

Prebiotic Inhibitory Activity of Protein-Like Molecules to the Template-Directed Formation of Oligoguanylate from Guanosine 5′-Monophosphate 2-Methylimidazolide on a Polycytidylic Acid Template

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The influence of thermal copolymers of amino acids (TCAA) as a primitive protein enzyme model has been investigated to detect catalytic and/or inhibitory activities on the template-directed formation of oligoguanylate (oligo(G)) from guanosine 5′-monophosphate 2-methylimidazolide (2-MeImpG) on a polycytidylic acid template (poly(C)) (TD reaction). Different amino acids and compositions were used to prepare TCAAs. These TCAAs were characterized by gel filtration HPLC and the composition analysis of amino acids using phenyl isothiocyanate. It was found that TCAA including His (TCAA-His) has inhibitory activity to the TD reaction, in which TCAA-His accelerates the hydrolysis of 2-MeImpG. Other types of TCAAs, including Gly, Ala, Val, Glu, and Asp, also accelerate the formation of P_1,P_2-bis(5′-guanosyl) diphosphate ($G^{5'}$ppG) and $G^{5'}$ppG incorporated oligo(G)s during the TD reaction at high concentrations of TCAAs. These reactions are considered as primitive enzyme models for the hydrolysis of nucleotide monomer and the formation of pyrophosphate-containing oligo(G).

The discovery of ribozymes suggests that RNA-like molecules played central roles for the emergence of life.[1–4] There have been several studies on the chemical evolution of RNA molecules; including studies on the prebiotic formation of RNA,[5–14] the stability of RNA under the primitive earth conditions,[15–22] and artificial ribozymes to design an in vitro RNA world.[4,23] In most of these studies, model reactions composed of RNA molecules were presented. However, this does not straightforwardly mean that the prebiotic catalytic network systems were made entirely of RNA molecules, though it was necessary that RNAs hold genetic information and catalytic ability under the primitive earth conditions. Some results imply that the chemical evolution of RNA was associated with other biologically important molecules, such as protein-like molecules. One must therefore take into account the relationships between RNA and protein-like molecules under the primitive earth conditions. First, RNAs and proteins do indeed work cooperatively in the present organisms. Second, the existence of in vitro viruses suggests that virus-type systems involve a system of the assignment between genotype RNAs and phenotype peptides, which had emerged after the RNA world-type systems.[24,25] Third, it has been elucidated that protein-like molecules could have formed under the primitive earth conditions; such formation could be much easier than nucleic acids.[26–35] Interactive chemical evolution between RNA and protein-like molecules should have been necessary in the course of the chemical evolution from the RNA world type system to the higher stages towards the origin-of-life system.[36–40] Thus, it is natural to assume that the chemical evolution of RNA and protein-like molecules had proceeded cooperatively rather than to consider that RNA world systems were formed entirely of RNA even at the early stage of the origins of life. In addition, it should be noted that this idea is not in conflict with the RNA world hypothesis. However, few studies have been carried out from the viewpoint of the cooperative chemical evolution between RNA and protein-like molecules.[34–37]

Although a number of studies other than the present study have been carried out to find possible catalytic activities of protein-like molecules, remarkable activities have not yet been detected.[26–31] Especially, catalytic functions of proteinoides had been explored in the 1960–1970s, in which a variety of catalytic abilities were demonstrated. However, there have been no investigations of these protein-like molecules in prebiotic polymerase model reactions.[28–31]

On the other hand, if the RNA world hypothesis is correct, then RNA or RNA-like molecules should have formed spontaneously under primitive earth conditions. There have been successful studies on the primitive polymerase reaction model of RNA, such as the template-directed formation of oligonucleotide using the activated nucleotide monomer on a polynucleotide template (TD reaction)[5–8] and the spontaneous formation of oligonucleotides in the presence of metal ion catalysts[9–11] or clay mineral catalysts.[12,13] In spite of these efforts, few investigations have been carried out on the role of prebiotic protein-like molecules in the prebiotic formation of RNA.

Thus, we have been studying possible roles of protein-like molecules, which are formed from mixtures of amino acids under the possible primitive earth conditions, in both the formation and the degradation of RNA molecules.[37,40] In this paper, the influence of thermal copolymers of amino acids (TCAA) was investigated to detect possible catalytic and/or inhibitory activities for the template-directed formation of oligoguanylate (oligo(G)) from guanosine 5′-monophosphate 2-methylimidazolide (2-MeImpG) on a polycytidylic acid (poly(C)) template.[37]

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The TD reaction of oligo(G) from 2-MeImpG on a poly(C) was chosen in this study for the following reasons. Previous studies on the TD reactions have used several activated nucleotide monomers,[5–8] homo- or mix-polynucleotide templates with different bases,[8,41,42] and hairpin oligonucleotides.[43] These studies have well established that the activated nucleotide monomers are to be directed by the template bases with Watson–Crick base-pairing, while the efficiency of the TD reactions is dependent on the combination of the type of activated nucleotide monomer and the type of polynucleotide template. For instance, the formation of oligo(G) from 2-MeImpG on a poly(C) template results a highest elongation efficiency and regioselectivity among the different TD reactions, in which 20–30-mer oligo(G)s with mainly 3′,5′-linkage are produced.[7] Further, extensive kinetics and mechanistic investigations on the TD reaction of oligo(G) on a poly(C) template have been reported.[44,45] Thus, the TD reaction of oligo(G) on a poly(C) template is regarded as one of the most useful model reaction to investigate the role of protein-like molecules for the prebiotic formation of RNA.

