Tetrahedron Letters,Vol.31,No.2,pp 235-238,1990 0040-4039/90 $3.00 + .00
Printed in Great Britain Pergamon Press plc

FACILE SYNTHESIS OF NUCLEOTIDES CONTAINING POLYPHOSPHATES BY Mn(II) AND Cd(II) ION-CATALYZED PYROPHOSPHATE BOND FORMATION IN AQUEOUS SOLUTION

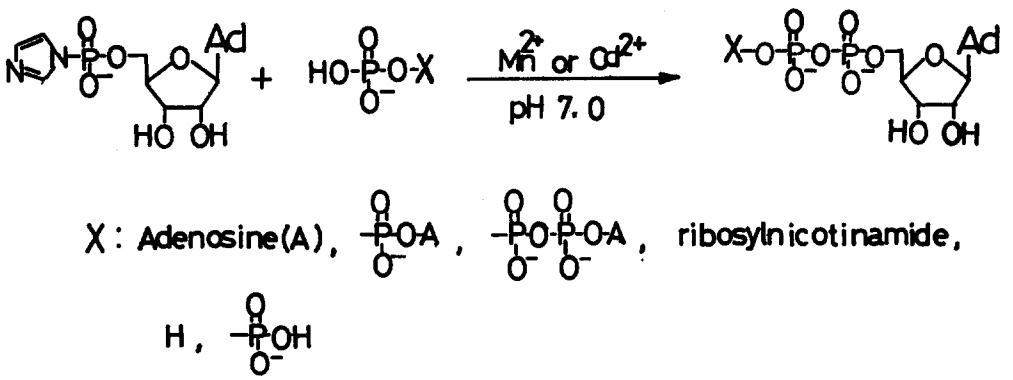
Masamitsu Shimazu, Kazuo Shinozuka and Hiroaki Sawai*
Department of Chemistry, Faculty of Engineering, Gunma University,
Kiryu, Gunma 376 Japan

Summary: Mn^{2+} and Cd^{2+} catalyzed pyrophosphate bond formation from adenosine-5'-phosphorimidazolide and nucleotides or phosphates in neutral aqueous solution, giving nucleotides containing polyphosphates.

The pyrophosphate bond is found in various kinds of nucleotides and coenzymes such as ATP, NAD, diadenosine polyphosphate[1], Ap_nA, and cap portion of messenger RNA[2]. These compounds play essential roles in many kinds of biochemical systems[1-3]. Several chemical methods have been developed to synthesize nucleotides with pyrophosphate bond from activated nucleotides and phosphates[4]. These methods, however, use anhydrous organic solvent as a reaction medium, thereby requiring a complicated procedure of solubilizing nucleotides in organic solvent. The methods need the removal of a trace amount of water by repeated azeotropic distillation with pyridine, the use of tertiary alkylammonium ion as a counter cation and of protecting groups for some base parts to perform the targeted reaction. In biochemical systems, the compounds are synthesized by condensation of nucleotides or phosphates with nucleoside-5'-triphosphate catalyzed by enzymes requiring Mg^{2+} or Mn^{2+} as a cofactor[5]. Orgel and his coworkers reported on a simple procedure for the synthesis of pyrophosphate-capped intermediate in aqueous solution from oligonucleotide with adenosine-5'-phosphorimidazolide (ImpA) in the presence of Mg^{2+} [6].

We attempted pyrophosphate bond formation in aqueous solution using metal ion catalysts mimicking enzymatic pyrophosphate formation, and found that Mn^{2+} and Cd^{2+} are effective as catalysts. Here we describe the metal ion-catalyzed pyrophosphate formation in neutral aqueous solution and its application to the synthesis of some biologically active nucleotides containing pyrophosphate bond.

We first examined the metal ion-catalyzed diadenosine diphosphate (AppA) formation from ImpA and adenosine-5'-monophsphate (pA) to search for an efficient catalyst for the pyrophosphate formation. ImpA was



prepared from pA and imidazole by a modified published method[7]. The AppA formation was carried out in a reaction mixture containing ImpA, pA and metal chloride in 0.2M N-ethylmorpholine-HCl buffer(pH 7.0) at room temperature for 8 days. The reaction mixture was treated with Versenol solution to remove the metal ion as Versenol-metal chelate prior to analysis by HPLC on a RPC-5 column or a ODS silica gel column. The HPLC analysis showed the formation of pA, AppA and a small amount of oligoadenylates. Table 1 summarizes the yield data of AppA formation.

