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Oligouridylates Formation with N-(O,O'-Diisopropyl)-Phosphoryl Alanine in the presence of Poly(β-(N^7-Adeninyl)Ethyl Methacrylate)

Hong Qi XIANG, Jian Jun HU, Yang JIANG, Yu Fen ZHAO*

Bioorganic Phosphorus Laboratory, Department of Chemistry, School of Life Science and Engineering, Tsinghua University, Beijing 100084

Abstract: The oligouridylates formation with N-(O,O-diisopropyl)-phosphoryl alanine in the presence of poly(β-(N^7-adeninyl)ethyl methacrylate) was studied. An increased yield by the presence of the polymeric nucleic acid analog was confirmed by anion exchange column HPLC, C18 reverse phase HPLC, and turbo ionspray MS.

Keywords: N-phosphoryl amino acid, nucleoside, interaction, polymeric nucleic acid analog.

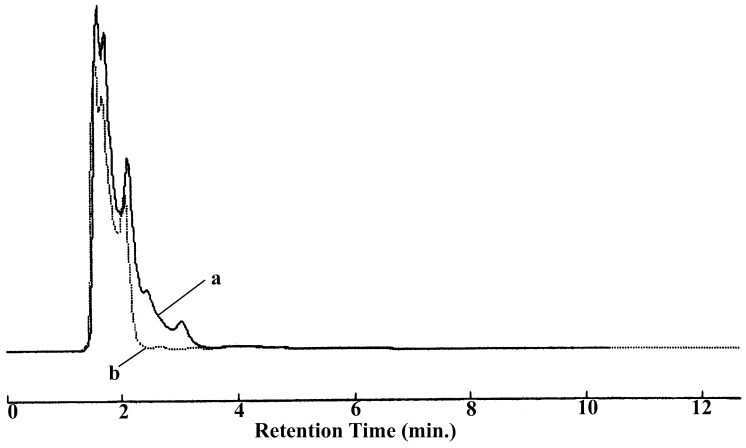
Phosphoryl amino acids are a series of novel molecules, which not only are capable of self-assembly into oligopeptides but also can act as the phosphoryl donor to phosphorylate nucleosides to nucleotides and oilgonucleotides[1]. A systematic study of the latter reaction opens a new way to search for the interaction and recognition between amino acids and nucleotides. Nevertheless, the poor yield of this reaction brings difficulties in separation, characterization and data analysis process.

Nucleic acid analogs have been studied for decades and a template effect of an adenine-containing nucleic acid analog on the polymerization of monomers containing complementary bases has been reported[2,3]. Considering the specific base-base interactions, it was our idea that the reaction of phosphoryl amino acid with nucleoside could be accelerated if a polymeric nucleic acid analog containing complementary bases is incorporated into the reaction system as a template. Here, the reaction between uridine and N-(O,O'-diisopropyl) phosphoryl alanine in the presence of poly(β-(N^7-adeninyl)ethyl methacrylate) was investigated.

Uridine (24 mg, 0.1 mmol), poly(β-(N^7-adeninyl)ethyl methacrylate) (25 mg, 0.1 mmol adenine, synthesized as ref. 4), and distilled water (0.5 mL) were mixed and heated at 50°C under an infrared lamp for six days. During this period, N-(O,O'-diisopropyl)-phosphoryl alanine (0.8 mmol, abbreviated as DIPP-Ala in the following, synthesized as ref. 5) and $NaHCO_3$ (0.8 mmol) were dissolved in 10 ml of distilled water and added to the above mixture in twenty separate portions. 0.5 mL of water was added to the final solid product. The solution portion was obtained by centrifuge and analyzed. As a control experiment, a sample was prepared by the same procedure in the absence of the poly(β-(N^7-adeninyl)ethyl methacrylate).

Because the wavelength of the UV detector was set at 254 nm and amino acids had little absorption at this wavelength, the peaks appeared in the anion exchange chromatograms in **Figure 1** must be corresponding to the derivatives from nucleosides. It can be seen that the intensities of the peaks were gradually reduced with the retention time. As the compound having more anions per molecule is more sluggishly eluted out on anion exchange column and more phosphate anions of an oligonucleotide means a longer chain length, the result shows that a series of compounds with a gradually-rising molecular weight were formed in the reaction system. And the yield of the oligonucleotide decreases with its chain length.