Experimental

Materials and Equipment. 2-MeImpG was synthesized using a modification of the procedure of Joyce et al. (purity > 95%).[41,46] Polycytidylic acid (poly(C)) was purchased from SIGMA and amino acids were purchased from Wako, Japan. All other reagents used were of analytical grade. Oligo(G) was prepared from the hydrolytic degradation of poly(G), in which poly(G) was degraded in a 0.1 M (1 M = 1 mol dm^{-3}) NaOH aqueous solution for 1 h at 37 °C and further treated in a pH 2 $HClO_4$ solution for 1 h at 37 °C to yield 3′- or 2′-phosphate terminal.

High-performance liquid chromatography (HPLC) was performed by a HPLC system LC10A (Shimadzu, Japan) with a DNA-NPR anion-exchange column from TOSOH Co., Japan and a ODS-2 column from GL Science Co., Japan.

Preparation and Characterization of Thermal Copolymers of Amino Acids. Preparation of TCAA was carried out using modifications of a preparation method reported in previous studies.[26,27] A standard type of TCAA was made from a mixture of 1 or 25 mmol each of Gly, L-Ala, L-Val, L-Glu, and L-Asp and optionally 1 or 25 mmol of L-His, L-Lys, L-Cys, L-Leu, L-Arg, L-Tyr, L-Trp, or L-Phe was added. TCAAs are abbreviated as TCAA-Std (including Gly, L-Ala, L-Val, L-Glu, and L-Asp), TCAA-His, TCAA-Lys, TCAA-Cys, TCAA-Leu, TCAA-Arg, TCAA-Tyr, TCAA-Trp, and TCAA-Phe. TCAA-(Glu–Asp) was prepared from a mixture of 25 mmol of L-Glu and L-Asp and 0.5 or 25 mmol of $MnCl_2 \cdot 4H_2O$. The mixture was heated for 2 or 24 h at 180 °C. These mixtures including TCAAs were dissolved in 10–100 mL water and the diluted mixtures were dialyzed using ultrafiltration filters (Spectrum, Spectra/Por MWCO 1000, 3500, or 12000–14000 tubing); then TCAA was lyophilized.

The molecular weight was determined by size exclusion chromatography (GPC) on a TSKgel G2000SWXL column (TOSOH Co., Tokyo) using a buffer containing 0.05 M NaH_2PO_4 and 0.3 M NaCl at pH 7.0. The compositions of amino acids in TCAA were determined using derivation with phenyl isothiocyanate (PITC) for the hydrolyzed products of TCAA, which were obtained in 6 M HCl for 24 h at 110 °C under N_2 atmosphere.[47] Samples were analyzed on a ODS-2 column using a gradient of 0.05 M CH_3COONH_4 in water at pH 6.8 mixed with buffer B containing 0.1 M CH_3COONH_4 in 80% CH_3OH at pH 6.8 at 52 °C. The concentration of buffer B was changed from 0% at 0 min to 20% at 12 min, 35% at 13 min, 40% at 30 min, 100% at 32 min, and 100% at 35 min. Detection of absorbance was carried out at 254 nm.

Template-Directed Formation of Oligoguanylate (TD Reaction). TD reaction of oligo(G) on a poly(C) template from 2-MeImpG was performed in a solution containing 0.015 M 2-MeImpG, 0.025 M poly(C), 1 M NaCl, 0.2 M $MgCl_2$, 0.1 M 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethansulfonic acid (HEPES) at pH 8.0 in the absence and presence of TCAA. The reaction was monitored for 0–7 d at 25 °C and an aliquot of the sample solution was withdrawn and immediately quenched in liquid nitrogen. Poly(C) template was degraded to cytidine 3′-monophosphate ($C^{3'}p$) using ribonuclease A (RNase A) 6000 unit/100 μL sample for 18 h at 37 °C. Time courses were analyzed on a DNA-NPR column using a gradient of 0.3–1.5 M NaCl at pH 11 buffer. The DNA-NPR column does not separate 2-MeImpG, guanosine 5′-monophosphate ($^{5'}pG$), and $C^{3'}p$, so that samples with and without RNase A treatment were further analyzed on a ODS-2 column using a gradient of 0.005 M NaH_2PO_4 in water at pH 3.5 mixed with 0.01 M NaH_2PO_4 in 40% CH_3OH at pH 4.0. Aggregation and/or quadrahelix formation of oligo(G)s are readily eliminated by these experimental procedures.

Hydrolysis of 2-MeImpG in the Absence and Presence of TCAA. The hydrolysis of 2-MeImpG without poly(C) was performed in an aqueous solution containing 0.015 M 2-MeImpG, 1 M NaCl, 0.2 M $MgCl_2$, and 0.1 M HEPES at pH 8.0 in the absence and presence of TCAA. The reaction was monitored for 0–7 d at 25 °C. Aliquots were withdrawn and analyzed on a ODS-2 column using a gradient of 0.005 M NaH_2PO_4 in water at pH 3.5 mixed with 0.01 M NaH_2PO_4 in 40% CH_3OH at pH 4.0.

Computer Simulation of the TD Reaction. The computer simulation was performed on Mathematica 2.2.2 software.

