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CATALYTIC ACTIVITIES OF [GADV]-PEPTIDES

Formation and Establishment of [GADV]-Protein World for the Emergence of Life

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Abstract. We have previously postulated a novel hypothesis for the origin of life, assuming that life on the earth originated from "[GADV]-protein world", not from the "RNA world" (see Ikehara's review, 2002). The [GADV]-protein world is constituted from peptides and proteins with random sequences of four amino acids (glycine [G], alanine [A], aspartic acid [D] and valine [V]), which accumulated by pseudo-replication of the [GADV]-proteins. To obtain evidence for the hypothesis, we produced [GADV]-peptides by repeated heat-drying of the amino acids for 30 cycles ([GADV]-P_{30}) and examined whether the peptides have some catalytic activities or not. From the results, it was found that the [GADV]-P_{30} can hydrolyze several kinds of chemical bonds in molecules, such as umbelliferyl-β-D-galactoside, glycine-*p*-nitroanilide and bovine serum albumin. This suggests that [GADV]-P_{30} could play an important role in the accumulation of [GADV]-proteins through pseudo-replication, leading to the emergence of life. We further show that [GADV]-octapaptides with random sequences, but containing no cyclic compounds as diketepiperazines, have catalytic activity, hydrolyzing peptide bonds in a natural protein, bovine serum albumin. The catalytic activity of the octapeptides was much higher than the [GADV]-P_{30} produced through repeated heat-drying treatments. These results also support the [GADV]-protein-world hypothesis of the origin of life (see Ikehara's review, 2002). Possible steps for the emergence of life on the primitive earth are presented.

Keywords: origin of life, [GADV]-protein world hypothesis, pseudo-replication of [GADV]-proteins, prebiotic synthesis, chemical evolution, peptide catalyst, primitive enzyme

1. Introduction

It is now well known that extant organisms live fundamentally through a genetic flow from DNA (genetic information carrier) to proteins (biocatalysts for many reactions in organisms) *via* RNA (messenger of the genetic information). Therefore, it is generally considered that replication of genetic materials is the most fundamental and important for organisms to survive. But, the genetic information in the DNA of extant organisms is propagated and maintained by proteineous replication enzymes. So, it is extremely difficult to explain the origin of life from the standpoint of present life, because DNA does not replicate without the enzymes with catalytic functions required to replicate DNA, whereas proteins cannot be produced without DNA

and proteins cannot be used as genetic materials. That is, there is an apparent contradiction between DNA and proteins, the so-called chicken-and-egg problem of the origin of life.

About 20 years ago, the RNA-world scenario was postulated to resolve the chicken-and-egg problem, based on the unanticipated discovery of RNA catalysts or ribozymes (Gilbert, 1986; Gesteland *et al.*, 1999). In this hypothesis, it is considered that self-replicating RNA preceded the emergence of functional proteins under the assumptions concerning prebiotic chemistry, such as facile synthesis of the building blocks of RNA or nucleotides, and the spontaneous assembly of these building blocks to form RNA sequences. The scenario has also assumed that at least one of the RNA sequences was a ribozyme that was able to self-replicate. From many recent experiments in prebiotic chemistry, however, there is a growing number of dissenting voices calling this optimistic picture into question (Shapiro, 1984, 1988, 2000; De Duve, 1992; Orgel, 1994; Joyce, 1994).

During the last decade we have proposed several hypotheses: origination of genes (Ikehara and Okazawa, 1993; Ikehara *et al.*, 1996), a primitive genetic code (Ikehara and Yoshida, 1998; Ikehara *et al.*, 2002) (Figure 1), and a 0th-order structure hypothesis on the origin of proteins (Ikehara, 2001, 2002). We have also provided a novel [GADV]-protein-world hypothesis which suggests that life originated from the [GADV]-protein-world (Ikehara, 2001, 2002). Where [G], [A], [D], and [V]

