Heterogeneous assembly of silver(I) and calcium(II) ions accompanying a dimer formation of *cyclo*(L-Ala–L-Met)$_3$†

Tomoko Okada,[a] Kentaro Tanaka,[ab] Motoo Shiro[c] and Mitsuhiko Shionoya*[a]

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Heterogeneous assembly of three Ag^+ and one Ca^{2+} ion has been accomplished in a dimeric structure of cyclic hexapeptides, *cyclo*(L-Ala–L-Met)$_3$.

Peptides have long been recognized as promising molecules with metal-dependent functions not only in biological systems but also in artificial systems. In particular, cyclic peptides have been extensively used for ion recognition,[1] ion channels,[2] metal arrays[3] and other applications.[4] These functions are closely related to the number and sequence of their amino acid constituents. Cyclic peptides bearing metal binding sites at side chains on the cyclic framework would provide a novel tool for construction of discrete metal-assembled supermolecules with unique chemical and physical properties.

In this study, heterogeneous assembly of two different types of metal ions, transition and alkaline earth metal ions, was accomplished by the use of cyclic hexapeptides with metal coordination sites at both amino acid side chains and the carbonyl groups on the cyclic framework. A cyclic hexapeptide having a repeating L-Ala–L-Met sequence, *cyclo*(L-Ala–L-Met)$_3$ (**1**), was designed for this purpose (Fig. 1). This peptide has metal binding sites at the thioether groups for softer metals (outer circle in pale grey) and the carbonyl groups for harder metals (inner circle in black). The roundly arranged carbonyl groups of cyclic hexapeptides are known to fit rather hard metal ions, such as alkaline or alkaline earth ions.[5] However, there are only a few reports on the detailed structures of their complexes. The cyclic hexapeptide (**1**), having two different types of metal binding sites, was found to quantitatively form a tetranuclear complex (**2**) with three Ag^+ and one Ca^{2+} ion in a capsule-like[6] dimeric structure (Fig. 2). It should be highlighted that the co-existence of both metal ions is essential for the quantitative dimer formation.

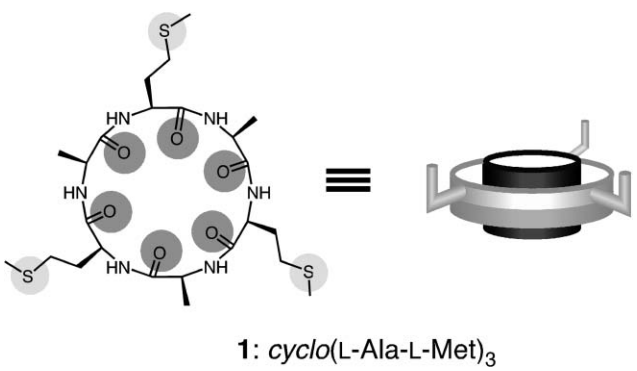

Fig. 1 Cyclic hexapeptide, *cyclo*(L-Ala–L-Met)$_3$ (**1**), used in this study.

The cyclic hexapeptide, *cyclo*(L-Ala–L-Met)$_3$ (**1**), was prepared from the corresponding linear hexapeptide, H–(L-Ala–L-Met)$_3$–OH, by intramolecular cyclization in dilute solution. ^{1}H and ^{13}C NMR spectra of this cyclic peptide (**1**) exhibited highly symmetrical patterns arising from its cyclic structure with a repeating L-Ala–L-Met sequence. The cyclic structure of the hexapeptide was finally confirmed by its electrospray ionization-time-of-flight (ESI-TOF) mass spectrometry (for the detailed data, see electronic supplementary information†).