Results and Discussion

Characterization of TCAA. Preparation, morphology, and prebiotic functions of TCAAs have been widely studied.[26–31] Thermal copolymers of amino acids formed by the procedure shown in the experimental section involve peptide bonding. Thus, in the present study, the characterization of TCAAs was carried out by the molecular weight analysis by GPC and the composition analysis (Tables 1, 2). The molecular weight in Table 1 indicates that about 100 sequences of amino acids were linked within TCAAs. The molecular weight of TCAAs was less dependent on the pore size of dialysis tubing used for the purification of TCAAs. The composition analysis succeeded as shown in Table 2; it was possible to cleave the peptide bonding of TCAA using a conventional hydrolytic procedure in a 6 M HCl solution. Besides, the success of the conventional composition analysis of amino acids of proteins using PITC and the recovery of amino acids by 6 M HCl hydrolysis support the conclusion that TCAAs involve peptide bonding. From the composition analysis of TCAA-Std and TCAA-His, the amino acids used as the starting material for the preparation were detected, but the extent of L-Asp was low. This is probably due to the fact that L-Asp was consumed as a dissolving medium of other amino acids.[12] Although L-Phe, L-Tyr, and L-Trp could not be determined in the simplified analytical method of amino acids, these amino acids should be incorporated.

Influence of TCAA to TD Reaction. The TD reaction is expressed by following pathways:[13,44,45,48]

Table 1. Molecular Weight of Thermal Copolymers of Amino Acids

	MW	MW(low)	MW(high)
TCAA-Std[a)]	13000	12700	15300
TCAA-His[a)]	12400	10400	15300
TCAA-Lys[a)]	12800	11600	15900
TCAA-Cys[a)]	12600	11600	13300
TCAA-Leu[a)]	12600	10400	14600
TCAA-Arg[a)]	12700	10400	14600
TCAA-(Glu–Asp)[a)]	15000	13600	16000
TCAA-Std/1000 MW-cut[b)]	15300	14000	16600
TCAA-Std/12000 MW-cut/Freeze dried[c)]	15600	13400	16300
TCAA-His/1000 MW-cut[b)]	15500	13100	15700
TCAA-His/12000 MW-cut/Freeze dried[c)]	15700	14600	16600
TCAA-Std/3500 MW-cut/Freeze dried[d)]	13400	10900	15300
TCAA-His/3500 MW-cut/Freeze dried[d)]	13300	9300	14500
TCAA-Phe/3500 MW-cut/Freeze dried[d)]	13800	8000	14800
TCAA-Tyr/3500 MW-cut/Freeze dried[d)]	13000	9800	15300
TCAA-Trp/3500 MW-cut/Freeze dried[d)]	13400	8000	15300

a) TCAA was prepared without further purification. b) TCAA was prepared with dialysis tubing at molecular weight 1000. c) TCAA was prepared with dialysis tubing at molecular weight 12000–14000 and lyophilized. d) TCAA was prepared with dialysis tubing at molecular weight 3500 and lyophilized.

Table 2. Amino Acid Compositions of Thermal Copolymers of Amino Acids

	Amino acids composition/%					
	Gly	Ala	Val	Glu	Asp	His
TCAA-Std	30	23	34	12	1	NA[a)]
TCAA-His	18	17	20	13	1	31
TCAA-Trp	20	30	26	18	6	NA[a)]
TCAA-Phe	20	30	26	15	9	NA[a)]

a) Not applied.

$$pG^* \rightarrow pG \quad (1)$$

$$pG + pG^* \rightarrow (pG)_2 \quad (2\text{-}2)$$

$$(pG)_2 + pG^* \rightarrow (pG)_3 \quad (2\text{-}3)$$

$$\cdots$$

$$(pG)_{n-1} + pG^* \rightarrow (pG)_n \quad (2\text{-}n)$$

where pG^* indicates 2-MeImpG and $(pG)_i$ indicates oligo(G)s in length i ($i = 2$–n). Equation 1 shows hydrolysis of pG^* and Eqs. 2-2–2-n show the elongation of oligo(G)s.

TCAAs prepared from different amino acids, such as anionic, cationic, hydrophobic, and hydrophilic amino acids, were selected to test whether or not they show some effects on the TD reaction (Table 3, Fig. 1). TCAAs did not show the catalytic ability to the TD reaction and TCAA-His somewhat catalyzed the 2-MeImpG hydrolysis. Based on our study of the influence of amino acids on the TD reaction, notable inhibitory activity was only detected for His among the 20 common amino acids.[37,40] Thus, the influence of TCAA-His prepared without any purification might contain the influence of remaining His. To clarify the influence of TCAAs, the TCAAs were dialyzed using dialysis tubing. Additionally, it was confirmed by our previous studies that amino acids themselves did not show notable influence except His.[37,40] Thus, the influence of amino acid monomers other than His involved in TCAA mixtures should be eliminated. TCAAs other than TCAA-His did not show any notable effect on the TD reaction, even when TCAAs were dialyzed using 1000 MWCO or 12000–14000 MWCO. However, in the presence of 0.25 M TCAAs, the extent of 2-MeImpG and oligo(G) did somewhat decrease, while the extent of $G^{5'}$ppG was enhanced. The increase of $G^{5'}$ppG will be discussed in a later section. The trend was also shown at pH 7.5 and the enhancement of the formation of $G^{5'}$ppG at lower pH is consistent with previous studies.[7] The TD reaction with TCAA-His at different reaction periods indicates that the extent of oligo(G) in the presence of TCAA-His was very small (Fig. 2). The reaction curves support the conclusions that TCAA-Std does not have notable activity and TCAA-His does have inhibitory activity to the TD reaction. Other types of aromatic amino acids than His did not show inhibitory activity; this fact implies that the activity is due to the activity of imidazole residue. The inhibition by TCAA-His became clearer when TCAA-His was dialyzed using 1000 MWCO or 12000–14000 MWCO.