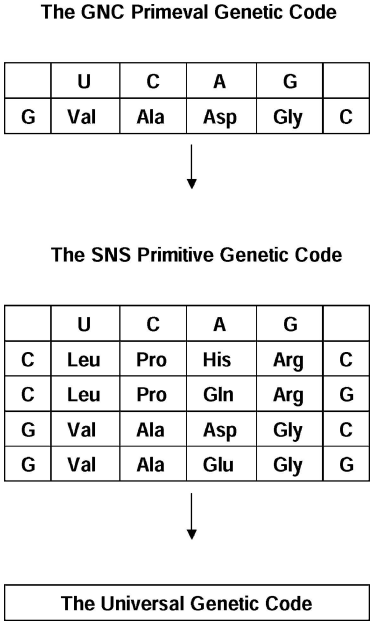

Figure 1. GNC-SNS primitive genetic code hypothesis, which we have proposed (Ikehara *et al.*, 2002). The hypothesis postulates that the universal genetic code originated from the GNC primeval code (4 codons and 4 amino acids) through the SNS primitive code (16 codons and 10 amino acids).

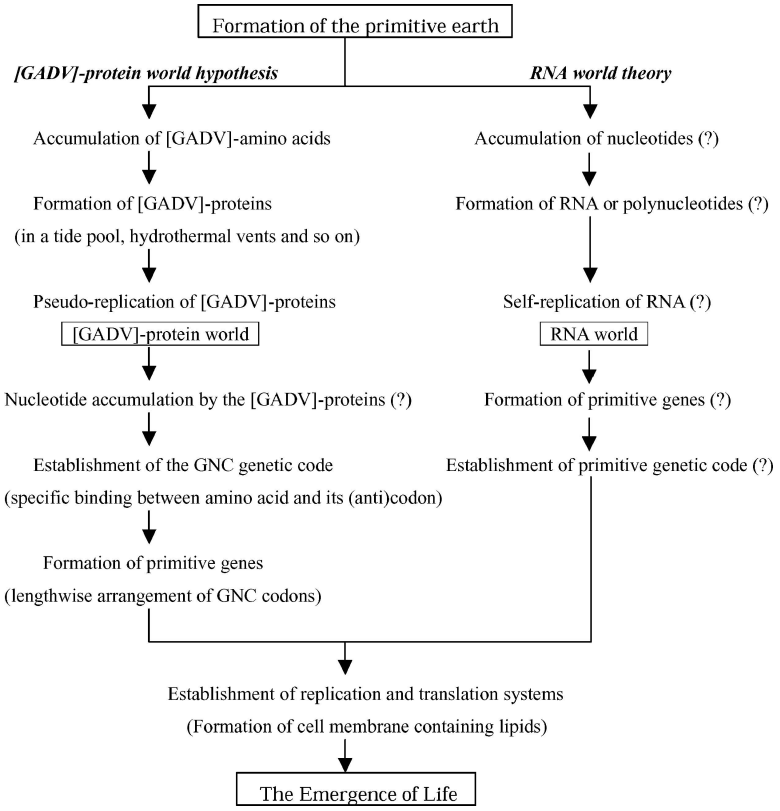

Figure 2. Two possible routes to the emergence of life, illustrating the [GADV]-protein world hypothesis and the RNA world theory. Left route: [GADV]-protein world hypothesis on the origin of life, which we have proposed. The hypothesis anticipates that life originated from [GADV]-protein world composed of [GADV]-proteins, which were amplified by pseudo-replication in the absence of genetic function. The simple amino acid composition of [GADV]-proteins could make it possible to pseudo-replicate the most primitive proteins. Right route: RNA world theory, which is widely accepted at the present time to explain the origin of life. The theory is based on self-replicability of RNA, but it is considered that there are many weak points (marked with (?)) on the route to the emergence of life.

mean Gly, Ala, Asp, and Val, respectively. The [GADV]-protein-world is composed of versatile [GADV]-proteins accumulated through pseudo-replication of the proteins in the absence of genes (Figure 2). The term, "pseudo-replication", means that not the same but similar [GADV]-proteins are synthesized by [GADV]-protein catalysts through random assembly of [GADV]-amino acids. This hypothesis is favored over the RNA-world theory (Gilbert, 1986) because many prebiotic syntheses of simple amino acids (Miller, 1953; Harada and Fox, 1964; Miller and Orgel, 1973; Sakurai and Yanagawa, 1984; Miyakawa *et al.*, 1998, Takano *et al.*, 2001) and peptides (Yanagawa and Kojima, 1985; Ito *et al.*, 1990; Imai *et al.*, 1999a, b) have been

demonstrated, while abiotic nucleotide syntheses and formation of phosphodiesters leading to RNA synthesis (Gesteland *et al.*, 1999) are totally lacking.

