Characterization by Electrospray Ionization (ESI) Mass Spectrometry of an Oligomeric, Anionic Thiomalato-silver(I) Complex showing Biological Activity

Kenji Nomiya,*[a] Yoshihiro Kondoh,[a] Hitomi Nagano[b] and Munehiro Oda[b]

[a] *Department of Materials Science, Faculty of Science, Kanagawa University, Hiratsuka, Kanagawa 259-12, Japan*
[b] *Meiji Cell Technology Center, Meiji Milk Products Co. Ltd., 540 Naruda, Odawara, Kanagawa 250, Japan*

The tetrameric anionic species $\{Na_3[Ag(Htma)]_4\}^-$ (*m/z* = 1092.8; H_3tma = thiomalic acid) is detected by negative-ion electrospray ionization (ESI) mass spectrometry as a stable constituent unit derived from an aqueous solution of the oligomeric thiomalato-silver(I) complex, recently isolated and characterized as $\{Na[Ag(Htma)]\cdot 0.5H_2O\}_n$ (*n* = 24–34) **1**, which displays antimicrobial activity against selected bacteria, yeast and moulds.

In medicinally active compounds of silver(I) and gold(I) most complexes formed with thiol ligands are believed to be polymeric,[1–5] but only a few structural studies have been performed. In mass spectrometry of silver-containing compounds, the presence of the two isotopes ^{107}Ag (51.8392%) and ^{109}Ag (48.1608%) is especially useful particularly for cluster compounds composed of several Ag atoms, because their mass spectra should give doublet peaks with almost equal intensity for monometallic species and triplets with about 1 : 2 : 1 intensity ratio for bimetallic species, and their isotopic distribution can then be utilized for identification of such compounds. In this work, we have successfully obtained the negative-ion ESI mass spectra[6] of the tetrametallic species derived from an aqueous solution of the oligomeric, thiomalato silver(I) complex $\{Na[Ag(Htma)]\cdot 0.5H_2O\}_n$ **1** [*n* = 24–34, *M* = 6910–9790, Htma = dianion of thiomalic acid $HO_2CCH(SH)CH_2CO_2H$], although the measurement of its fast-atom-bombardment (FAB) mass spectra was unsuccessful. Complex **1**,[7] which shows remarkable antimicrobial activity against selected bacteria, yeast and moulds, and is also effective in the life-prolongation of cut flowers such as chrysanthemums, roses and carnations, was recently prepared from the reaction of aqueous $AgNO_3$ with H_3tma dissolved in aqueous NaOH and has been characterized by complete elemental analyses, TG/DTA, FTIR, ^1H, ^{13}C and ^{109}Ag NMR spectroscopies, and molecular mass determination.† The sodium salt of this complex has been isolated as a light- and thermally-stable, yellow powder: other salts with different counterions such as Li, K, Cs, NH_4 or Ba, could also been obtained but not in a crystalline state.

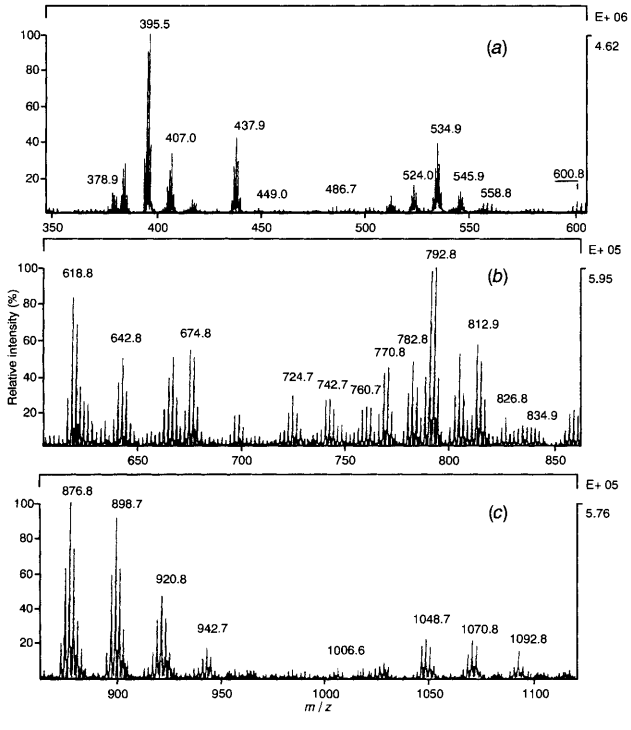

Fig. 1 Negative-ion ESI mass spectra of **1** measured in aqueous solution in the range *m/z* 350–600 (*a*), 600–860 (*b*) and 850–1120 (*c*)