The TCAA prepared from the mixture of Glu and Asp was briefly investigated in the presence of Mn^{2+}, since Mn^{2+} is required for the RNA polymerase actions.[49] The extent of oligo(G) was enhanced slightly with Mn^{2+}; the extent of 2-MeImpG remaining decreased and that of 2-mer increased. Besides, addi-

tion of poly(Lys) reduced the efficiency of the TD reaction, where a white precipitate was observed and the hydrolysis of 2-MeImpG was enhanced (Table 3). Based on our previous study on the influence of amino acid to the TD reaction, Lys did not show notable inhibitory activity. Thus, the influence of poly(Lys) is not due to the Lys residue and the precipitate might have enhanced the hydrolysis of 2-MeImpG.

Our previous study showed that His monomer has catalytic ability for the hydrolysis of 2-MeImpG.[37,40] Thus, the result means that His residue has hydrolytic activity for the hydrolysis of 2-MeImpG even though it was incorporated in TCAA. To evaluate this assumption, we monitored the degradation of 2-MeImpG in the presence of TCAA; the reaction curves are shown in Fig. 3a. Here, the fact that oligo(G) formation in

Table 3. Extents (%) of $^{5'}$pG, 2-MeImpG, G$^{5'}$ppG, and Oligo(G)s Formed by the Template-Directed Reaction with Different Types of Thermal Copolymers of Amino Acids

Conditions	$^{5'}$pG	2-MeImpG	G$^{5'}$ppG	$(pG)_2$	$(pG)_3$	$(pG)_4$	$(pG)_{5+}$
no TCAA, 3 d	37.6	36.5	0.9	6.3	2.0	1.9	14.8
no TCAA, 7 d	48.9	13.5	1.5	9.6	3.3	2.7	20.5
0.01–0.012 M TCAA, 3 d[a)]							
TCAA-Std	38.6	36.3	0.9	5.6	2.1	1.7	14.9
TCAA-His	43.0	37.8	1.1	3.5	1.7	1.4	11.6
TCAA-Lys	38.9	36.1	0.9	4.0	2.1	1.8	16.1
TCAA-Cys	39.1	35.4	0.9	1.9	1.6	1.7	19.4
TCAA-Leu	41.2	34.1	1.0	5.5	2.0	1.7	14.6
TCAA-Arg	40.1	35.6	1.0	4.6	1.9	1.7	15.0
0.01–0.012 M TCAA, 7 d[a)]							
TCAA-Std	55.0	15.4	1.8	6.0	2.6	2.1	17.1
TCAA-His	56.3	15.5	2.3	4.9	2.5	2.1	16.4
TCAA-Lys	59.6	17.7	1.8	3.8	1.8	1.5	13.8
TCAA-Cys	58.2	15.4	1.7	5.0	2.2	1.8	15.8
TCAA-Leu	55.7	16.1	1.7	5.6	2.3	1.9	16.7
TCAA-Arg	56.3	16.2	1.6	5.4	2.2	1.9	16.4
0.05–0.06 M TCAA, 3 d[b)]							
TCAA-Std	34.9	27.4	1.4	7.1	2.6	3.3	23.4
TCAA-His	57.7	29.2	3.3	1.5	1.0	1.4	5.8
TCAA-Lys	40.5	33.3	1.3	5.4	1.7	2.1	15.7
TCAA-Cys	40.0	31.2	1.3	7.2	2.1	2.2	15.9
TCAA-Leu	39.5	34.0	1.3	5.2	1.7	2.2	16.0
TCAA-Arg	40.7	33.9	1.3	4.6	1.7	2.2	15.7
TCAA-(Glu–Asp)[b)]	38.4	27.9	3.0	9.8	3.3	0.5	17.0
+ 0.0005 M Mn^{2+}[c)]	38.2	18.5	5.7	12.5	5.0	4.2	15.9
+ 0.025 M Mn^{2+}[c)]	34.2	19.0	2.9	16.7	4.3	3.4	19.4
poly-Lys[b,d)]	88.6	8.7	1.5	0.1	0.1	0.1	0.9
TCAA dialyzed with 1000 MW, 3 d[e)]							
0.05 M TCAA-Std[f)]	35.7	43.6	1.6	3.8	2.2	2.3	10.8
0.06 M TCAA-His[f)]	75.4	16.9	4.9	0.3	0.7	1.0	0.9
0.25 M TCAA-Std[f)]	60.7	23.1	10.7	0.3	1.3	1.2	2.7
0.30 M TCAA-His[f)]	84.8	0.7	14.6	0.0	0.0	0.0	0.0
TCAA dialyzed with 12000–14000 MW, 3 d[g)]							
0.05 M TCAA-Std	41.4	38.9	2.0	3.0	1.8	1.9	11.1
0.05 M TCAA-His	62.5	26.3	3.6	0.4	1.0	1.3	4.7
0.0015 M G$^{5'}$ppG[h)]	35.4	43.0	5.9	2.4	1.5	1.7	10.2
0.05–0.06 M, pH 7.5, 3 d[i)]							
no TCAA	64.9	16.6	4.9	3.4	2.4	2.5	5.6
TCAA-Std	67.2	15.6	6.8	3.0	1.7	1.8	4.1
TCAA-His	73.7	12.3	12.9	0.6	0.4	0.1	0.0
TCAA-Tyr	72.4	13.3	6.2	1.8	1.5	2.0	2.8
TCAA-Trp	68.8	13.4	5.8	3.0	2.6	2.8	3.6
TCAA-Phe	71.8	14.6	6.1	2.0	1.3	1.9	2.3
0.15 M TCAA-Std[j)]	67.3	16.9	10.9	1.3	1.1	1.0	1.6
0.18 M TCAA-His[j)]	76.6	5.1	15.2	1.9	1.0	0.3	0.1