Interactions between the cyclic hexapeptide (**1**) with Ag^+ and Ca^{2+} ions were firstly examined by ^{1}H NMR titration with $Ag(CF_3SO_3)$ using a solution of **1** in acetone-d_6–CD_3OD (5 : 1) in the presence of 0.5 equiv. of $Ca(CF_3SO_3)_2$ (Fig. 3). With an increasing concentration of Ag^+ ions, another set of signals gradually appeared, and the spectral changes completed when 1.5 equiv. of Ag^+ ions were added (**1**–Ag^+–Ca^{2+} = 2 : 3 : 1, Fig. 3e). The singlet signal for the methyl groups of –SCH_3 was divided into two singlet signals (δ 2.52 and 2.49 ppm) with a downfield shift ($\Delta\delta$ = *ca.* 0.5 ppm),[7] indicating the coordination of the thioether groups to Ag^+ ions. The spectral changes of other proton signals were also observed probably due to the conformational changes of the cyclic framework upon metal complexation. Furthermore, the ESI-TOF mass spectrum[8] of the solution (**1**–Ag^+–Ca^{2+} = 2 : 3 : 1) strongly suggested the formation of the dimeric structure of $[Ag_3Ca\mathbf{1}_2]^{5+}$ (**2**), as shown in Fig. 2, in which three Ag^+ and one Ca^{2+} ion are simultaneously encapsulated between the sulfur donors of L-Met and the carbonyl groups of the cyclic framework, respectively. In the reverse titration with Ca^{2+} ions using a solution of **1**–Ag^+ = 2 : 3, the same spectrum was obtained as Fig. 3e, when the ratio of **1**–Ag^+–Ca^{2+} reached 2 : 3 : 1.

The ^{1}H NMR spectrum of the dimer complex (**2**) (Fig. 3e) displayed two sets of signals for L-Ala–L-Met in a 1 : 1 integral

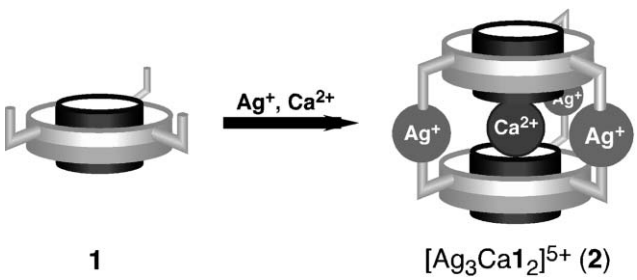

Fig. 2 A scheme for the formation of a tetranuclear complex (**2**) from *cyclo*(L-Ala–L-Met)$_3$ (**1**), three Ag^+ and one Ca^{2+} ion.

† Electronic supplementary information (ESI) available: synthetic data for **1** and **2**. See http://www.rsc.org/suppdata/cc/b4/b415567j/

*shionoya@chem.s.u-tokyo.ac.jp

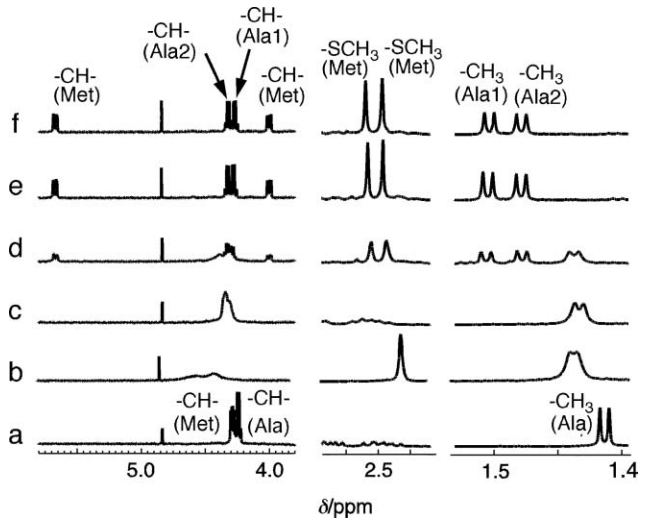

Fig. 3 ^{1}H NMR spectra of (a) cyclic hexapeptide (**1**) only, (b) **1** + Ag^+ (1.5 equiv.), (c) **1** + Ca^{2+} (0.5 equiv.), (d) **1** + Ca^{2+} (0.5 equiv.) + Ag^+ (0.75 equiv.), (e) **1** + Ca^{2+} (0.5 equiv.) + Ag^+ (1.5 equiv.) and (f) **1** + Ca^{2+} (0.5 equiv.) + Ag^+ (3.0 equiv.) in acetone-d_6–CD_3OD (5 : 1), [**1**] = 2 mM at 293 K.

ratio, while that of the metal-free ligand (**1**) showed only one set of L-Ala–L-Met signals. For example, the signals of the methyl protons of L-Ala appeared at 1.51 and 1.46 ppm (Ala1 and Ala2), and those of the methine protons of L-Ala at 4.22 and 4.16 ppm (Ala2 and Ala1). Then, the NOE study was carried out to know how the two cyclic peptides are stacked in the dimer complex (**2**) in solution. As a result, the orientation of the two cyclic peptides (**1**) in $[Ag_3Ca\mathbf{1}_2]^{5+}$ (**2**) was proven to be in a head-to-tail manner.[9] The appearance of the two sets of signals as shown above can be best explained in terms of the head-to-tail stacking of two cyclic peptides whose amino acid residues are unequivalently placed against the ring plane.