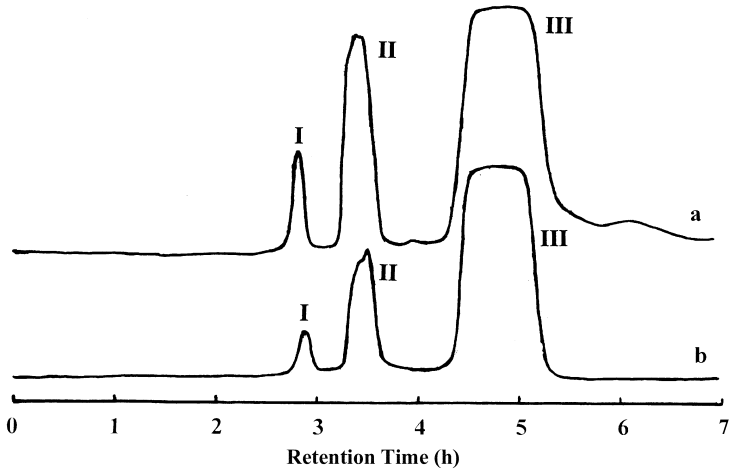
Figure 1 Anion exchange column HPLC of reaction products of uridine with DIPP-Ala obtained in the presence (a) and in the absence (b) of poly(β-(N^7-adeninyl)ethyl methacrylate). Column: ZORBAX Bio Series Oligo, 80×6.2 mm, Mobile Phase: 0.01 mol/L NaH_2PO_4, pH7.0, Flow Rate: 1.0 ml/min, Detector: UV, 254 nm.



With respect to the control sample, the sample obtained in the presence of the adenine-containing polymer showed two extra shoulder peaks at longer retention time. It indicates that, with the presence of the polymeric nucleic acid analog, products with more anions per molecule were formed. That is, in the case of this reaction system, oligonucleotides with longer chain were synthesized.

Both samples showed three fractions in the open column chromatograms by Sephadex G-15 gel. The last fraction was identified to be the unreacted uridine as proved by HPLC and FAB-MS. The first two were attributed to the compounds with a larger fluid dynamics volume than that of uridine[6]. As shown in the attached table of **Figure 2**, in the presence of the polymeric analog, the content percents of the first two fractions were increased from 4% to 5% and 16% to 24%, respectively, while that of unreacted uridine was decreased from 80% to 71%. Furthermore, by comparing with authentic compounds, the products of UMP, 2'-5'UpU, and 3'-5'UpU were identified in the first fraction by C_{18} reverse phase HPLC.

Figure 2 Gel permeation chromatogram of reaction product of uridine with DIPP-Ala obtained in the presence (a) and in the absence (b) of poly(β-(N^7-adeninyl)ethyl methacrylate). Column: Sephadex G-15, 850×18 mm, Mobile Phase: deionized water, pH7.0, Flow Rate: 0.8 ml/min, Detector: UV, 254 nm.



Sample	Content percent (%)		
	Fraction I	Fraction II	Fraction III
In the presence	5	24	71
In the absence	4	16	80

Table 1 Positive ion MS data of the samples obtained by the reaction of uridine with N-(O, O'-diisopropyl)-phosphoryl alanine in the presence and absence of the poly(β-(N^7-adeninyl)ethyl methacrylate), respectively.

Possible structure formed	*m/z*	Relative intensity	
		Presence	Absence
UMP	325	4	1
UpU	551	10	3
cyclo-UpU	613	4	
UpUp(i-PrO)	673		2
UpUp(Ala) (i-PrO)	744	3	
cyclo-Up(Ala)Up(Ala)	755	3	
UpUpU	857	7	2
Up(Ala)Up(Ala)U	999	4	

Table 1 listed the m/z value and relative intensity of the molecular ion peaks observed from the first fraction by turbo ionspray mass spectroscopy. The products UMP, UpU, UpUpU, and Up(Ala)Up(Ala)U were all observed in the reaction. Compared to the control sample, the relative amount of UpU and UpUpU in the sample obtained in the presence of nucleic acid analog were increased from 3% to 10% and 2% to 7%, separately.

In order to discriminate the polymer backbone effect, the poly(ethyl methacrylate) without adenine was added into the reaction system and there were no such effects. These results indicate that poly(β-(N^7-adeninyl)ethyl methacrylate) did act as a template

in the reaction system of uridine with N-phosphoryl alanine and accelerate the formation of oligouridylates, which provides a strategy to study the chemical relation of amino acid and peptide with nucleotides and oligonucleotides.

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References

1. Y. F. Zhao, P. S. Cao, Chemical Evolution: *Physics of the Origin and Evolution of Life*, J. Chela-Flores and F. Raulin eds., Kluwer Academic Publishers, Netherlands, **1996**, 279.
2. Y. Inaki, *Prog. Polym. Sci.*, **1992**, *17*, 515.
3. W. T. Smith Jr, *Prog. Polym. Sci.*, **1996**, *21*, 209.
4. G. J. Ji, C. B. Xue, J. N. Zeng, L. P. Li, W. G. Chai, and Y. F. Zhao, *Synthesis*, **1988**, *6*, 444.
5. M. Akashi, Y. Kita, Y. Inaki, K. Takemoto, *Makromol. Chem.*, **1977,** *178*, 1211.
6. M. J. He, W. X. Chen, X. X. Dong, *Polymer Physics*, Fudan University Press, Shanghai, **1994**, 199.

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