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0.05–0.06 M, pH 7.5, 7 d[i)]							
no TCAA	72.9	6.3	6.8	2.5	2.4	3.0	6.0
TCAA-Std	74.3	7.3	7.9	2.6	2.1	2.2	3.7
TCAA-His	81.0	3.1	14.3	0.9	0.6	0.3	0.1
TCAA-Tyr	74.7	5.5	6.7	2.1	2.5	4.0	4.5
TCAA-Trp	80.2	5.7	7.1	1.9	1.6	1.5	2.0
TCAA-Phe	79.8	6.4	7.2	1.2	1.7	1.8	2.0
0.15 M TCAA-Std[j)]	77.2	7.2	10.1	1.6	1.2	1.0	1.7
0.18 M TCAA-His[j)]	80.3	1.3	13.7	4.4	0.2	0.1	0.0

The percentages are the uncorrected HPLC absorbance readings. The concentrations of TCAA and poly(Lys) are based on the amino acid monomer unit. a) 0.01 M TCAA-Std or 0.012 M other TCAA was added to the TD reaction. b) 0.05 M TCAA-Std or 0.06 M other TCAA was added to the TD reaction. c) TCAA-(Glu–Asp) was prepared in the presence of $MnCl_2$. The concentrations indicate the concentrations of Mn^{2+} at the TD reaction. d) 0.05 M Poly(Lys) was added to the TD reaction instead of TCAA. e) TCAAs were purified using 1000 molecular weight cut off dialysis tubing. f) 0.05–0.30 M TCAA-Std or TCAA-His was added to the TD reaction. g) TCAAs were purified using 12000–14000 molecular weight cut off dialysis tubing and lyophilized. h) 0.015 M $G^{5'}$ppG was added to the TD reaction. i) 0.05 M TCAA-Std or 0.06 M other TCAA was added to the TD reaction and the TD reaction was performed at pH 7.5. TCAAs were purified using 3500 molecular weight cut off dialysis tubing and lyophilized. j) 0.15 M TCAA-Std or 0.18 M TCAA-His was added to the TD reaction.

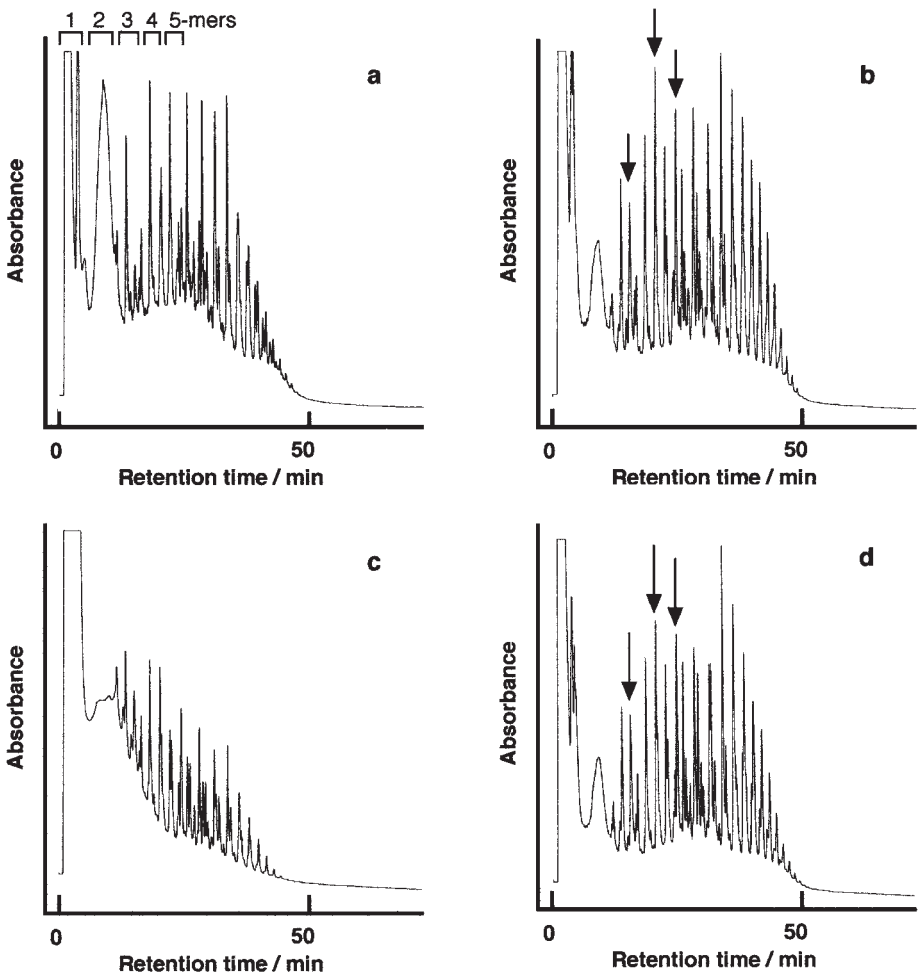

Fig. 1. HPLC profiles for the template directed formation of oligo(G)s in the absence and presence of thermal copolymers of amino acids. [2-MeImpG] = 0.015 M, [poly(C)] = 0.025 M, [NaCl] = 1.0 M, [$MgCl_2$] = 0.2 M, [HEPES] = 0.1 M, pH = 8.0, 25 °C, 3 d. (a), no TCAA; (b), 0.05 M TCAA-Std; (c), 0.06 M TCAA-His; (d), 0.0015 M $G^{5'}$ppG. The concentrations of TCAA are based on monomer unit of amino acids. Arrows indicate $G^{5'}$ppG-capped oligo(G)s.

