Polymer Bulletin 1, 85–89 (1978)

Polymer Bulletin

Synthesis of Nucleic Acid Base Substituted Poly-L-lysine

Tomohiro Ishikawa, Yoshiaki Inaki and Kiichi Takemoto

Department of Petrochemistry, Faculty of Engineering, Osaka University, Suita, Osaka, Japan

Introduction

A series of nucleic acid analogs has been designed and widely prepared, and their functionalities have been estimated in relation to special base-pairing properties. (TAKEMOTO 1976). In these investigations, however, it was difficult to know the conformation of these polymers in solution, because most of them consist of a vinyl-type backbone and pendant nucleic acid bases. In this respect, a study on nucleic acid analogs having poly-α-amino acid backbone is of interest. This communication concerns the synthesis of ε,N-substituted polylysine with nucleic acid bases, in orer to get detailed informations about the conformational influence of the nature of their backbone structure on the complex formation of the nucleic acid analogs.

Experimental

p-Nitrophenyl 3-(6-aminopurine-9-yl)propionate (Ade-PNP) (1), and *p*-nitrophenyl 3-(2,4-dioxo-5-methyl 1,2-dihydropyrimidine-1-yl)propionate (Thy-PNP) (2)

These *p*-nitrophenyl esters were prepared by the reaction of *p*-nitrophenyl trifluoroacetate with the corresponding carboxyethyl derivatives of adenine and thymine in pyridine solution according to the method by Overberger and Inaki (OVERBERGER, INAKI 1978).

p-Nitrophenyl 3-(2,4-dioxo-1,2-dihydropyrimidine-1-yl)propionate (Ura-PNP) (3)

The *p*-nitrophenyl ester was prepared by the reaction of *p*-nitrophenyl trifluoroacetate with the carboxyethyl derivative of uracil using the same procedure described for Thy-PNP (2) in 97 % yield. mp 197-199°C. ANAL. Calcd. for $C_{13}H_{11}O_6N_3$: C, 51.15 %; H, 3.63 %; N, 13.77 %. Found: C, 51.16 %; H, 3.60 %; N, 13.60 %.

Poly(L-lysine trifluoroacetate) (4)

Poly(ε,N-trifluoroacetyl-L-lysine) (poly(ε,N-Tfa-L-lysine)) was prepared from the polymerization of ε,N-trifluoroacetyl-α,N-carboxy-L-lysine anhydride in dry dioxane with the use of triethylamine as an initiator, according to the method of Sela (SELA 1963).

0170-0839/78/0001/0085/$01.00

The intrinsic viscosity determined in dichloroacetic acid at 25°C was 0.36, which corresponds approximately to a molecular weight of 40,000.

The trifluoroacetyl protecting group was removed using piperidine in methanol, and the resulted clear solution was evaporated to dryness in vacuum to afford poly(L-lysine trifluoroacetate) (4). The polymer obtained was dried thoroughly and was used for further polymer reactions.

Poly(ε,N-3-(6-aminopurin-9-yl)propionyl-L-lysine):(poly(ε,N-Ade-L-lysine))(5)

To the solution of poly(L-lysine trifluoroacetate)(4)(400 mg, 1.7 mmol) in 10 ml of dimethyl sulfoxide (DMSO), Ade-PNP (1)(850 mg, 2.6 mmol) and triethylamine (0.3 ml, 2.1 mmol) were added, and the solution was stirred at 25°C for 3 days. The resulted mixture was poured into excess ethyl ether to give oily polymer. Acetone was added to the polymer to precipitate it, which was purified by the repeated precipitation from dimethylformamide (DMF)-ethyl ether or DMSO-acetone. The polymer precipitated was washed with ethyl ether and dried in vacuum to give a colorless powder, poly(ε,N-Ade-L-lysine)(5)(425 mg, 79 % yield).