Thus, we firstly examined whether [GADV]-peptides formed by repeated heat-drying treatments for n cycles ([GADV]-P_n), which simulated phenomena occurring in a tide-pool on the primitive earth, could exhibit some catalytic activities, especially protease activity, or not. The suffix, n, means the number of repeated heat-drying treatments. However, the [GADV]-P_n should contain cyclic compounds as diketopiperazines. Of course, the detection of peptide bond-hydrolytic activity on the [GADV]-P_n would be important to confirm the possibility of the [GADV]-protein world in an era before the emergence of the most primitive genetic code such as the GNC-primeval genetic code (Figure 1), because, during the era, [GADV]-peptides should be produced by physical and chemical processes, and the earliest [GADV]-peptides would inevitably contain cyclic compounds as diketopiperazines. That protease activity also indicates that [GADV]-P_n could catalyze the formation of [GADV]-peptides or [GADV]-proteins owing to the principle of microscopic reversibility of a catalytic reaction. The activity of the [GADV]-P_n, therefore, produced by repeated heat-drying cycles, could have contributed to the formation of a [GADV]-protein world (Figure 2). It also suggests that the cyclic compounds, as diketopiperazines, incorporated into [GADV]-P_n might play a role in both hydrolysis and formation of peptide bonds.

On the other hand, [GADV]-peptides or [GADV]-proteins, which were synthesized in the era after the establishment of the most primitive genetic code as GNC code (Figure 1), must be formed only with peptide bonds among [GADV]-amino acids. Therefore, it is also important to learn if cyclic compounds as diketopiperazines were required for the catalyitic acivity found in the [GADV]-P_{30}, because the repeated heat-drying cycles used to obtain the [GADV]-peptides would be expected to also form diketopiperazines from cyclizations of [GADV]-amino acids, as described above. The present study was undertaken to provide experimental evidence bearing on this question. We did this through random formation of [GADV]-peptides under conditions where cyclic compounds do not form. We were able to show that, while the cyclic compounds as diketopiperazines possibly may contribute to the catalytic activity, its presence in the [GADV]-peptides was not required. This indicates that catalytic activity of the [GADV]-random peptides for polymerizing amino acids as the reverse reaction of hydrolysis of peptide bonds could make it possible to establish the [GADV]-protein world for the emergence of life (Figure 2).

2. Experimental Procedures

2.1. CHEMICALS

Glycine, alanine, aspartic acid and valine for [GADV]-P_n synthesis were purchased from Wako-Jyunyaku Cemicals Co. Fmoc (9-fluorenylmethyloxycarbonyl) wang

resin (C-terminal amino acid on resin), Fmoc-glycine, Fmoc-alanine, Fmoc-aspartic acid, Fmoc-valine, 1-[Bis(dimethylamino)methylene]-1*H*-benzotriazolium 3-oxide hexafluorophosphate (HBTU: coupling reagent), 6-Chloro-1-hydroxy-benzotriazole (6-Cl-HOBT: coupling reagent), N,N-diisopropylethylamide (DIEA: coupling reagent), piperidine (deblocking reagent), and dimethyl-formamide (DMF: solvent for cleaning reaction products), which are reagents for solid phase peptide synthesis, were purchased from Wako Jyunyaku Chemicals Co.

Substrates for measurements of catalytic activities of [GADV]-P_n, 4-methylumbelliferyl-β-D-galactopyranoside (MetU-Gal) and glycine-*p*-nitroanilide (Gly-pNA), were also obtained from Wako-Jyunyaku Cemicals Co. Substrate for detection of protease activity of [GADV]-peptides, bovine serum albumin (BSA), was purchased from Nakarai Tesque, Inc.

2.2. Synthesis of [GADV]-peptides

To produce [GADV]-P_n by repeated heat-drying treatments, glycine, alanine, aspartic acid and valine were solubilized at a concentration of 10 μmole/ml in 1 ml aqueous solution containing 36 μmole/ml $CuCl_2$ and 0.3 mmole/ml NaCl and were repeatedly dry-heated (Shuwannachot and Rode, 1998). In each cycle, the solution was heated at 90 °C for more than 3 h to evaporate it to dryness, and 0.6 ml of water was added to the dried sample. The treatment was repeated for 30 times, unless otherwise described. Peptide bond formation among glycine, alanine, aspartic acid and valine was followed by the Lowry method (Lowry *et al.*, 1951).

