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Sodium Chloride Enhanced Oligomerization of L-Arginine

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Abstract: *NaCl significantly enhanced the longer oligoarginine formation in the oligomerization of L-arginine activated by* N,N′*-carbonyldiimidazole (CDI) in homogeneous aqueous solution. The optimal concentration of NaCl for the highest yield of longer oligoarginine formation is around 1*M. *It is suggested that the weak interactions of Cl^- with the positive-charged guanidinium group of the oligoarginines formed in the oligomerization of L-arginine are responsible for the enhancement by NaCl.* © 2005 Wiley Periodicals, Inc. Biopolymers 81: 1–7, 2006

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Keywords: *L-arginine; oligoarginines; oligomerization; chloride ion, enhanced peptide formation*

INTRODUCTION

The peptide formation in homogeneous aqueous solution is extremely unfavorable from both the thermodynamic and kinetic point of view.[1,2] Therefore in homogeneous aqueous solution it is rarely possible to convert a significant proportion of the input-activated monomers to oligomers beyond 10-mer in significant yield[3,4] because of the competition of hydrolysis. The mineral-catalyzed oligomerization in aqueous solution gave rise to production of longer peptides at the cost of utility of the activated monomers.[3] We incidentally found that the oligomerization of L-glutamic acid activated by *N*,*N*′-carbonyldiimidazole (CDI) was significantly enhanced by alkali cations.[5] The novelty of NaCl-enhanced oligomerization of L-glutamic acid is the production of significantly more longer peptides and less shorter peptides compared to that without the additive. Here we found that the longer oligoarginine formation in the oligomerization of *N*-carboxyanhydride–L-arginine was also significantly enhanced by NaCl.

MATERIALS AND METHODS

L-amino acids, glycine, *N*,*N*′-carbonyldiimidazole and other major compounds were from Sigma. The oligomerization reactions were based on the established procedures.[6] The reaction was started by adding the solution (pH 8.0, 2°C) of amino acids prepared by titrating the free base of L-Arg (Sigma) using 1*M* HCl to twice excess of solid CDI. After quick vortexing and storage at 2°C for 5 min, the tubes were kept in a 20°C water bath for 24 h.

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The samples were injected for analysis using high performance liquid chromatography (HPLC) (Hitachi L7100 pump with UV-VIS L7420 detector) on Zorbax 300-SCX (4.6 × 250 mm, 5 μ) using a $NH_2CH_2CH_2NH_2{\cdot}2HCl$ gradient (0–100% B in 60 min; buffer A: 40% methanol in 0.02*M* NaH_2PO_4 at pH 5.2; buffer B is 1*M* NH_2CH_2-$CH_2NH_2{\cdot}2HCl$ in buffer A at pH 3.2). The resolution of oligoarginines using the method reported here is significantly better than the previous one.[7] The separation of oligomers of glycine was based on the modified method.[8] The oligomers were eluted using a gradient of acetonitrile (0–20% B in 100 min; buffer A: 10 m*M* $C_6H_{13}SO_3Na$ pH 2.5, buffer B: 50% acetonitrile in buffer A) on a Alltima C18 column (4.6 × 260, 5 μ). The HPLC elutions were detected at 214 nm except that of glycine, which was detected at 200 nm.