Table 1. AppA Formation from ImpA and pA Catalyzed by Metal Ions

Metal salt	Yield(%)*	Metal salt	Yield(%)*
$MgCl_2$	29.3	$CuCl_2$	4.3
$CaCl_2$	17.8	$FeCl_2$	18.4
$BaCl_2$	10.2	$CoCl_2$	26.2
$ZnCl_2$	12.8	$NiCl_2$	18.4
$CdCl_2$	41.4	None	5.6
$MnCl_2$	43.6		

* Yields were based on the starting ImpA.
The reactions were run at 25°C for 8 d using 20mM ImpA, 60mM pA and 20mM metal chloride

The Mn^{2+} and Cd^{2+} are the most effective catalysts, giving AppA in more than 40% yield. Mg^{2+}, Co^{2+}, Ni^{2+} and Ca^{2+} demonstrated moderate activities. We examined the time course of the reaction of ImpA with pA by Mn^{2+} ion catalyst and confirmed that the reaction completed in 10 days, 2 days and 4 h at 4°C, 25°C and 50°C, respectively. The yield of AppA was 41-44%, independent of the reaction temperature. However, the reaction rate was markedly accelerated by a high temperature. The pH of the reaction medium had a considerable effect on the pyrophosphate formation. Hydrolysis of the phosphorimidazolide bond of ImpA was predominant below pH 6.0. The high pH above 7.5 stabilized the phosphorimidazolide bond and decreased the yield of the AppA. A neutral condition gave the best result.

In a similar way, ImpA reacted with adenosine-5'-triphosphate (ATP), adenosine-5'-diphosphate (ADP) and nicotinamide mononucleotide (NMN) to form the corresponding polyphosphates in the presence of metal ion catalysts. The Mn^{2+} and Cd^{2+} enhanced the pyrophosphate formation, and the reaction completed in one day at room temperature. Table 2 lists the yield data of ApppA, AppppA and NAD formation from ImpA with ATP, ADP and NMN. A small amount of AppA was also formed as a by-product in the reactions. We assigned the structure of AppppA and ApppA using ^{1}H and ^{31}P NMR[8]. These compounds were isolated in large amounts by QAE-Sephadex A-25 column chromatography with a linear gradient elution of 0.2-1.0 M triethylammonium bicarbonate buffer. The isolated yields were nearly the same as those obtained by HPLC. The yield of Ap_nA with Cd^{2+} ion catalyst was higher than that of the conventional method which used an anhydrous organic solvent[9]. Formation of NAD was small compared with that of diadenosine polyphosphate.

Table 2 Synthesis of ApppA, AppppA and NAD from ImpA and Nucleotides

Metal salt	Nucleotide	Product	Yield(% based on the nucleotide)	
$MnCl_2$	ADP	ApppA	59.0*	(31.8)**
$CdCl_2$			63.4	(30.8)
None			3.5	(1.7)
$MnCl_2$	ATP	AppppA	54.0	(39.6)
$CdCl_2$			59.0	(39.6)
None			5.5	(trace)
$MnCl_2$	NMN	NAD	32.4	
$CdCl_2$			34.9	
None			0	

* The yield obtained from the reaction of 20mM nucleotide, 60mM ImpA in the presence of 20mM metal chloride at 25°C for 4 d.
** The yield obtained from the reaction of 20mM nucleotide with 20mM ImpA in the presence of 20mM metal chloride at 25°C for 1 d.

We further examined the formation of ADP or ATP, from ImpA and inorganic orthophosphate or pyrophosphate using Mg^{2+} and Cd^{2+} ions as a catalyst. The Mg^{2+} ion exhibited the highest activity in the ADP and ATP formation, though the yield was smaller than that of diadenosine polyphosphates. The catalytic activity of metal ions was stronger in the order of $Mg^{2+} > Mn^{2+} > Cd^{2+} >>$ none. The yield of ADP and ATP was 32% and 12% based on ImpA, respectively, when the reaction was performed at room temperature for 4 days with 20mM ImpA, 200mM inorganic phosphates and 20mM $MgCl_2$. Without metal ions, neither ADP nor ATP was formed.