The negative-ion ESI mass spectra,‡ [Fig. 1(*c*)], shows several prominent five-line peaks in the range *m/z* 850–1120, each of which consists of an approximate 1 : 4 : 6 : 4 : 1 intensity ratio indicative of tetrametallic silver(I) species. Observed isotopic distributions of each fragment *e.g.* $\{Na_3[Ag_4(Htma)_4]\}^-$ (*m/z* 1092.8) and $\{[Ag_4(Htma)_2(H_2tma)]\}^-$ (*m/z* 876.7) were in good agreement with calculated values (1092.521 and 876.576, respectively). Fragmentation based on a deprotonation and/or an attachment of Na^+ ion among tetrametallic silver(I) species readily acounts for the observed species (Table 1). In the ESI mass spectra shown in Fig. 1(*b*), several quartet peaks, with *ca.* 1 : 3 : 3 : 1 intensity ratios, observed in the range *m/z* 600–860, can be assigned to uninegative ions of trimetallic silver(I) species such as $\{Na[Ag_3(Htma)_2(H_2tma)]\}^-$ (*m/z* 792.8) and $\{[Ag_3(Htma)_2]\}^-$ (*m/z* 618.8). On the other hand, prominent peaks in the region *m/z* 350–600 shown in Fig. 1(*a*), are quintets with *ca.* 1 : 4 : 6 : 4 : 1 intensity ratio, but with line-to-line separation of only 0.5 *m/z*, indicating that such species species are dinegative ions of tetrametallic silver(I) species, *e.g.* $\{Na_2[Ag_4(Htma)]_4\}^{2-}$ (*m/z* 534.9) and $\{[Ag_4(Htma)_3]\}^{2-}$ (*m/z* 437.9).

The complete elemental analysis of **1**[7] indicated mole ratios $Na^+ : Ag^I : Htma^{2-}$ of 1 : 1 : 1 and suggests that the oligomeric complex **1** is cyclic. On the other hand, the present ESI mass spectrometric studies have demonstrated the presence of the tetrametallic silver(I) species with compositions consistent with the analytical data, strongly indicating that the cyclic, tetramer $\{Na_4[Ag_4(Htma)_4]\}$ is a stable, subunit of the oligomer **1**. However, this tetramer does not constitute the oligomeric complex **1** itself, because of the observed $[Na^+]$ values and

Table 1 ESI mass assignments of tetrameric silver(I) species

Found (*m/z*)	Formula
876.8	$\{[(Ag^+)_4(H_2tma^-)(Htma^{2-})_2]\}^-$
898.7	$\{Na[(Ag^+)_4(Htma^{2-})_3]\}^-$
920.8	$\{Na_2[(Ag^+)_4(Htma^{2-})_2(tma^{3-})]\}^-$
924.7	$\{Na_3[(Ag^+)_4(Htma^{2-})(tma^{3-})_2]\}^-$
1048.7	$\{Na[(Ag^+)_4(H_2tma^-)_2(Htma^{2-})_2]\}^-$
1070.8	$\{Na_2[(Ag^+)_4(H_2tma^-)(Htma^{2-})_3]\}^-$
1092.8	$\{Na_3[(Ag^+)_4(Htma^{2-})_4]\}^-$

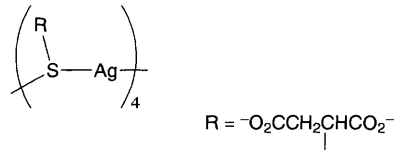

Fig. 2 Suggested cyclic tetrameric structure of a subunit of **1**

dissociation degree α = 0.689–0.701.[7] Oligomer **1** is probably $\{Na_4[Ag(Htma)]_4\}_x$ (x = 6–8) formed by a coupling of cyclic tetramers, on the basis of the measured n value (n = 24–34).