The 2-mer, 3-mer, and 4-mer of L-Arg were prepared from the oligomerization products of 50 m*M* L-Arg activated by 100 m*M* CDI using Zorbax 300-SCX HPLC. The fractions were concentrated and then desalted using reverse phase (RP) HPLC (Alltima C18, 4.6 × 260, 5 μ; buffer A: 0.1% CF_3COOH; buffer B: 50% acetonitrile in buffer A). After lyophilization, satisfactory mass spectra (Beijing Mass Spectroscopic Center, Institute of Chemistry, Chinese Academy of Sciences, Beijing) were obtained in both $[M + H]^+$ and $[M - H]^-$ modes for the 2-mer, 3-mer, and 4-mer. For 2-mer of L-Arg: calcd. for $C_{12}H_{26}N_8O_3$ MW 330.4, detected 331.3$[M + H]^+$, 166.2$[M + 2H]^{2+}$, 329.6$[M - H]^-$, 443.4$[M + CF_3COO]^-$, 557.2$[M + CF_3COOH + CF_3COO]^-$; for 3-mer: calcd. for $C_{18}H_{38}N_{12}O_4$ MW 486.6, detected 487.4$[M + H]^+$, 244.3$[M + 2H]^{2+}$, 485.4$[M - H]^-$, 599.1$[M + CF_3COO]^-$, 712.9$[M + CF_3COOH + CF_3COO]^-$; for the 4-mer: calcd. for $C_{24}H_{50}N_{16}O_5$ MW 642.8, detected 643.5$[M + H]^+$, 215.3$[M + 3H]^{3+}$, 641.1$[M - H]^-$, 754.9$[M + CF_3COO]^-$, and 868.8$[M + CF_3COOH + CF_3COO]^-$. Matrix assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectroscopic analysis of the products from oligomerization of 50 m*M* L-arginine for 24-h detected peaks corresponding to the 2-mer through the 9-mer of L-Arg; detected 331.3 [330.4], 487.3[486.6], 643.3[642.8], 799.4[799], 955.6[955.2], 1111.6[1111.4], 1267.7[1267.8], 1423.7[1424], 1579.8[1580.2].

The concentration of the prepared Arg–Arg and Arg–Arg–Arg was determined by first hydrolysis of Arg–Arg and Arg–Arg–Arg in 6*M* HCl at 110°C for 40 h and the subsequent amino acid determination using classical ninhydrin method.

The yields of the elongation products of 50 m*M* L-Arg and Arg–Arg by around 5 m*M* activated L-Arg both with and without 1*M* NaCl were determined using HPLC.[5] Every 20 min the products from a system of 50 m*M* L-Arg with about 5 m*M* activated L-Arg or 50 m*M* Arg–Arg with about 5 m*M* activated L-Arg kept in a 20°C water bath were analyzed using Zorbax 300-SCX HPLC. The rate constants for the peptide formation in 4 h were determined using the established methods.[9]

In the screening of the effect of other salts on the oligomerization, the pH of the amino acid solutions containing the additives was adjusted to pH 8 before the reaction with CDI. Unless specified, all the additives of anions are Na^+ form.

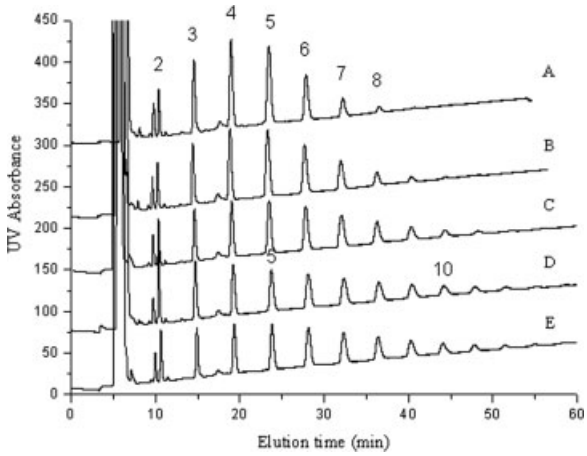

FIGURE 1 Oligoarginine formation in CDI-induced oligomerization of 50 m*M* L-Arg with different concentrations of NaCl. A: Control reaction; B–E: eluates in the presence of 0.1, 0.4, 1, and 3*M* NaCl.