In addition, when a half amount of Ca^{2+} ions was added for the dimer complex (**1**–Ag^+–Ca^{2+} = 2 : 3 : 0.5), signals for both the dimer complex and the other uncomplexed product were individually observed in a 1 : 1 integral ratio (Fig. 3d). This result suggests the quantitative formation of the dimer complex, in which their ligand exchange rates were slower than the NMR timescale. Moreover, the co-existence of three Ag^+ and one Ca^{2+} ion was essential for the quantitative dimer complexation, as the signals for the reference solution of **1**–Ag^+ = 2 : 3 or **1**–Ca^{2+} = 2 : 1 were significantly broadened (Figs. 3b and c) with continuous shift upon addition of metal ions. Complexation between Ag^+ ions and the thioether groups thus reinforces the encapsulation of Ca^{2+} ions into the cavity formed by the six carbonyl oxygens in the dimeric structure. Sr^{2+} and Ba^{2+} ions also formed dimeric structures with the aid of Ag^+ ions only when excess amounts of these metal ions were added. Other alkaline earth, alkaline and lanthanide ions including Na^+ and La^{3+} with an ionic radius similar to Ca^{2+} did not form dimer complexes under the same conditions.[10]

We tried to crystallize complex **2** from a solution of **1**–Ag^+–Ca^{2+} = 2 : 3 : 1,[11] but the obtained single crystals were proven to be a disproportionated product probably due to its lower solubility and crystal packing. Its X-ray crystal analysis revealed that the resulting complex (**3**) is a Ca^{2+}-linked dimer of **2**, $[(Ag_3Ca\mathbf{1}_2)_2Ca]^{12+}$ (**3**) (Fig. 4).‡ In the partial structure corresponding to **2** in complex **3**, one Ca^{2+} ion is coordinated by six carbonyl oxygens in a slightly distorted octahedral geometry, and each Ag^+ ion is bound by two sulfur atoms. As observed in solution, two cyclic peptides were stacked in a head-to-tail orientation with the aid of three Ag^+ and one Ca^{2+} ion.[12] Since the ^{1}H NMR spectrum of the solution of **1**–Ag^+–Ca^{2+} = 2 : 3 : 2 was identical to that of the solution of **1**–Ag^+–Ca^{2+} = 2 : 3 : 1, the third Ca^{2+} ion connecting two dimer units in the solid state should be released as a solvated ion in the solution.

In summary, quantitative, heterogeneous metal assembly was accomplished using a pre-designed cyclic hexapeptide, *cyclo*(L-Ala–L-Met)$_3$, as a template. Peptides with desired numbers and sequences of metal binding sites would provide template-directed arrays of metal-containing devices.

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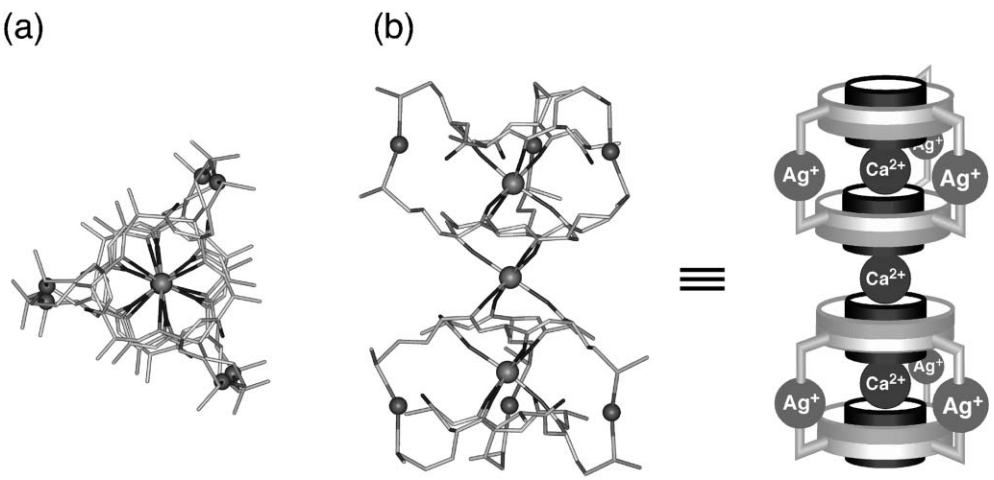

Fig. 4 The X-ray structure of $[(Ag_3Ca\mathbf{1}_2)_2Ca]^{12+}$ (**3**); (a) a top view and (b) a side view. Hydrogen atoms are omitted for clarity.