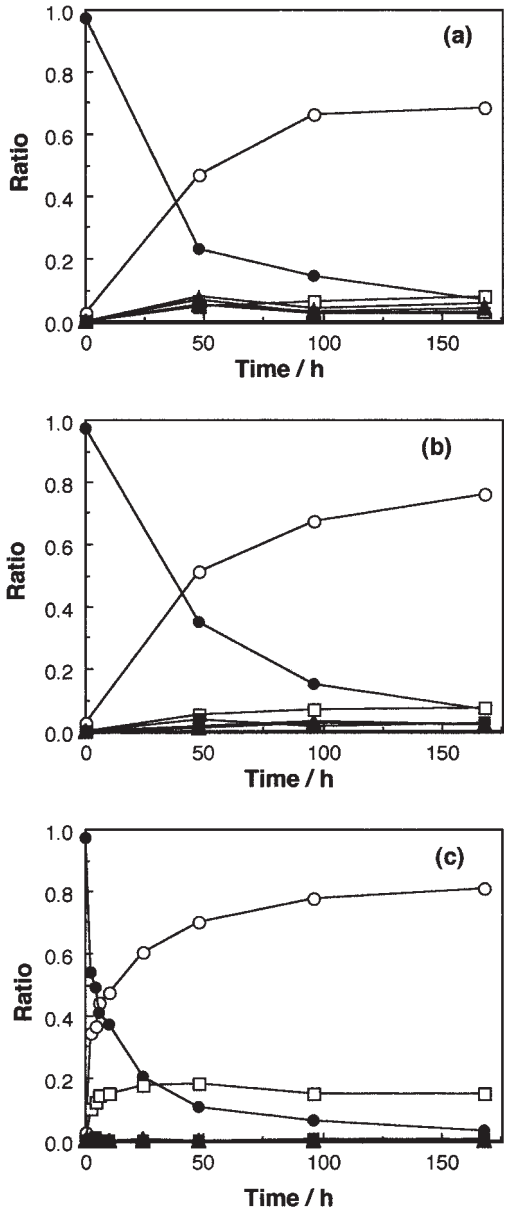

Fig. 2. Reaction curves of the TD reactions. The reaction conditions are the same as shown in Fig. 1. (a), no TCAA; (b), 0.05 M TCAA-Std; (c), 0.06 M TCAA-His. Open circles: $^{5'}$pG, closed circles: 2-MeImpG, open squares: G$^{5'}$ppG, closed squares: 2-mers, open triangles: 3-mers, closed triangles: 4-mers, +: 5-mers and longer oligomers.

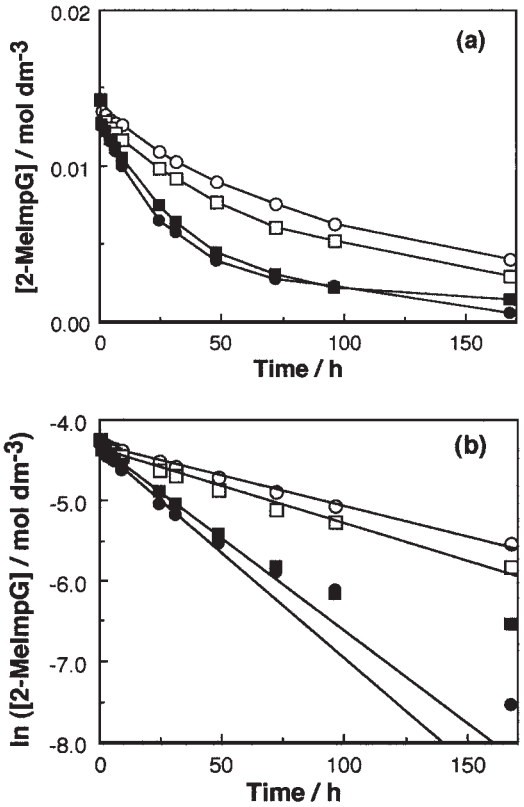

Fig. 3. Reaction curves and pseudo-first-order rate plots for the hydrolysis of 2-MeImpG in the absence and presence of TCAA. [2-MeImpG] = 0.015 M, [NaCl] = 1.0 M, [$MgCl_2$] = 0.2 M, [HEPES] = 0.1 M, pH = 8.0, 25 °C. (a), reaction curves; (b), pseudo-first-order rate plots. Open circles: no TCAA; open squares: 0.05 M TCAA-Std prepared by heating for 2 h at 180 °C, purified with 12000–14000 molecular cut-off dialysis tubing, and lyophilized; closed circles: 0.05 M TCAA-His prepared by heating for 2 h at 180 °C, purified with 12000–14000 molecular cut-off dialysis tubing, and lyophilized; closed squares: 0.05 M TCAA-His prepared by heating for 24 h at 180 °C, purified with 3500 molecular cut-off dialysis tubing, and lyophilized.

the absence of a poly(C) template is very poor reflects the fact that the oligo(G) formation is a template-directed reaction, which is consistent with the conclusions shown in previous studies.[3–8,41–43] Thus, the reaction curves indicate that the hydrolytic degradation of 2-MeImpG was accelerated with TCAA-His, but that it was less accelerated with TCAA-Std. These results reflect that TCAA-Std did not show the notable inhibitory activity but TCAA-His showed somewhat strong inhibitory activity. The half-lives of the 2-MeImpG were 80 h in the absence of TCAA, 57 h in the presence of TCAA-Std, and 21–27 h in the presence of TCAA-His. Pseudo-first-order rate plots of the disappearance of 2-MeImpG suggest that the reaction obeys a first-order-process at the beginning of the reaction (Fig. 3); later it seems to deviate somewhat from the first-order process. The pseudo-first-order rate constants of the 2-MeImpG hydrolysis were $(2.1 \pm 0.1) \times 10^{-6}$ s^{-1} in the absence of TCAA, $(2.6 \pm 0.1) \times 10^{-6}$ s^{-1} in the presence of TCAA-Std, and (5.9 ± 0.2)–$(6.3 \pm 0.3) \times 10^{-6}$ s^{-1} in the presence of TCAA-His. The second-order rate plots for the 2-MeImpG hydrolysis with TCAA-His yielded somewhat better fits at the end of the reaction. This may indicate that the hydrolysis of 2-MeImpG in the presence of TCAA-His involves a second-order rate process. This fact would be consistent with the result that the hydrolysis of 2-MeImpG follows a second-order rate process in the presence of free His.[40]