RESULTS AND DISCUSSION

The reaction of CDI with amino acids results in the activation of the α-amino group, yielding N-[imidazolyl-(1)-carbonyl]-(L)-amino acid, which subsequently cyclizes to form an N-carboxyanhydride.[10] The typical oligomerization of 50 m*M* L-Arg activated by 100 m*M* CDI carried out at 20°C for 24 h gave rise to a series of successive oligoarginines with 9-mer as the longest oligomer (Figure 1A), in agreement to the previous results.[7] The assignment of the oligoarginines was based on coinjection of elecctrospray ionization–mass spectroscopy (ESI-MS) identified 2-mer, 3-mer, and 4-mer with oligomerization samples respectively. MALDI-TOF MS analysis of the oligomerization products gave rise to a similar pattern of oligoarginine formation to the corresponding HPLC profiles (Figure 1A) in both distribution and intensity of oligoarginine peaks, supporting the above assignment.

Similar to Na^+-enhanced oligomerization of L-glutamic acid,[5] the presence of 0.1*M* NaCl in 50 m*M* L-Arg activated by 100 m*M* CDI significantly increased the yields of the 7-mer and the longer peptides, and decreased the yields of 5-mer and the shorter oligomers (Figure 1B). Increasing the concentration of NaCl to 0.2*M*, 0.4*M* (Figure 1C), 0.6*M*, and 0.8*M* gave rise to ever-increasing yields of the 6-mer and longer oligomers and decreased yields of the short oligomers. The formation of the longest oligoarginines is optimal when the concentration of NaCl is around 1*M* (Figure 1D), where the longest peptides detected is a 15-mer, and the peak areas of the 3-mer, 4-mer, and 5-mer are 61, 47, and 50%, respectively, of the

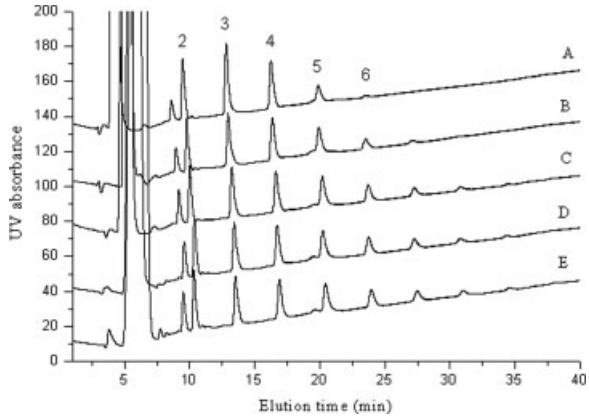

FIGURE 2 Oligoarginine formation from CDI-induced oligomerization of 5 m*M* L-Arg with different concentrations of NaCl. A: Control reaction; B–E: eluates in the presence of 0.1, 0.5, 1, and 2*M* NaCl.

corresponding peak areas without NaCl. The yield of the 8-mer and the longer oligomers to total peak areas of the oligoarginines with 1*M* NaCl was increased to 24.1 from 1.58% without the additive. Further increase the concentration of NaCl to 3*M* (Figure 1E) and 4*M* resulted in a similar pattern of peptide formation to that with 1*M* NaCl and slightly inhibited yields of the oligoarginines. In terms of longest oligoarginine formation, the enhancement by 1*M* NaCl in homogeneous aqueous solution is significantly more efficient than illite, and at least comparably efficient to that of FeS_2, the known best solid catalyst for the oligomerization of L-Arg.[7]

More evident increase of longer peptide formation was observed when the concentration of L-Arg was reduced to 5, 10, and 20 m*M*. The longest oligoarginine formed without the additive is the 6-mer, 7-mer, and 8-mer, respectively. Similar to the enhancement of 50 m*M* L-Arg oligomerization with NaCl, increasing the concentration of NaCl resulted in the formation of significantly more longer peptides and less shorter peptides, although the total peak areas of the oligoarginines on HPLC profiles did not change significantly. The longest peptides formed are 10-mer, 11-mer, and 14-mer when the oligomerization of 5, 10, and 20 m*M* L-Arg was carried out with addition of 0.75 or 1*M* concentration of NaCl (Figure 2).