[GADV]-random octapeptides were synthesized from C-terminal Fmoc-amino acid on resin with Shimadzu peptide synthesizer, PSSM-8, by stepwisely adding equal amounts of four Fmoc-amino acids (Fmoc-glycine, Fmoc-alanine, Fmoc-aspartic acid and Fmoc-valine). The treatments were automatically repeated seven times in the synthesizer.

Molecular weight and amino acid composition of the products were analyzed by DE Biosystems Voyager System MALDI TOF Mass spectrometer and Hitachi L8500 amino acid analyzer, respectively.

2.3. Absorption and fluorescence spectroscopy

UV absorption and visible-ultraviolet absorption spectra of [GADV]-P_n and [GADV]-random octapeptides were obtained with Shimadzu UV-2400PC spectrophotometer. Changes induced during the treatments of the [GADV] solution were monitored by UV light absorption at 245 nm in the experiments described in this paper. In addition, fluorescent emission from the [GADV]-P_{30} was observed with Hitachi F2500, using 320 nm UV light for excitation of the peptides.

2.4. Gel filtration chromatography

To estimate approximate molecular size of the [GADV]-P_{30} or [GADV]-random octapeptides, gel filtration chromatography of the sample in 0.2 ml of H_2O was performed with Sephadex G25. The inside diameter and the total volume of the column were 0.5 cm and 10 cm^3, respectively. The sample was eluted from the column with distilled water. 10 drops of the eluate were collected in every test-tube with an automated fraction collector, and absorption at 245 nm for [GADV]-P_n or 230 nm for [GADV]-octapeptides was measured after addition of 0.7 ml of water to the tubes, respectively.

2.5. Catalytic activity mesurements of [GADV]-peptides

Reaction mixtures were prepared by addition of MetU-Gal and Gly-pNA into [GADV]-P_{30} solution at a final concentration of 1.6 mg/ml and 0.1 mg/ml, respectively. Hydrolysis of MetU-Gal by the [GADV]-P_{30} was traced by the fluorescence emission spectrum of 4-methylumbelliferon (MetU) accumulated in the solution. The amount of *p*-nitroaniline (pNA) accumulated by hydrolysis of Gly-pNA was measured by UV-absorption at 390 nm.

Hydrolytic activity of the [GADV]-P_{30} or [GADV]-random octapeptides toward natural peptide bonds was analyzed by disappearance of intact protein band and the concomitant appearance of peptide fragments released from a natural protein, bovine serum albumin (BSA). After the chromatography, 16 μl of BSA (1 mg/ml) and 10 μl of 0.4 M $CuCl_2$ were added into 0.16 ml of the fraction. Detection of bands of the intact BSA and the fragments was carried out with SDS polyacrylamide gel electrophoresis, followed by Coomassie brilliant blue staining.

3. [GADV]-Peptides Produced by Heat-Drying Cycles

3.1. Synthesis of [GADV]-P_n

After repeated heat-drying of [GADV] solution containing $CuCl_2$ and NaCl, the color of the mixture gradually changed from faint blue to yellowish green and fluorescence emission from the sample intensified as the number of heat-drying cycles increased (Figure 3). This suggests that fluorochromes must be formed from [GADV]-amino acids through cyclic molecules as diketopiperazines in the solution, in addition to [GADV]-P_n, and that various compounds could be produced from [GADV]-amino acids complexed with metal ions in the solution during the heat-drying treatments. Accompanied by the color development, the number of peptide bonds, which were measured by Lowry method, increased, as heat-drying treatments were repeated (Figure 4). Peptide bond formation upon the repeated heat-drying process decreased in order of glycine, alanine and aspartic acid and

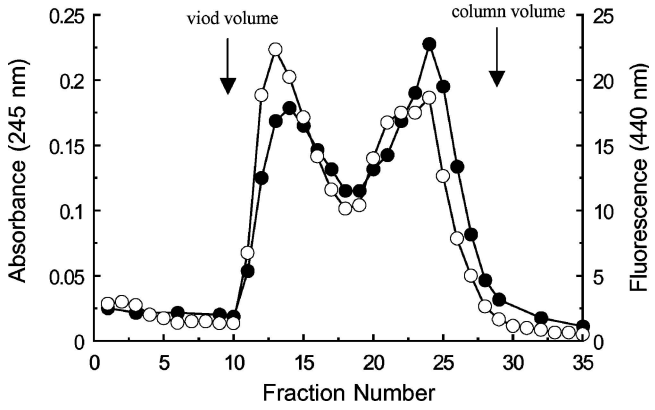

Figure 3. Sephadex G25 gel filtration chromatography of [GADV]-peptides produced by the repeated heat-drying for 30 times. Open circles and closed circles indicate absorbance at 245 nm (left axis) and fluorescence at 440 nm (right axis), respectively.