In order to evaluate how the difference of the hydrolysis rate constants reflects the difference of the yield of oligo(G), a computer simulation was performed on the basis of a previous kinetic analysis. The reaction models expressed by Eqs. 1 and 2-2–2-*n* were used and the values of k_{hy} for the reaction (1)

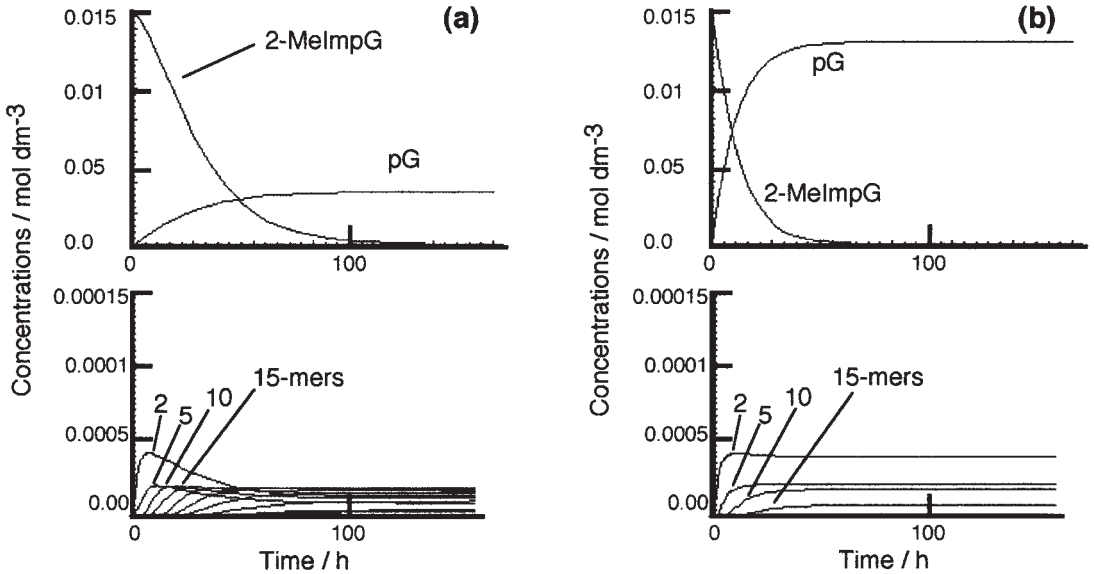

Fig. 4. Computer simulations of the TD reactions. Calculations were carried out on the basis of the reaction model shown in Eqs. 1, 2-2–2-*n*. Oligo(G)s up to 45-mer in length were involved in the model. The values of the rate constants: (a) $k_{hy} = 2 \times 10^{-6}$ s^{-1}, $k_2 = 3 \times 10^{-5}$ M^{-1} s^{-1}, $k_3 = 1 \times 10^{-2}$ M^{-1} s^{-1}, k_4–$k_{45} = 2 \times 10^{-2}$ M^{-1} s^{-1}; (b) $k_{hy} = 2 \times 10^{-5}$ s^{-1} and all others are the same as (a).

was varied to demonstrate the extent of oligo(G) when the values of k_2, k_3, and k_{4+} for the processes given in Eqs. 2-2–2-*n* were fixed. The reaction rate constants were simplified on the basis of the previous data.[44] The extent of oligo(G) was notably reduced where 10 times of k_{hy} was applied. The simulation shown in Fig. 4 supports the conclusion that the decrease of the yield of oligo(G) in the presence of TCAA-His is due to the enhancement of hydrolysis of 2-MeImpG by TCAA-His. While 2-MeImpG is not a biologically essential compound in living organisms, it is considered as a prebiotic molecule.[50,51] Thus, the hydrolysis of 2-MeImpG catalyzed by TCAA-His can be regarded as an example of a prebiotic enzymatic reaction model composed entirely of prebiotic molecules corresponding to RNA monomer and protein.

The extent of $G^{5'}$ppG increased in the presence of TCAAs; additional peaks were observed in HPLC charts when TCAA was added (Fig. 1d, Table 3). The enhancement of $G^{5'}$ppG was observed for both TCAA-Std and TCAA-His, especially at high concentrations. Although the extent of oligo(G) was very low in the presence of TCAA-His, the yield of $G^{5'}$ppG was as high as that observed in the presence of TCAA-Std. We suspected that oligo(G)s would have formed and showed HPLC peaks which are due to $G^{5'}$ppG and $G^{5'}$ppG capped oligo(G)s. This was confirmed by the TD reaction that was analyzed in the absence of TCAA but in the presence of 0.0015 M $G^{5'}$ppG as a starting additive. The comparison of HPLC profiles in the presence of TCAA and $G^{5'}$ppG supports the conclusion that the HPLC peaks that newly appeared in the presence of TCAA are consistent with those that appeared in the presence of $G^{5'}$ppG. The enhancement of the formation of $G^{5'}$ppG may be a general trend using TCAAs prepared in this study to the TD reaction, since the extents of $G^{5'}$ppG in the presence of other types of TCAAs were also high. This fact indicates that the structures of TCAA-Std and TCAA-His are similar. In addition, the extent of $G^{5'}$ppG somewhat increased without poly(C) in the presence of TCAA. Conclusively, the formation of $G^{5'}$ppG is enhanced by TCAA where oligo(G) elongates from $G^{5'}$ppG on a poly(C) template.