The effect of the other anions on oligomerization of 50 m*M* L-Arg was screened. The longest oligoarginine formation and the corresponding anion concentration were summarized in Table I. In terms of the longest oligoarginine formation, a similar pattern of enhancement by NaBr and NaI to that of NaCl was also observed, although NaBr and NaI was slightly less efficient than NaCl. However, the effect as high as 0.25*M* NaF on the oligomerization was very limited compared to that of NaCl. The Na^+ form of linear anions N_3^- and SCN^- enhanced the longer peptide formation less efficiently than that of NaCl, and up to 0.25*M* of Na^+ form of $[Fe(CN)_6]^{4-}$ enhanced the oligomerization slightly. The presence of trigonal planar NO_3^- increased the peptide formation close to that of NaCl. The effect of Na^+ form of tetrahedral ClO_4^- and SO_4^{2-} is noteworthy. The oligomerization of 50 m*M* L-Arg with 0.1–3*M* $NaClO_4$ is less efficient than the corresponding NaCl; however, the peptide formation of 50 m*M* L-Arg with 0.1*M* Na_2SO_4 is comparable to that with 1*M* NaCl. Increasing the concentration to 0.25 and 0.5*M* yielded the 15-mer as the longest oligomer. The organic anions acetate and succinate yielded medium enhancement. Changing Na^+ form of Cl^- to the K^+ and Li^+ form gave rise to a similar enhancement. Although the enhancement by anions is obviously selective, we did not find the direct relevance of the phenomenon to ionic strength, to geometries of the anions, nor to the Hofmeister effect of the anions.

The presence of wide spectra of anions in the oligomerization of L-Arg does result in the formation of more longer oligoarginines and less shorter oligoarginines. The oligomerization with different concentration of salts in terms of number (DPn) and weight (DPw) average degree of polymerization is summarized in Table II. For the oligomerization of 50 m*M* L-Arg, the corresponding DPn (DPw) is increased with the increase of NaCl concentration, from 3.84 (4.34) without NaCl to 4.34 (5.47) with 1*M* of NaCl. In the oligomerization of 5 m*M* L-Arg, DPn (DPw) is increased from 2.78 (3.03) without NaCl to 3.13 (3.72) with 1*M* of NaCl. The enhancement by the presence of the other anions in terms of DPn and DPw (Table II) was also evidently suggested. With the increase of the concentration of the salts, both DPn and DPw tend to increase except that of $MgCl_2$. Moreover, the effect on DPn and DPw by the presence of NaF, $Na_4Fe(CN)_6$, and CH_3COONa is very limited, although the presence of the above salts did yield significant longer oligoarginines (Table I). In general, the concentration dependence of the enhancement of salts in terms of DPn and DPw is similar to that in terms of the longest oligoarginine formation. However, it is noted that the effect of di-, tri-, and tetravalent anions tested is not much greater than that of the monovalent anions such as Cl^-.

From Figures 1 and 2 it is clear that the sum of the peak areas of oligoarginines with different concentration of NaCl detected using HPLC is similar. The sum of peak areas of the oligoarginines formed in the oligomerization of 50 m*M* L-Arg with the presence of

Table I The Effect of Anions on Oligomerization of 50 m*M* L-Arg Activated by 100 m*M* CDI

Anion	Cation	Geometries of Anions	Anion Conc. (*M*)[a]	Longest Peptide[b]
Cl^-	Na^+	Spherical	1–3	15
	Li^+		1	14
	K^+		2	15
	Me_4N^+		1–3	14
	Et_4N^+		0.8–1	14
	Pr_4N^+		0.6–0.8	14
	Bu_4N^+		0.5	13
	Mg^{2+}		0.5–1	13
F^-	Na^+	Spherical	0.25	11
Br^-	Na^+	Spherical	1–2	15
I^-	Na^+	Spherical	0.75–1	14
N_3^-	Na^+	Linear	0.75–2	13
SCN^-	Na^+	Linear	1–2	15
NO_3^-	Na^+	Trigonal planar	1	14[d]
ClO_4^-	Na^+	Tetrahedral	1	14
SO_4^{2-}	Na^+	Tetrahedral	0.5[c]	15
PO_4^{3-}	Na^+	Tetrahedral	0.08[c]	9
$Fe(CN)_6^{4-}$	Na^+	Octahedral	0.25[c]	13
CH_3COO^-	Na^+		1	13
Sodium succinate	Na^+		0.25[c]	12

