Histidine and Thermal Copolymers of Amino Acids Containing Histidine as Prebiotic Inhibitor for the Template-Directed Formation of Oligoguanylate on a Poly(C) Template

Kunio Kawamura* and Kazuhiro Kuranoue
Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, Sakai, Osaka 599-8531

(Received May 2, 2001; CL-010406)

Possibility of the cooperative chemical evolution of nucleic acids and proteins has been investigated using the template-directed formation of oligoguanylate with amino acids and thermal copolymers of amino acids, in which strong inhibition by histidine containing thermal copolymer and histidine itself was observed. The inhibition is regarded as prebiotic enzymatic activities for the hydrolysis of activated nucleotide monomer and the formation of pyrophospho-capped oligoguanylate.

Discovery of the catalytic activity of ribonucleic acids (RNA) has suggested that RNA-like molecules played a central role for the emergence of life (the RNA world hypothesis).[1] If this hypothesis is correct then RNA-like molecules formed spontaneously from prebiotic RNA monomers under primitive earth conditions. There have been a number of successful studies of the condensation of activated nucleotide monomers to form oligonucleotides in the presence[2] and absence[3] of RNA templates.[3] For instance, the template-directed formation of oligoguanylate (oligo(G)) from guanosine 5'-monophosphate 2-methylimidazolide (2MeImpG) on a polycytidylic acid (poly(C)) template (TD reaction) has been extensively investigated.[2] Further, possible pathways from bases to the activated nucleotides have been experimentally elucidated.[4] Thus, the condensation reaction using nucleotide imidazolide is an important approach, which has been frequently used for primitive RNA polymerase models.[2,3] On the other hand, it is widely accepted that amino acids and protein-like molecules formed under primitive earth conditions.[5,6] In addition, both nucleic acids and proteins are essential for modern organisms. Thus, it is reasonable that RNA and protein-like molecules evolved cooperatively under primitive earth conditions. However, there has been surprisingly less studies on simultaneous chemical evolution of nucleic acids and proteins. Thus, in this study, thermal copolymers of amino acids (TC), which are well known as proteinoids formed under possible primitive earth conditions,[6] were tested whether to show some catalytic abilities for the TD reaction. Consequently, it was found that histidine and TC containing histidine showed notable inhibition, and TC also promoted the formation of pyrophospho-capped oligo(G).

Influence of the 20 common amino acids and 5 types of thermal copolymers of amino acids for TD reaction was tested. TD reactions were performed at 25 °C for 3–7 days in a buffer solution containing 1.0 M (1 M = 1 mol dm^{-3}) NaCl, 0.2 M $MgCl_2$, 0.1 M 2-[4-(2-hydroxyethyl)-1-piperazynyl]ethanesulfonic acid (HEPES), 0.015 M 2MeImpG, 0.025 M poly(C) (as monomer unit) at pH 8.0 in the presence of amino acid or TC, and then analyzed by anion-exchange and reversed-phase HPLC. Preparation of five types of TC was followed by previous procedures.[6] A standard type of TC (TC-std) was made from a mixture of 1 mmol each of glycine, L-alanine, L-valine, L-gultamic acid, and L-aspartic acid and optionally 1 mmol of L-histidine (TC-his), L-lysine (TC-lys), L-cystein (TC-cys), L-leucine (TC-leu), or L-arginine (TC-arg) was added. The mixture of amino acids was heated for 2 h at 180 °C and dialyzed using ultrafiltration filters (Spectrum, Spectra/Por MWCO 1000 or 12000–14000 tubing), and then TC was lyophilized.

Among the 20 common amino acids, only L-histidine showed notable inhibition activity for the TD reaction (Table 1). Especially, oligo(G) did not form at all with 0.05 M L-histidine. Moreover, it was confirmed that D-histidine has also inhibition activity for the formation of oligo(G). Here, the extent of pG increased and that of 2MeImpG decreased with histidine. This fact suggests that the inhibition of oligo(G) formation is due to the histidine catalyzed hydrolysis of 2MeImpG. To evaluate the catalysis for 2MeImpG hydrolysis by histidine, the rate of 2MeImpG hydrolysis without poly(C) at 0.01 M histidine was measured. The reaction curves show that the hydrolysis of 2MeImpG is accelerated by both L- and D-histidine (Figure 1), in which the half-life of 2MeImpG was 7–11 times reduced with histidine. Pseudo-second-order rate plots were well fitted for the hydrolysis of 2MeImpG with histidine and this may be due to a similar mechanism observed in a previous study.[7]

Table 1. Yields (%) of oligo(G) in the presence of amino acids

Amino acid	2MeImpG	pG	G5'ppG	2mer	3mer	4mer	5mer+
none[a]	36	38	0.9	6.3	2.0	1.9	15
av[b]	33	40	0.9	5.6	2.2	2.0	17
L-his[c]	12	74	0.8	0.9	1.5	1.6	8.6
L-his[d]	1.7	97	1.1	0.0	0.0	0.0	0.0
D-his[c]	23	70	1.9	0.4	0.7	0.8	3.8

The percentages are the uncorrected HPLC absorbance readings. Reaction conditions: [NaCl] = 1.0 M, [$MgCl_2$] = 0.2 M, [HEPES] = 0.1 M, [2MeImpG] = 0.015 M, [poly(C)] = 0.025 M, pH = 8.0, 25 °C, 3 d. [a]The average of 4 runs of TD reaction without amino acids. [b][Amino acid] = 0.01 M, average values of the 20 common amino acids except histidine. [c][L- or D-his] = 0.01 M. [d][L-his] = 0.05 M.