^{13}C and ^{109}Ag NMR spectroscopy[7] has shown that all the $Htma^{2-}$ ligands and Ag^I ions are equivalent in this oligomeric complex, and both ^{13}C NMR and FT-IR measurements have indicated that each Ag^I is only coordinated by the soft thiol S atom, but not with the hard carboxylic oxygens. Oligomerization thus results from S atoms bridging between Ag^I atoms (Fig. 2). FT-IR measurements[7] have also suggested that, in the solid state, one of two carboxylic groups in the monomeric unit is protonated and that the other interacts with the Na^+ ion. On the other hand, the observed dissociation degree (α = 0.689–0.701) and the almost neutral pH ($\approx$ 5.8) of the oligomeric complex **1** in aqueous solution indicates that about 70% of the attached Na^+ ions in the solid state are dissociated in aqueous solution, but that none of the carboxylic protons are dissociated.

Thus, either the carboxylic protons or the attached Na^+ ions should participate, both in the solid state and in aqueous solution, in the coupling between cyclic tetramers $\{Na_4[Ag_4(Htma)_4]\}$ leading to the formation of the oligomeric complex **1**.

We thank M. Kanai, Finnigan Mat Instruments, Inc., for her helpful discussion of the ESI mass spectra.

Received, 11th April 1995; Com. 5/02328I

Footnotes

† The molecular weight and/or polymerization degree n have been determined[7] to be 6910–9790 (n = 24–34) from the relationship MM(1 + $n\alpha$) = 279n by a combination of the mol mass value MM = 382 (within ± 5%), cryoscopically observed using 7.95 mg of the complex dissolved in water (0.40457 g) (Mikroanalytisches Labor Pascher, Remagen, Germany), with a dissociation degree α = 0.689–0.701 determined from the slope of the line of $[Na^+]_{obs}$ *vs.* c_{Na} obtained from $[Na^+]$ measurement using an Na^+-ion selective electrode for seven different concentrations c_{Na} of the complex in aqueous solution in the range c_{Na} 0.285–14.23 mmol dm^{-3}. This comparatively large degree of polymerization has been also suggested by the temperature-dependent ^1H NMR spectra.

‡ The ESI spectra were measured on a MAT 900 mass spectrometer with an ESI source (Finnigan Mat, Bremen, Germany) in the range m/z of 100–1200; 10 to 20 scans were summed using a scan rate of 10 s $decade^{-1}$. The electrospray interface was heated to 270 °C (or capillary temperature), the spray voltage was 2.6 kV, the sheath N_2 gas pressure was 4.5 bar and an aqueous solution of **1** (60 pmol μl^{-1}) was infused into the ESI source at flow rate of 20 μl min^{-1} using a syringe pump (Harvard Apparatus, MA, USA).

References

1 P. D. Cookson and E. R. T. Tiekink, *J. Coord. Chem.*, 1992, **26**, 313.
2 C. W. S. Harker, E. R. T. Tiekink and M. W. Whitehouse, *Inorg. Chim. Acta*, 1991, **181**, 23.
3 C. F. Shaw, III, G. Schmitz, H. O. Thompson and P. Witkiewicz, *J. Inorg. Biochem.*, 1979, **10**, 317.
4 M. A. Mazid, M. T. Razi, P. J. Sadler, G. N. Greaves, S. J. Gurman, M. H. J. Koch and J. C. Phillips, *J. Chem. Soc., Chem. Commun.*, 1980, 1261.
5 K. Nomiya, Y. Kondoh, K. Onoue, N. C. Kasuga, H. Nagano, M. Oda, T. Sudoh and S. Sakuma, *J. Inorg. Biochem.*, 1995 in the press. The described n = 12–14 should be corrected to n = 21–27.
6 For recent papers on mechanisms of the ESI process see: J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Mass Spectrom. Rev.*, 1990, **9**, 37; R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga and H. R. Udseth, *Anal. Chem.*, 1990, **62**, 882; M. G. Ikonomou, A. T. Blades and P. Kebarle, *Anal. Chem.*, 1991, **63**, 1989.
7 K. Nomiya, K. Onoue, Y. Kondoh, N. C. Kasuga, H. Nagano, M. Oda and S. Sakuma, *Polyhedron*, 1995 in the press. The described n = 15–19 should be corrected to n = 24–34.