[a] The concentration listed is the optimal concentration of anions giving rise to the longest peptide formation. For the Cl^- form of Li^+, K^+, Pr_4N^+, Bu_4N^+, and the Na^+ form of Br^-, I^-, N_3^-, SCN^-, and ClO_4^-, up to 2*M* of anions were screened.

[b] The number represents the unit of L-Arg residues of the longest peptides in the presence of corresponding concentration of anions determined by HPLC elution of the products; the longest oligoarginine formed without additive is 9-mer.

[c] The highest concentration used in screening.

[d] The presence of 0.5*M* and higher concentration of $NaNO_3$ resulted in the precipitation of 5-mer and longer oligoarginines; therefore the peptide formation was determined by dilution of the products first using buffer A.

0, 0.1, 0.5, 1, and 3*M* of NaCl (Figure 1) varies within just 4.1%. In addition, the sum of peak areas with different concentrations of NaCl in the oligomerization of 5 m*M* L-Arg is just within 7.9% (Figure 2). This observation is applicable to that of the oligomerization of 50 m*M* L-Arg in the presence of the other salts listed in Table II. Therefore the effect of the salts seems to mainly change the oligomer distribution, namely increase the yields of the longer peptides and decrease the yields of the short peptides relative to that without additive. In this regard, the enhancement on the oligomerization of L-Arg by the presence of the salts is similar to NaCl-enhanced oligomerization of L-glutamic acid.[5]

It should be noted that the oligoarginine distribution is not the same for the oligomerization within different salts. For example, the presence of NaCl resulted in the production of 15-mer oligoarginines, slightly more than that of Me_4NCl and Et_4NCl (Table I). However, both DPn and DPw in the presence of 1*M* and higher concentration of Me_4NCl are higher than that of NaCl owing to their differences in oligoarginine distribution. In addition to the major role of anions on the salt-enhanced oligomerization of L-Arg, the effect of cation is not negligible, as shown in Table II. For example, both DPn and DPw in the presence of $MgCl_2$ are significantly less than that of NaCl. This indicates the complications of the interactions of the ions in aqueous solution.

The yields of peptides of 50 m*M* L-Arg at pHs from pH 5 to pH 9 were quite similar in both the yield and the pattern of the oligomer distribution. This eliminated the slight pH difference owing to the presence of the additives as a cause of the enhancement. The presence of 0.1–2*M* Cl^- of extreme weak coordinative cations, such as $(CH_3)_4N^+$, $(CH_3CH_2)_4N^+$, $(CH_3CH_2CH_2)_4N^+$, and $(CH_3CH_2CH_2CH_2)_4N^+$ gave rise to a oligoarginine formation similar to that with NaCl (Table I), suggesting the essential role of Cl^- for the enhancement.

The CDI-induced oligomerization of 50 m*M* L-histidine and L-lysine gave rise to complicated unidentified products. MALDI-TOF MS analysis of the products of 50 m*M* L-histidine with 1*M* NaCl and 50 m*M* L-lysine with 1*M* NaCl detected products with molecular weights significantly more than those without additive. The oligomerization of 2.5 m*M* glycine activated by 5 m*M* CDI yielded oligoglycines from the 2-

Table II DPn and DPw of the Oligoarginines Formed in the Oligomerization of 50 m*M* L-Arg with Different Concentrations of Salts[a]