Tomoko Okada,[a] Kentaro Tanaka,[ab] Motoo Shiro[c] and Mitsuhiko Shionoya*[a]
[a]*Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: shionoya@chem.s.u-tokyo.ac.jp; Fax: +81 3 5841 8061; Tel: +81 3 5841 8061*
[b]*PRESTO, Japan Science and Technology Agency (JST), Japan*
[c]*Rigaku Corporation, 3-9-12 Matsubaracho, Akishima, Tokyo 196-8666, Japan*

Notes and references

‡ Abbreviations used: Ala, alanine; Met, methionine; ESI-TOF, electrospray ionization-time-of-flight. Crystal data for **3**: $[(Ag_3Ca\mathbf{1}_2)_2Ca]Ca(CF_3SO_3)_{14}(H_2O)_7$, M = 5447.79, cubic, $P2_13$, a = 29.86(2) Å, V = 26615(32) Å^3, T = 93 K, Z = 4, D_{calc} = 1.359 g cm^{-3}, 17387 reflections, $R1$ = 0.062 ($I > 2\sigma(I)$), $wR2$ = 0.193 (all reflections)). There were no hydrogens located for the water molecules. CCDC 248985. See http://www.rsc.org/suppdata/cc/b4/b415567j/ for crystallographic data in .cif or other electronic format. Intensity data for complex **3** were collected on a Rigaku RAXIS-RAPID imaging plate area detector with MoKα radiation (λ = 0.71075 Å). All calculations were performed using the Crystal Structure crystallographic software package except for refinement, which was performed using SHELXL-97[13].

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7 The signals for the methyl groups of Met (–SCH_3) in the metal-free cyclic peptide (**1**) were overlapped around 2.0 ppm with the residual signals of acetone used as the solvent. However, the presence of a singlet signal for –SCH_3 was confirmed at 2.09 ppm in a spectrum measured in CD_3OD.
8 ESI-TOF mass data: *m*/*z* 1180.9 $[Ag_3Ca\mathbf{1}_2{\cdot}(CF_3SO_3)_3]^{2+}$ (calcd. 1180.9), 2173.3 $[Ag_3Ca\mathbf{1}_2{\cdot}(CF_3SO_3)_4]^{+}$ (calcd. 2173.0).
9 Since this cyclic peptide has two different faces, as colored differently in greyscale in Fig. 1, due to its asymmetric centers, there are three possible orientations when two cyclic peptides are stacked together to form a dimer complex, that is, in a head-to-head, tail-to-tail or head-to-tail orientation. The COSY and NOESY spectra determined the head-to-tail arrangement as was indicated by the interaction between methyl protons of Ala (Ala1) in one cyclic peptide and methine protons of Ala (Ala2) in the other one.
10 Ion selectivity for the encapsulation will be reported elsewhere in detail.
11 $Ag(CF_3SO_3)$ (1.9 mg, 7.4 μmol) and $Ca(CF_3SO_3)_2$ (0.8 mg, 2.5 μmol) were added to a solution of *cyclo*(L-Ala–L-Met)$_3$ (**1**) (3.0 mg, 4.9 μmol) in acetone–CH_2Cl_2 (0.5 mL–0.1 mL). Crystals (**3**) suitable for X-ray analysis were grown from the solution which was overlaid by diethyl ether (2 mL) at 277 K for 3 days.
12 All three Ca^{2+} ions are aligned along the C_3 axis of the molecule. Six sulfur atoms adopt an *R* configuration, whereas the remaining six sulfur donors take an *S* configuration. The distances of Ag^+–Ag^+ and Ca^{2+}–Ag^+ are *ca.* 11 and 6.6 Å, respectively. The bond angle of –(CH_3)S–Ag^+–S(CH_3)– is deviated from the linear coordination, and a carbonyl oxygen points toward the Ag^+ ion (O–Ag^+ 2.55 Å) to form a distorted trigonal-planar geometry.
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