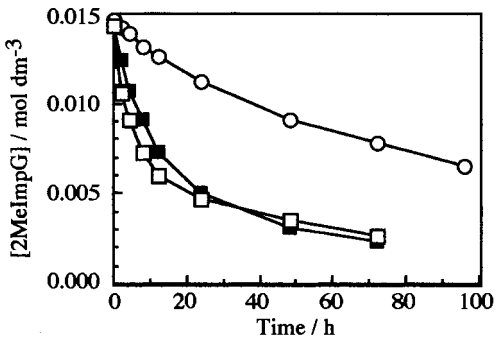

Figure 1. Reaction curves of the hydrolysis of 2MeImpG in the presence and absence of histidine.
[NaCl] = 1.0 M, [$MgCl_2$] = 0.2 M, [HEPES] = 0.1 M, [2MeImpG] = 0.015 M, [histidine] = 0.01 M, pH = 8.0, 25 °C. ○: no histidine, □: L-histidine, ■: D-histidine.

On the other hand, the influence of TC was investigated for TD reaction. Although TC prepared without histidine did not show notable influence on the TD reaction products, the yield of oligo(G) was fairly reduced at 0.06 M TC-his, in which the concentrations of TC were calculated on the basis of amino acid unit (Table 2). Further, the extent of P_1,P_2-bis(5'-guanosyl) diphosphate ($G^{5'}$ppG) was also increased with TC-his. To demonstrate more clearly the inhibition by TC, TC-std and TC-his were dialyzed and then used for the TD reaction. Table 2 indicates that the formation of oligo(G) was inhibited and the hydrolysis of 2MeImpG was enhanced at 0.06 M TC-his dialyzed using either MWCO 1000 or 12000–14000 tubing, while TC-std changed less the yield of oligo(G).

Table 2. Yields (%) of oligo(G) in the presence of thermal copolymers of amino acids

TC	2MeImpG	pG	$G^{5'}$ppG	2mer	3mer	4mer	5mer+
without dialysis							
TC-std	38	37	0.8	6.0	1.8	1.9	14
TC-his	29	58	3.3	1.5	1.0	1.4	5.9
dialyzed with MWCO 1000							
TC-std	44	36	1.6	3.8	2.2	2.3	11
TC-his	17	75	4.9	0.3	0.7	1.0	0.8
dialyzed with MWCO 12000 – 14000							
TC-std	39	41	2.0	3.0	1.8	1.9	11
TC-his	26	63	3.6	0.4	1.0	1.3	4.7

The percentages are the uncorrected HPLC absorbance readings. Reaction conditions are the same as Table 1. The concentrations of TC: [TC-std] = 0.05 M, [TC-his] = 0.06 M (based on monomer unit of amino acids).

The catalytic ability of histidine and TC-his for the 2MeImpG hydrolysis is unexpected finding since it has been observed in previous kinetic studies that simple bases, such as imidazole and 2-methylimidazole, do not have catalytic ability for the hydrolysis of activated nucleotides.[8] Further, the results in the present study indicate that histidine incorporated in TC is essential for the catalysis of 2MeImpG hydrolysis, where the histidine residues would be important. Thus, details how histidine is incorporated as the catalytic site of TC-his will be helpful to understand the reaction mechanism in future.

On the other hand, the extent of $G^{5'}$ppG considerably increased in the presence of TC (Table 2). Further, HPLC chromatogram of TD reaction at 0.05 M TC-std dialyzed with MWCO 12000–14000 tubing shows additional peaks (Figure 2). Besides, it is known that $G^{5'}$ppG-capped oligo(G) forms from TD reaction under several conditions.[9] Thus, these facts indicate that the formation of $G^{5'}$ppG-capped oligo(G) may contribute to the inhibition of TD reaction by TC. To evaluate this assumption, the TD reaction with 0.0015 M $G^{5'}$ppG in the absence of TC was carried out, in which the TD reaction products showed very similar HPLC pattern to that observed in the presence of TC. This fact suggests that oligo(G) formed with TC-std and TC-his involves $G^{5'}$ppG-capped oligo(G) isomers (Figure 3).