NaCl	0*M* 3.84/4.34	0.1*M* 4.05/4.69	0.2*M* 4.16/4.91	0.4*M* 4.32/5.25	0.6*M* 4.40/5.46	0.8*M* 4.37/5.45	1.0*M* 4.35/5.47	—	3.0*M* 4.07/5.28	4.0*M* 3.79/4.86
LiCl	0*M* 3.88/4.39	0.5*M* 4.33/5.34	1.0*M* 4.23/5.30	2.0*M* 3.74/4.69	—	—	—	—	—	—
KCl	0*M* 3.88/4.39	0.5*M* 4.50/5.52	1.0*M* 4.57/5.74	2.0*M* 4.58/5.92	—	—	—	—	—	—
Me_4NCl	0*M* 3.89/4.37	0.1*M* 4.10/4.74	0.2*M* 4.12/4.81	0.4*M* 4.46/5.35	0.6*M* 4.52/5.48	0.8*M* 4.69/5.78	1.0*M* 4.64/5.74	2.0*M* 4.75/5.94	3.0*M* 4.74/5.89	4*M* 4.28/5.25
Et_4NCl	0*M* 3.92/4.43	0.1*M* 4.15/4.78	0.2*M* 4.30/5.04	0.4*M* 4.36/5.16	0.6*M* 4.46/5.34	0.8*M* 4.72/5.78	1.0*M* 4.73/5.81	2.0*M* 4.46/5.50	3.0*M* 3.03/3.53	—
Pr_4NCl	0*M* 3.96/4.49	0.1*M* 4.21/4.88	0.2*M* 4.38/5.17	0.4*M* 4.57/5.51	0.6*M* 4.63/5.64	0.8*M* 4.61/5.64	1.0*M* 4.45/5.46	2.0*M* 2.47/2.69	—	—
Bu_4NCl	0*M* 3.96/4.49	0.1*M* 4.17/4.84	0.5*M* 4.34/5.22	1.0*M* 3.49/4.05	2.0*M* 2.06/2.09	—	—	—	—	—
$MgCl_2$	0*M* 3.99/4.53	0.1*M* 4.06/4.76	0.25*M* 4.05/4.90	0.5*M* 3.99/4.91	0.75*M* 3.80/4.74	1.0*M* 3.62/4.49	2.0*M* 3.00/3.59	—	—	—
NaF	0*M* 3.82/4.34	0.1*M* 3.95/4.55	0.25*M* 3.95/4.63	—	—	—	—	—	—	—
NaBr	0*M* 3.87/4.39	0.1*M* 4.14/4.83	0.25*M* 4.31/5.19	0.5*M* 4.42/5.48	0.75*M* 4.45/5.60	1.0*M* 4.41/5.62	2.0*M* 4.21/5.44	—	—	—
NaI	0*M* 3.87/4.38	0.1*M* 4.11/4.81	0.25*M* 4.23/5.10	0.5*M* 4.36/5.39	0.75*M* 4.35/5.47	1.0*M* 4.25/5.39	2.0*M* 3.74/4.68	—	—	—
NaSCN	0*M* 3.82/4.30	0.1*M* 4.13/4.79	0.5*M* 4.42/5.43	0.75*M* 4.38/5.47	1.0*M* 4.34/5.47	2.0*M* 4.30/5.45	—	—	—	—
$NaClO_4$	0*M* 3.86/4.37	0.1*M* 4.18/4.90	0.25*M* 4.35/5.22	0.5*M* 4.34/5.35	0.75*M* 4.31/5.17	1.0*M* 4.10/5.31	2.0*M* 3.77/4.70	3*M* 3.19/3.82	—	—
Na_2SO_4	0*M* 3.87/4.37	0.01*M* 4.02/4.67	0.1*M* 4.43/5.53	0.25*M* 4.50/5.73	0.5*M* 4.27/5.47	—	—	—	—	—
Na_3PO_4	0*M* 3.92/4.42	0.033*M* 3.84/4.46	0.08*M* 3.53/4.09	—	—	—	—	—	—	—
$Na_4Fe(CN)_6$	0*M* 3.82/4.30	0.1*M* 3.31/3.88	0.25*M* 3.94/5.00	—	—	—	—	—	—	—
CH_3COONa	0*M* 3.88/4.39	0.5*M* 4.08/4.90	1.0*M* 4.01/4.93	—	—	—	—	—	—	—
Sodium succinate	0*M* 3.79/4.27	0.1*M* 3.68/4.40	0.25*M* 3.58/4.33	0.5*M* 3.36/4.08	—	—	—	—	—	—
NaCl*	O*M* 2.78/3.03	0.1*M* 2.96/3.35	0.5*M* 3.10/3.65	0.75*M* 3.15/3.76	1.0*M* 3.13/3.72	2.0*M* 3.09/3.67	3.0*M* 3.10/3.73	—	—	—