The mechanism of the pyrophosphate formation by Mn^{2+} and Cd^{2+} is as yet unclear. We postulate that, by coordination, the Mn^{2+} and Cd^{2+} ion orient ImpA and nucleotides or phosphates in a way to promote pyrophosphate bond formation. The stereochemistry of the complex formed

by different metal ions with ImpA and nucleotides or phosphates is apparently the most important factor for pyrophosphate formation. The activation of the phosphate residue by metal ions also seems to be an important factor determining the course of the reaction. Meanwhile, Pb^{2+}, Zn^{2+} and UO_2^{2+} ions which could activate the OH group of ribonucleotide enhanced the phosphodiester bond formation from ImpA in aqueous solution[10]. The nature of the metal ions controls the types of the bond formation.

In comparison with the conventional method, the present method provides a simple and short procedure for the synthesis of nucleotides containing the pyrophosphate bond. The condensation reaction proceeds in neutral aqueous solution to give products in a considerably high yields and no protecting group is required.

Applications of this method to the synthesis of other nucleotides such as GppppG, cap portion of messenger RNA are now in progress.

Acknowledgment. This work was partially supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

References and Notes

1 E. Rapaport and P. C. Zamecnik, Proc. Nat. Acad. Sci. USA, 73, 3984 (1976); E. Rapaport, P. C. Zamecnik and E. F. Baril, J. Biol. Chem., 265, 12148 (1981).
2 K. Miura, Adv. Biophysics, 14, 205 (1981).
3 A. L. Lehninger, in " Biochemistry", second ed., Worth Publishers Inc., 1975, Part 2.
4 J. G. Moffat and H. G. Khorana, J. Am. Chem. Soc., 83, 649 (1961); D. E. Hoard and D. G. Ott, J. Am. Chem. Soc., 87, 1785 (1965); A. F. Cook, M. J. Holman and A. L. Nassbaum, J. Am. Chem. Soc., 91, 1522 (1969); T. Hata. K. Furusawa and M. Sekine, J. Chem. Soc. Chem. Commun., 1975, 196; T. Kamimura, Y. Osaki, M. Sekine and T. Hata, Tetrahedron Lett., 25, 2683 (1984); R. Woerd, C. G. Bakker and A. W. Schwarz, Tetrahedron Lett., 28, 2763 (1987).
5 T. G. Spiro, in "Inorganic Biochemistry" Vol.1, ed. G. L. Eichorn, Elsevier Publishing Co., Amsterdam, 1973, P.578; J. R. Knowles, Ann. Rev. Biochem., 49, 877 (1980); A. Guranowsky, G. Just, E. Holler and H. Jakubowsky, Biochemistry, 27, 2759 (1988).
6 B. C. F. Chu and L. E. Orgel, Biochim. Biophys. Acta, 782, 103 (1984).
7 R. Lohrmann and L. E. Orgel, Tetrahedron, 34, 853 (1978).
8 1-H NMR shifts of ApppA and AppppA relative to TMS (ppm): ApppA, 8.29(8-H), 8.10(2-H), 6.01 J=4.4Hz (1'-H); AppppA, 8.38(8-H), 8.14(2-H), 6.03 J=5.8Hz (1'-H).
31-P NMR shifts of ApppA and AppppA relative to 85% H_3PO_4 (ppm): ApppA, 12.41(α-P), 23.97(β-P); AppppA, 11,50(α-P), 23.36(β-P).
9 P. Feldhaus, T. Frohlich, R. S. Goody, M. Isakov and R. H. Shirmer, Eur. J. Biochem, 57, 197 (1975).
10 H. Sawai, J. Am. Chem. Soc., 98, 7037 (1976); H. Sawai, M. Ohno and T. Shibata, Tetrahedron, 37, 481 (1981); H. Sawai, K. Kuroda, and T. Hojo, Bull. Chem. Soc. Jpn, 62, 2018 (1989).

(Received in Japan 2 August 1989)