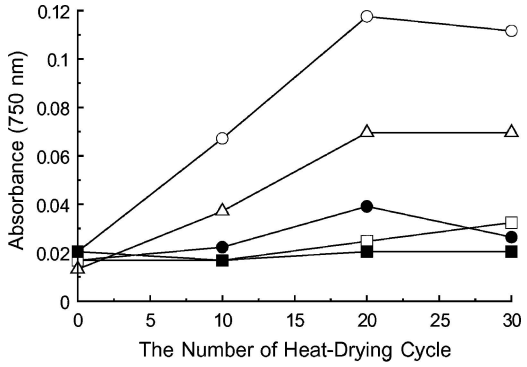

Figure 4. Peptide bond formation among amino acids during repeated heat-drying process. The peptide formation was monitored by Lowry method. Absorbance at 750 nm of the samples obtained by heat-drying treatments of Gly (open circles), Ala (closed circles), Asp (open squares) and Val (closed squares) was shown in this figure. Open triangles indicate the results of [GADV]-peptides obtained by the same experimental procedures.

valine solutions. This suggests that steric hindrance between amino acids might inhibit the peptide bond formation.

3.2. Estimation of apparent molecular weight of [GADV]-P_{30} with gel filtration chrpmatography

Gel filtration column chromatography was carried out to estimate apparent molecular weight of [GADV]-P_{30}, which was obtained by heat-drying treatments for 30 times. Two peaks were observed when the [GADV]-P_{30} were subjected to a

Sephadex G25 column. In addition, when the elution position of the aggregates were compared with molecular size markers (blue dextran and riboflavin) and the fractionation range (5,000∼1,500) of the Sephadex gel, it was estimated that molecular weights of the [GADV]-P_{30} are at least more than 1,500 Da (Figure 3). Judging from the elution profiles showing two peaks and molecular weights (525, 539, 657) of the main products in [GADV]-P_{30} measured by MALDI-TOF MASS, it is suggested that several molecules of peptides formed aggregates in an aqueous solution.

3.3. CATALYTIC ACTIVITIES OF [GADV]-P_{30}

Next, we examined whether the [GADV]-P_{30} has catalytic abilities to hydrolyze the β-galactoside bond in MetU-Gal and amide bond (peptide bond) in Gly-pNA or not. The results clearly show that the [GADV]-P_{30} can hydrolyze the chemical bonds in the substrates, resulting in production of MetU (Figure 5A) and pNA (Figure 5B), respectively. In the control experiments carried out in the absence of [GADV]-P_{30}, both the glycosidic linkage and the amide bond were not substantially hydrolyzed.

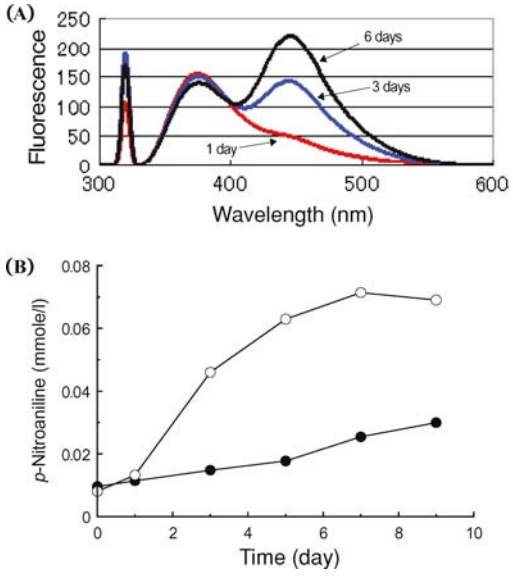

Figure 5. (A) Hydrolysis of β-glycoside bond in MetU-Gal with [GADV]-P_{30} produced by repeated heat-drying. Fluorescence spectra of the samples excited with UV light at 320 nm, which were obtained after holding a mixture of [GADV]-peptides and the substrate at 37 °C for 1, 3 and 6 days, respectively, are shown. (B) Hydrolytic activity of amide (peptide) bond in Gly-pNA with [GADV]-P_{30}. Open and closed circles represent absorbance at 390 nm after incubation of the substrate in the presence and absence of the peptides, respectively.

