-carboxy-anhydride and Related Heterocycles", Springer-Verlag, Berlin 1987, pp. 3–58.
- [2] S. K. Wolk, G. Swift, Y. H. Paik, K. M. Yocom, R. L. Smith, E. S. Simon, *Macromolecules* **1994**, *27*, 7613.
- [3] K. Matsubara, T. Nakato, M. Tomida, *Macromolecules* 1997, 30, 2305.
- [4] T. Nakato, M. Yoshitake, K. Matsubara, M. Tomida, *Macromolecules* 1998, 31, 2107.
- [5] A. Nakato, A. Kusuno, T. Kakuchi, J. Polym. Sci., Part A 2000, 38, 117.
- [6] T. Kakuchi, M. Shibata, S. Matsunami, T. Nakato, M. Tomida, J. Polym. Sci., Part A 1997, 35, 285.
- [7] M. Tomida, T. Nakato, M. Kuramochi, M. Shibata, S. Matsunami, T. Kakuchi, *Polymer* 1996, 37, 4435.
- [8] M. G. Meirim, E. W. Neuse, F. Parisi, Angew. Makromol. Chem. 1990, 175, 141.
- [9] W. C. Shen, Biochim. Biophys. Acta. 1990, 122, 1034.
- [10] H. L. Jiang, K. J. Zhu, J. Appl. Polym. Sci. 2001, 80, 1416.