Poly(ε,N-3-(2,4-dioxo-5-methyl-1,2-dihydropyrimidin-1-yl)propionyl-L-lysine):(poly(ε,N-Thy-L-lysine))(6)

From poly(L-lysine trifluoroacetate)(4) and Thy-PNP (2) using the same procedure described for poly(ε,N-Ade-L-lysine)(5), the polymer (6) was obtained in 90 % yield.

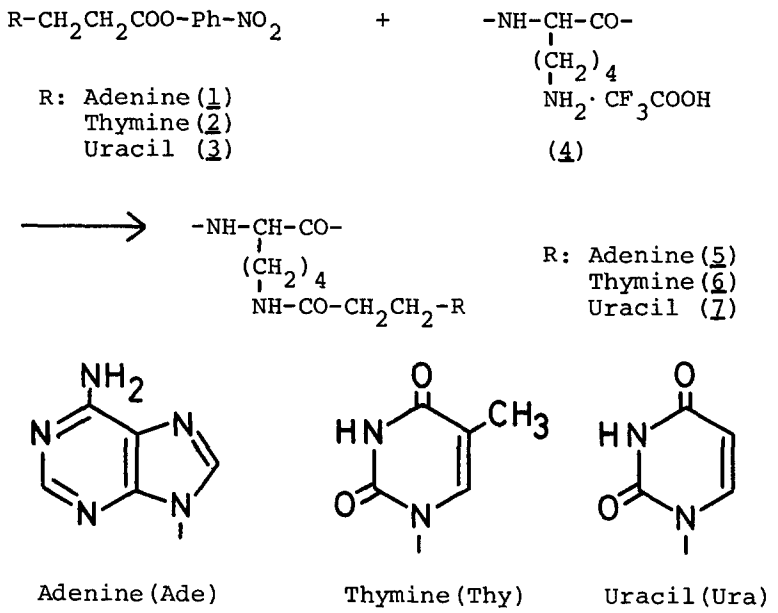
Poly(ε,N-3-(2,4-dioxo-1,2-dihydropyrimidin-1-yl) propionyl-L-lysine):(poly(ε,N-Ura-L-lysine)) (7)

Poly(ε,N-Ura-L-lysine)(7) was prepared also by the similar procedure from poly(L-lysine trifluoroacetate)(4) and Ura-PNP (3) in 97 % yield.

Results and Discussion

The derivatives of poly-L-lysine containing the nucleic acid bases, that is, adenine, thymine and uracil in the side groups were prepared as shown in SCHEME 1. Carboxyethyl derivatives of the nucleic acid bases were grafted onto poly-L-lysine by using the activated ester method (ANAND et al. 1971). Poly(L-lysine trifluoroacetate)(4) was prepared according to the method of Sela (SELA 1963). The *p*-nitrophenyl esters (1)-(3) were prepared by the reaction of the corresponding carboxyethyl derivatives of the nucleic acid bases with *p*-nitrophenyl trifluoroacetate according to the method of Overberger and Inaki (OVERBERGER, INAKI 1978), and purified by recrystallization.

SCHEME 1.



Adenine (Ade) Thymine (Thy) Uracil (Ura)

The reaction of the *p*-nitrophenyl esters with the polymer (4) was carried out in DMSO solution in the presence of triethylamine at 25°C. The poly-L-lysine derivatives obtained have different infrared absorption spectra from those of the starting compounds, and have absorptions assigned to the nucleic acid bases. Poly(ε,N-Ade-L-lysine) (5) was soluble in DMSO and ethylene glycol, and also in water below pH 3, where adenine was protonated form. In DMF, the polylysine containing 53 mol % adenine units (5a) was soluble, while the polymer containing 74 mol % adenine units (5b) was insoluble. Poly(ε,N-Thy-L-lysine) (6) and poly(ε,N-Ura-L-lysine) (7) were soluble in DMSO, DMF and 6N-hydrochloric acid.