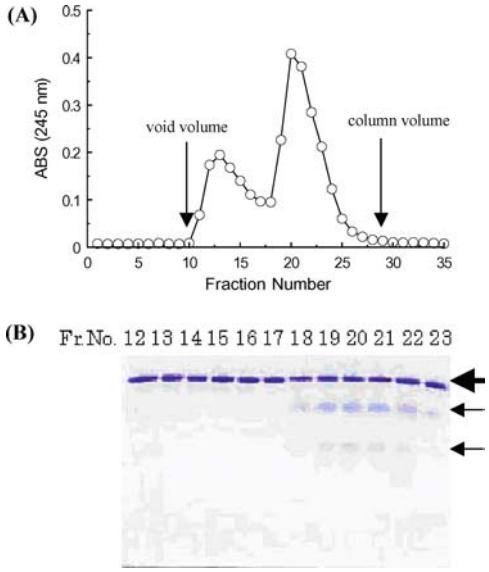

Figure 6. Hydrolytic activity of [GADV]-peptides, which were produced by repeated heat-drying for 30 times, toward peptide bonds in a natural protein (BSA). The mixtures of BSA and the each fraction from the Sephadex G25 chromatography (A) were incubated at 37 °C for six days. The results of SDS polyacrylamide gel electrophoresis are given in (B). Numbers written at the top of the gel indicate the fraction number of the chromatography. Bold arrow and thin arrows show positions of the native protein and hydrolytic fragments of the protein, respectively.

Furthermore, to confirm a catalytic activity of the [GADV]-P_{30} hydrolyzing peptide bonds in a natural protein, a mixture of BSA and the [GADV]-P_{30} in each fraction eluted from the Sephadex column was held at 37 °C for several days. As it was expected that [GADV]-octapeptides have a low catalytic activity, Sephadex gel filtration of the peptides was carried out to remove possible contaminants of proteases and low molecular weight chemical compounds, which might have catalytic activity toward peptide bonds,. The treatments were also effective to confirm reproducibility of a probable low catalytic activity of the peptides (Figure 6A). The results analyzed with SDS-polyacrylamide gel electrophoresis after holding the mixture for 6 days are given in Figure 6B. Figure 6B clearly shows that [GADV]-P_{30} in an unaggregated form possesses hydrolytic activity toward the peptide bonds in the natural protein, BSA.

4. [GADV]-Random Octapeptides Produced by Solid Phase Synthesis

4.1. Properties of [GADV]-random octapeptides

[GADV]-octapeptides with random sequences were synthesized with a peptide synthesizer to mimic [GADV]-peptides, which could be synthesized on the primitive

earth under the earliest genetic code, GNC (Figure 1). Absorption of the sample decreased monotonically as the wavelength decreases (data not shown). The reason why we synthesized octapeptides in the experiments is that eight is a multiple of four of [GADV]-amino acids, so that they contain equal amounts of the four amino acids, and there is not any other reason.

Peaks with mass differences 13–16 from each other were observed around main peaks with mass numbers 625–739 by mass spectrometry of the octapeptides (data not shown). This indicates that the octapeptides are a mixture of peptides with random sequences composed of the [GADV]-amino acids, as was expected, from the calculated molecular weight (702) of $[G_2A_2D_2V_2]$-octapeptides.

Amino acid analysis of the [GADV]-octapeptides showed that the octapeptides as predicted contained about equal amounts of the four amino acids, Gly, Ala, Asp and Val (data not shown). In contrast to the [GADV]-random peptides produced by repeated heat-drying described in the above section, the [GADV]-peptides synthesized with a peptide synthesizer did not emit any fluorescence under application of ultraviolet light (data not shown), indicating that the peptides produced with the solid phase synthesis did not contain any fluorochromophore, or cyclic compound.