[a] The grids in this table show the concentration of anions (*M*) and the corresponding DPn/DPw. The number (MWn) and weight (MWw) average molecular weights of polymerization of the corresponding oligomerization of L-Arg in the presence of the salts were first calculated based on the HPLC profiles of oligomerization products; the DPn and DPw were calculated based on: DPn(DPw) = [MWn(MWw) − 174.2]/156.2 + 1. The asterisk denotes the oligomerization of 5 m*M* L-Arg as a function of NaCl concentration as shown in Figure 2. Because the presence of NaN_3 yielded a series of unidentified products in addition to the usual oligoarginines and the precipitates were found during the oligomerization of L-Arg with $NaNO_3$ their DPn and DPw were not included.

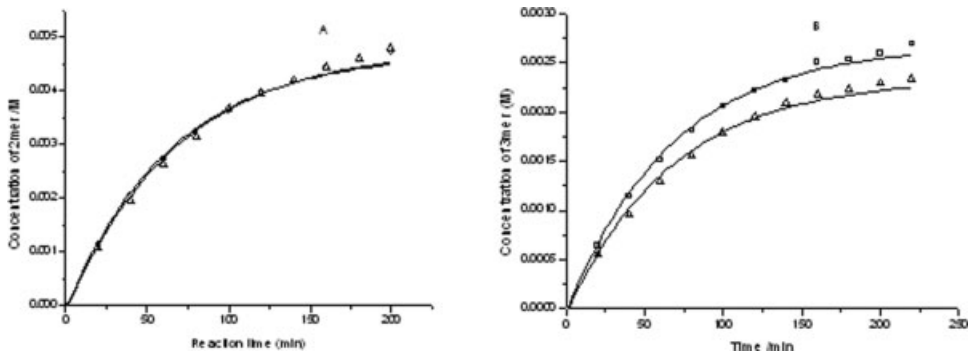

FIGURE 3 Elongation of 50 m*M* L-Arg (A) and 50 m*M* Arg–Arg (B) by about 5 m*M* activated L-Arg (square) and that in the presence of 1*M* Cl^- (triangle).

mer to 16-mer. It was observed that the presence of 0.01, 0.05, and 0.1*M* NaCl affected neither the yields nor the distribution of oligoglycines. Although higher concentration of NaCl (0.5*M* and higher) contained in oligomerization samples interferes with the separation and accurate quantification of oligoglycines shorter than the 7-mer, we did not observe significant effect on the formation of the 8-mer through 16-mer of glycine by the presence of 0.5, 1, and 3*M* NaCl.