The contents of the nucleic acid bases in the polylysine derivatives were determined by ultraviolet spectra of the polymers after hydrolysis: The polymers were hydrolyzed in 6N-hydrochloric acid at 105°C for 24 hr, into lysine dihydrochloride and the carboxyethyl derivatives of the nucleic acid bases. The quantitative calculation was made relative to the standard sample of the carboxyethyl derivative of the nucleic acid bases. The analytical data are listed in TABLE 1. The thymine and uracil derivatives were found to be completely substituted to polylysine. Low value in case of the ade-

TABLE 1. Poly-L-lysine containing nucleic acid bases

	Base	mol%[1]	UV[2] $\lambda_{max,nm}$	UV[2] ε_{max}	$[\alpha]_D$[3]	$[\eta]$[4]
(5a)	Ade	53	266	12,200	+ 4.8°	0.76[5]
(5b)	Ade	74	268	12,400	+ 4.7°	0.34[6]
(6)	Thy	97	273	9,000	+ 1.0°	0.40[5]
(7)	Ura	97	269	8,800	+ 2.7°	0.26[6]

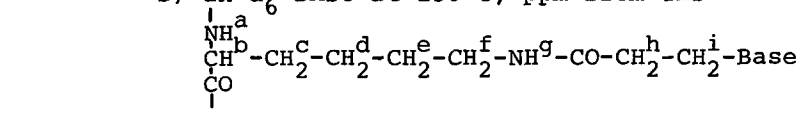
1) From UV spectra of the hydrolyzed samples
2) in DMSO at 25°C. ε value is corrected based on the nucleic acid bases
3) in DMSO (c=1) at 22°C
4) in DMSO at 25°C
5) $[\eta]$=0.4 in DMSO at 25°C for the original polymer; poly(ε,N-Tfa-L-lysine)
6) $[\eta]$=0.3 in DMSO at 25°C for the original polymer; poly(ε,N-Tfa-L-lysine)

TABLE 2. NMR spectra of the poly-L-lysine derivatives[1]

	Base	CH_2 c, d, e	CH_2 f	CH b	NH a, g	CH_2 h	CH_2 i
(5a)	Ade	1,40, 1.76	3.04	4.15	7.34, 7.52	2.72	4.40
(5b)	Ade	1.39, 1.74	3.04	4.08	7.36, 7.58	2.71	4.39
(6)	Thy	1.43, 1.8	3.07	4.15	7.34, 7.5	2.50	3.89
(7)	Ura	1.44, 1.77	3.09	4.21	7.36, 7.5	2.52	3.92

	8-H	2-H	6-NH_2	5-CH_3	5-H	6-H	3-NH
(5a)	8.03	8.23	5.28				
(5b)	8.01	8.22	5.54				
(6)				1.77		7.35	10.43
(7)					5.52	7.52	10.47

1) in d_6-DMSO at 150°C, ppm from TMS



nine base in the polymer may be attributed to unstability of the activated ester, Ade-PNP, and may also be explained in terms of the steric interaction among bulky side groups of the polymer. When the polylysine containing about 50 mol % adenine units was again treated with Ade-PNP, the adenine unit content in the polymer increased up to 74 mol % (5b)

The spectral data of these polymers are tabulated in TABLE 1 and 2. From these data, it was concluded that the activated ester of Ade-PNP (1) reacted only with ε-NH_2 group of poly-L-lysine, and did not react with amino group of the adenine base.

Summary

As a new type of the nucleic acid analogs, poly-L-lysine derivatives containing adenine, thymine and uracil were prepared. By using the activated ester method, carboxyethyl derivatives of the nucleic acid bases were grafted onto poly-L-lysine. The thymine and uracil derivatives were completely substituted to poly-L-lysine. The content of adenine unit in the polymer, however, was less than that of the thymine analog.

References

N.ANAND, N.S.R.K.MURTHY, F.NAIDER and M.GOODMAN: Macromolecules, 4, 564 (1971)
C.G.OVERBERGER and Y.INAKI: J.Polym.Sci., Polym.Chem. Ed., (1978), in press
M.SELA, R.ARNON and I.JACOBSON: Biopolymers, 1, 517 (1963)
K.TAKEMOTO: J.Polym.Sci., Polym.Symp., No.55, 105 (1976)

Received July 3, 1978