4.2. Gel filtration chromatography of the [GADV]-random octapeptides

Sephadex G25 column chromatography of the random octapeptides was carried out to estimate apparent molecular weight and aggregated state of the peptides. A single main peak at fraction number 14–15 was observed when the [GADV]-random peptides were subjected to Sephadex G25 chromatography (Figure 7A). By comparison of the peak position with those of molecular size markers (blue dextran and riboflavin) and the fractionation range of the Sephadex gel, it was estimated that the apparent molecular weight of the peptides is larger than that of the peptides produced by repeated heat-drying (more than 1500 Da). From the elution position and the apparent molecular weight, it was estimated that more than three molecules of the peptides (average molecular weight; 702) formed aggregates in an aqueous solution probably through hydrophobic interaction among side chains of valine in the peptides. The hypothesis was confirmed by Sephadex gel filtration chromatography of the [GADV]-random octapeptides in the presence of 4 M urea (Figure 7A), indicating that aggregates were dissociated into monomers by the addition of 4 M urea. Judging from that only about 30% of the aggregates were dissociated by 4 M urea, it is also supposed that [GADV]-random octapeptides formed rather stable aggregates like native proteins.

4.3. Catalytic activities of [GADV]-random octapeptides

Next, to examine whether the [GADV]-random octapeptides have catalytic activity hydrolyzing peptide bonds in a natural protein, a mixture of BSA and the

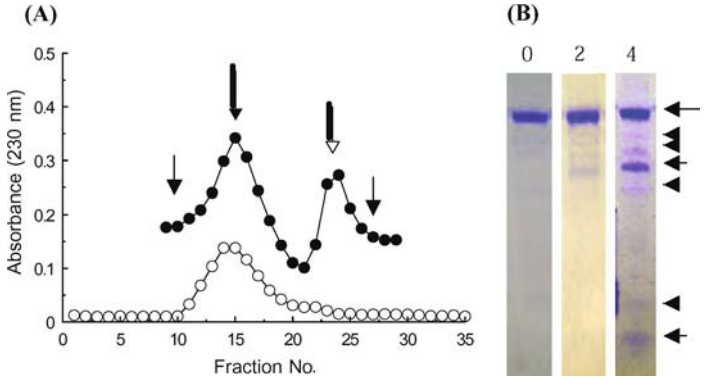

Figure 7. (A) Sephadex G25 gel filtration chromatography of [GADV]-random octapeptides. Open and closed circles indicate absorbance at 230 nm of the octapeptides in the absence of and the presence of 4 M urea, respectively. Bold arrows with open and closed arrow heads denote the positions of aggregated and dissociated forms of the random octapeptides, respectively. Left and right thin arrows indicate the elution positions of void volume and column volume estimated with blue dextran and riboflavin, respectively. (B) Hydrolytic activity of [GADV]-octapeptides, which were produced by a protein synthesizer, toward peptide bonds in a natural protein (BSA). The mixture of BSA and the peptides in a peak fraction (fraction number 14) from the Sephadex G25 chromatography was incubated at 37 °C for 0, 2 and 4 days. Numbers written at the top of the electropherograms indicate the number of days, for which the sample was held at 37 °C. Long arrow, short arrows, and arrow heads indicate the bands of native BSA, major and minor fragments of the protein, respectively.

[GADV]-peptides in a fraction (fraction number; 14) from Sephadex G25 column chromatography was held at 37 °C for several days. The results obtained by SDS-polyacrylamide gel electrophoresis of the samples, which were withdrawn after holding the mixture for 2 and 4 days, are given in Figure 7(B). From this analysis, it was found that several fragments of BSA hydrolyzed by the octapeptides were observed in the samples analyzed after 4 days incubation at 37 °C. The results indicate that the [GADV]-random octapeptides possess hydrolytic activity toward peptide bonds in the protein. Hydrolytic activities were also observed in fractions around the peak position (fraction number 14) of the column chromatogram (data not shown). In addition, it was found that the hydrolytic activity is much higher than that of [GADV]-peptides prepared by the repeated heat-drying treatments (Figure 6B). From the data, it can be concluded that even [GADV]-peptides without cyclic compounds as diketopiperazine can exhibit catalytic function of hydrolysis toward peptide bonds. A high hydrolytic activity of the random octapeptides suggests that appearance of the [GADV]-proteins produced after the GNC primeval genetic code would accelerate the steps to the emergence of life at a much higher rate than that by [GADV]-proteins produced before establishment of the primeval genetic code.