Based on HPLC peak areas of the activated L-Arg formed in 50 m*M* L-Arg activated by 100 m*M* CDI with and without 1*M* NaCl, we found actually the same quantity formation of the activated L-Arg. In order to clarify if the reactivity of the intermediates is affected by the presence of NaCl, dimer formation from a system of 50 m*M* L-Arg (Figure A) and trimer formation from a system of 50 m*M* Arg–Arg (Figure 3B) with 5 m*M* activated L-Arg were determined. The apparent first-order rate constant for dimerization is $1.51 \times 10^{-2} \pm 0.06\ \text{min}^{-1}$, similar to that with 1*M* NaCl ($1.45 \times 10^{-2} \pm 0.06\ \text{min}^{-1}$). The apparent first-order rate constant for 3-mer formation in Arg–Arg elongation experiments is $1.47 \times 10^{-2} \pm 0.04\ \text{min}^{-1}$, similar to that with 1*M* NaCl ($1.49 \times 10^{-2} \pm 0.05\ \text{min}^{-1}$). Therefore both the activation and the reactivities of the intermediates of L-Arg are not affected by the presence of 1*M* NaCl.

The oligomerization proceeds as a stepwise elongation process, giving rise to oligoarginines as the dominant products.[6,10] Therefore the oligomerization reactions are defined as

$$\text{G} \xrightarrow{k_2[\text{M}]} \text{G}_2 \xrightarrow{k_3[\text{M}]} \text{G}_3 \xrightarrow{k_4[\text{M}]} \text{G}_4 \longrightarrow \text{G}_{i-1} \xrightarrow{k_i[\text{M}]} \text{G}_i$$

where M stands for the activated L-Arg; G_2, G_3, . . . , G_i are oligoarginines of length 2, 3, . . . , i, and k_2, k_3, . . . , k_i are the individual second-order rate constants for elongation. Because the rate constant for dimerization (k_2) and trimer formation (k_3) were not affected by the presence of 1*M* NaCl (Figure 3), it is assumed that the enhanced elongation of the longer oligoarginines formed during the oligomerization must be responsible for the enhancement.

It is known that the binding of Cl^- to charged guanidinium is electrostatic in origin.[11,12] We suggest that the enhancement could be attributed to the weak coordination of Cl^- on positive-charged guanidinium groups of L-Arg residues of the formed oligoarginines, which causes either oligoarginines to be more ordered molecular clusters or changes of the conformations, so facilitating the elongation. The multiple coordination is known to lead to a linear increase of the complexation strength, e.g., Coulomb increment of $\Delta G = 5 \pm 1$ kJ/mol per single salt bridge[13]; therefore the coordination of Cl^- on molecules with multiple binding sites such as longer oligoarginines can be expected to be much stronger than that of Cl^- on L-Arg and Arg–Arg. This explains the null effect of 1*M* Cl^- on k_2 and k_3.

CONCLUSIONS

Although the mechanisms of the Cl^--enhanced oligomerization are not fully understood and clearly need further investigations, the peptide formation enhanced by Cl^-, the most abundant anions in nature, is especially significant for the origins of life with regard to the fact that peptides required to be the candidates of peptide catalysts are around 20 residues.[14,15] This phenomenon indicates that the prebiotic synthesis of longer peptides of basic amino acids in primeval oceans might be much easier than we previously expected in view of the inevitable presence of Cl^- in the primitive oceans and plausibly high concentrations of these ions in localized areas. The similarity of Cl^--enhanced oligomerization of L-Arg reported here to Na^+-enhanced oligomerization of

L-glutamic acid[5] demonstrated the mechanistic similarity of ion-enhanced oligomerization of the opposite-charged amino acids.

Cl^- is the dominant intracellular anion (~110 m*M*) and oligoarginines are novel molecules with varieties of biological functions, such as the notable function of a cell penetrating into mammalian cells.[16] The results strongly indicate the significant interactions between Cl^- and the longer oligoarginines.

It should be emphasized that although the weak interactions of the common ions in water with proteins and peptides are the basic aspects of protein chemistry and biochemistry (such as ion channels), the origin of many ion-specific effects is still considered largely a mystery.[17] For example, the Hofmeister effect found in 1888 is still unexplained and is a topic of intensive mechanistic investigations after more than one century.[18] This is one more case showing the complications of the weak interactions between ions and the charged molecules in aqueous solution and the effect on reactions.

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