It is widely believed that amino acids formed abundantly on the primitive earth.[4] Besides, there are hypotheses that primitive proteins were constructed from only 4 kinds of amino acids, that is, glycine, alanine, valine, and aspartic acid, so TC used in this study may resemble to primitive enzymes in the compositions of amino acids.[10] Thus, finding of inhibition by TC-his and histidine itself for the TD reaction may oppose to the RNA world hypothesis. Further investigations on the cooperative chemical evolution of nucleic acids and proteins are being in progress.

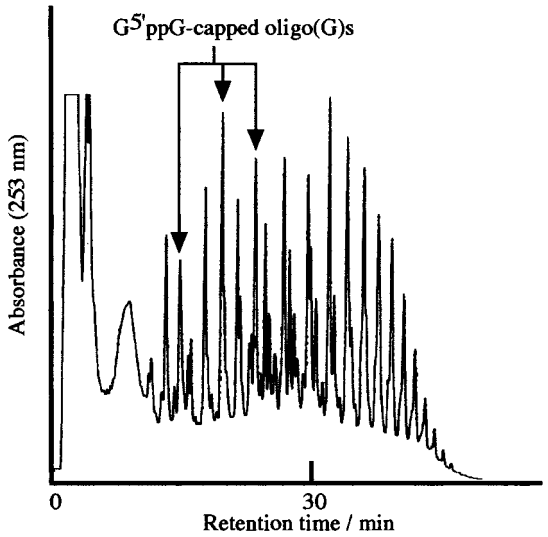

Figure 2. HPLC profile of the TD reaction in the presence of thermal copolymer of amino acids. [NaCl] = 1.0 M, [$MgCl_2$] = 0.2 M, [HEPES] = 0.1 M, pH = 8.0, [2MeImpG] = 0.015 M, [poly(C)] = 0.025 M, 25 °C, 3 d. Thermal copolymer (0.05 M) prepared from gly, ala, val, asp, glu was dialyzed with MWCO 12000–14000 tubing.

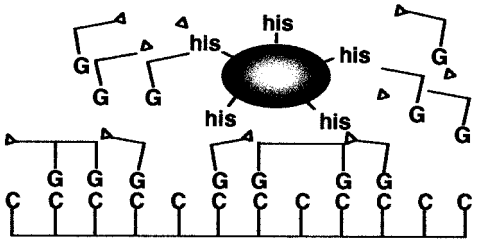

Figure 3. A model of catalytic hydrolysis of activated nucleotide monomer and formation of $G^{5'}$ppG-capped oligo(G) by thermal copolymer of amino acids containing histidine during template-directed formation of oligo(G).

We thank Professor Taketoshi Nakahara of Osaka Prefecture University for the use of HPLC apparatus. This research was partially supported by the Sumitomo Foundation 1998, Japan.

References

1 W. Gilbert, *Nature*, **319**, 618 (1986).
2 R. Lohrmann and L. E. Orgel, *J. Mol. Biol.*, **142**, 55 (1980); T. Inoue and L. E. Orgel, *Science*, **219**, 859 (1983).
3 H. Sawai, K. Kuroda, and H. Hojo, *Bull. Chem. Soc. Jpn.*, **62**, 2018 (1989); J. P. Ferris and G. Ertem, *Science*, **275**, 1387 (1992).
4 R. Lohrmann and L. E. Orgel, *Nature*, **244**, 418 (1973); L. E. Orgel and R. Lohrmann, *Acc. Chem. Res.*, **7**, 368 (1974); R. Lohrmann, *J. Mol. Evol.*, **10**, 137 (1977); S. Miyakawa, K. Murasawa, K. Kobayashi, and A. B. Sawaoka, *Origins Life Evol. Biosphere*, **30**, 557 (2000).
5 S. L. Miller, *Science*, **117**, 528 (1953); K. Kobayashi, T, Kaneko, T. Saito, and T. Oshima, *Origins Life Evol. Biosphere*, **28**, 155 (1998).
6 S. W. Fox and K. Harada, *Science*, **128**, 1214 (1958); S. W. Fox and K. Harada, *J. Am. Chem. Soc.*, **82**, 3745 (1960).
7 K. Kawamura and M. Umehara, *Bull. Chem. Soc. Jpn.*,**74**, 927 (2001).
8 A. Kanavarioti and M. T. Rosenbach, *J. Org. Chem.*, **56**, 1513 (1991); A. Kanavarioti, C. F. Bernasconi, D. L. Doodokyan, and D. J. Alberas, *J. Am. Chem. Soc.*, **111**, 7247 (1989); F. J. Ruzicka and P. A. Frey, *Bioorg. Chem.*, **21**, 238 (1993).
9 T. Inoue and L. E. Orgel, *J. Mol. Biol.*, **162**, 201 (1982).
10 M. Eigen, W. Gardiner, P. Schuster, and R. Winkler-Oswatisch, *Sci. Am.*, **244**, 88 (1981); K. Ikehara, *Seibutsu Kagaku*, **51**, 43 (1999).