5. Discussion

Several papers have previously reported the formation of peptides from amino acids by heat-drying and by a flow reactor simulating deep-sea hydrothermal vents, etc. (Yanagawa and Kojima, 1985; Ito *et al.*, 1990; Imai *et al.*, 1999a, b). But, no report has yet appeared on catalytic activity toward natural peptide bonds by the [GADV]-P_n as described in this paper. The [GADV]-P_{30} contained chromophores absorbing UV light and emitting fluorescent light, suggesting that aromatic compounds may have been produced during the repeated heat-drying process. But, it is important to note that the [GADV]-P_{30} containing the cyclic compounds have several kinds of catalytic activities for hydrolyzing β-galactoside bond and amide bond (peptide bond). That is because the complex compounds except pure [GADV]-peptides must be also formed from [GADV] amino acids in the absence of genetic code and genes, for example, in a tide pool on the primitive earth. The hydrolytic activity of [GADV]-P_{30} toward natural peptide bonds in BSA (Figure 6B) implies that [GADV]-P_{30} could form peptide bonds using surrounding [GADV]-amino acids and could effectively accumulate [GADV]-peptides or [GADV]-proteins, since catalysts including proteineous enzymes, which accelerate a forward reaction, can generally catalyze the reverse reaction through micro-reversibility.

In this paper, we also confirmed the existence of hydrolytic activity of the [GADV]-random octapeptides, which were produced with a peptide synthesizer, toward natural peptide bonds in BSA (Figure 7B), suggesting that the peptides also could synthesize peptide bonds using surrounding [GADV]-amino acids, resulting in effective accumulation of [GADV]-peptides or [GADV]-proteins due to the micro-reversibility of catalysts as described above.

Therefore, polymerization of [GADV]-amino acids into [GADV]-peptides or -proteins by the [GADV]-peptides could be regarded as a kind of pseudo-replication of [GADV]-proteins on the primitive earth, because the simple amino acid composition of the [GADV]-proteins could make it possible to synthesize [GADV]-proteins similar to the catalysts in the absence of genes. Effective accumulation of [GADV]-proteins by the pseudo-replication might accelerate the formation of nucleotides through its high catalytic activity, which could lead to formation of RNA-protein world and to the emergence of life (Figure 2).

Even if [GADV]-proteins in the [GADV]-protein world could accumulate [GADV]-proteins through their pseudo-replication in the absence of genes as we have proposed (Ikehara, 2001, 2002), the [GADV]-proteins in the protein world were not even the simplest life itself, since the proteins could not evolve in the protein world in the absence of genes. But, it is important to note that the [GADV]-proteins should be essential chemical components to lead to the emergence of life. On the other hand, we think that life never originated from the RNA world. That is because it is very difficult to produce nucleotides as components of RNA at a meaningful concentration on the primitive earth without the protein world, and to replicate RNA without effective catalysts or proteineous enzymes (Shapiro, 1984;

Shapiro, 1988). It is obvious that one molecule can not play principally two roles in both a catalyst with a stable structure and a template without three dimensional structure. Moreover, it is also very difficult to explain the origin of a primitive genetic code from the stand point of the RNA world theory, because it is also almost impossible for self-replicated RNA to carry genetic information for the production of active proteins, which is independent of a catalytic activity for RNA replication (Figure 2).

In contrast, considered from the stand point of the [GADV]-protein world hypothesis, it can be reasonably expected that [GADV]-proteins produced in the [GADV]-protein world could accumulate nucleotides through their high catalytic activities and create the most primitive genetic code, GNC (Figure 2). Furthermore, it is also conjectured that the most primitive genes could be created by lengthwise arrangement of the GNC genetic code. Although sufficient evidence for a way to the emergence of life was not obtained as yet, the [GADV]-protein world hypothesis should be superior to the RNA world theory, because reasonable steps to the emergence of life on the way from simple chemical compounds to more complex states could be conjectured from the standpoint of the [GADV]-protein world hypothesis (Figure 2). But, it is difficult to explain from the stand point of the RNA world theory (Figure 2). Thus, it can be concluded that the experimental results and considerations described in this paper support the [GADV]-protein world hypothesis on the origin of life.

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