On the Cooperative Chemical Evolution of Proteins and Nucleotides. There were two findings on the activity of TCAAs, that is, (1) the inhibitory activity by TCAA-His and (2) the enhancement of the formation of $G^{5'}$ppG and $G^{5'}$ppG capped oligo(G) by the different TCAAs. A number of studies other than the present study have been carried out to find possible catalytic activities of protein-like molecules and some catalytic abilities were demonstrated. Conclusively, these catalytic abilities were normally weak, in contrast to those of the modern enzymes. The difference of the catalytic activities between real enzymes and protein-like molecules is large; a real enzyme indeed accelerate reactions 10^{17} times at maximum.[52] Although artificial ribozymes made by in vitro selection techniques and clay minerals as prebiotic catalysts provide fairly large catalytic abilities, which achieve about 10^5 fold acceleration for the case of clay catalyst and about 10^7 fold acceleration for the case of artificial ribozymes,[13,53–55] these activities are still much smaller than those achieved by protein enzymes.

Thus, based on the fact that few large catalytic activities have been discovered by laboratory experiments, it is reasonable to consider that there should have been connecting pathways of the amplification of enzymatic activities of prebiotic protein-like molecules. In our previous study, we hypothesized that proficient enzymatic activities could not have emerged unless the catalytic molecules were made by highly organized synthetic methods of the catalysts. This presumption is supported by the present study.

Thus, at the same time, it would be important to assume connecting pathways of the synthetic method of catalysts between the modern proteins and the primitive protein-like molecules. As a connecting mechanism, it would be reasonable to assume an amplification mechanism of the catalytic activities of pro-

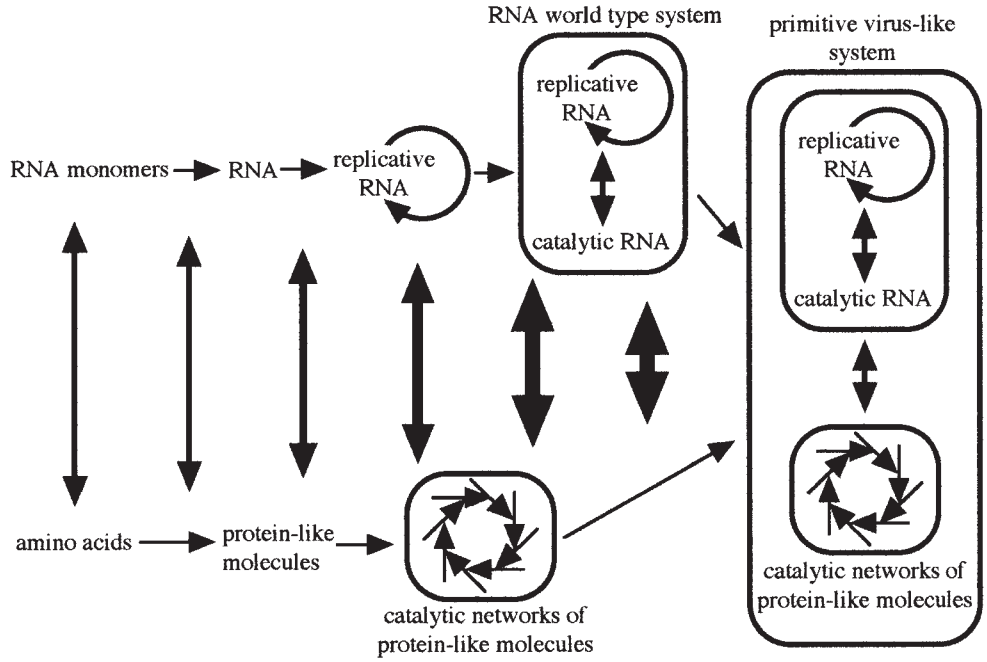

Fig. 5. Schematic diagram of a scenario of the chemical evolution of nucleic acids and proteins.

tein-like molecules during chemical evolution. A scenario of the chemical evolution of the synthesis assembly of catalysts is illustrated in Fig. 5. First, RNA molecules spontaneously formed and then RNA molecules formed a RNA world type system. On the other hand, protein-like molecules spontaneously formed and resulted in a closed network that consisted of catalytic reactions, independently from the construction of RNA reaction networks.[36,38,56,57] In this type of network, it should be hypothesized that the network should have more or fewer amplification pathways of catalytic abilities through the catalytic network. As the second stage, the cooperation of the chemical evolution between amino acids and nucleic acids has gradually increased. A rigid assignment method between nucleic acids and proteins could have formed in virus-like systems, which have been presumably constructed by the continuous association between nucleic acids and protein-like molecules.[24,25]

Conclusions

There have been few studies on the role of protein-like molecules on the prebiotic formation of RNA. In this study, the influence of TCAA to the template-directed formation of oligo(G) on a poly(C) template was investigated and the characterization of TCAAs was performed. The inhibitory activity of TCAA-His was detected to the template-directed formation of oligo(G) on a poly(C) template; other types of TCAA do not have strong activity for either acceleration or inhibition of the TD reaction. This systematic study included a variety of thermal copolymers of amino acids, so it would provide insight into designing more sophisticated experiments to evaluate the relationship between the chemical evolution of protein and RNA. The present study has provided a hypothetical scenario on the possible relationship between the chemical evolution of RNA and protein.

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