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Antimicrobial activity and thermostability of silver 6-benzylaminopurine montmorillonite

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ABSTRACT

Novel antimicrobial materials were constructed by the intercalation of organometallic cations into montmorillonite. The silver chelate complex of 6-benzylaminopurine was intercalated into montmorillonite by a cation exchange reaction in aqueous medium. The basal spacing of 1.8 nm was attributed to an almost planar arrangement of the silver chelate complex. Most of the interlayer cations were replaced by the silver chelate complexes. Thermogravimetric/differential thermal analyses indicated that the degradation temperature of the chelating ligands was increased by complexation with the silver ions and electrostatic binding in the interlayer space. *Ca.* 0.15% of the silver chelate complex was released in water after 7 days followed by steady-state release up to 22 days. The intercalated montmorillonite exhibited antibacterial and antifungal activity compared with the commercial silver supported products.

1. Introduction

Cytokinins are plant growth substances active in promoting cell division, and are also involved in cell growth, differentiation, and other physiological processes (Miller et al., 1955; Skoog and Armstrong, 1970). 6-Benzylaminopurine (6-BAP) is the first generation synthetic cytokinin, has proven to accelerate plant cell growth and division and has been recently developed as a color preserver in vegetables such as asparagus, broccoli, brussels sprouts, lettuce, and celery for extended color retention during harvesting, shipping and storage by using chlorophyll retention. The success of 6-BAP in increasing size and multiple budding characteristics in a variety of tropical and subtropical fruits and vegetables has attracted great interest in the agricultural economies (Sugiura, 2004).

We attempted to develop antimicrobial and antifungal agents derived from clay minerals, and have previously studied the properties of the silver ions or silver chelate complexes intercalated montmorillonite. These materials exhibited antibacterial and antifungal activities as compared with silver supported zeolite (Oya et al., 1991; Ohashi and Oya, 1992). The thermal stability up to 500 °C in air was also investigated (Ohashi and Oya, 1996). The thermal degradation of the intercalated species was significantly decreased. The present study is an investigation of the bioactivity of silver chelate complex with 6-BAP intercalated into montmorillonite.

2. Experimental methods

2.1. Materials

A commercial Na-montmorillonite (Mont, trade name Kunipia-F) was used as a host material was supplied by Kunimine Ind. Co (Japan). The cation exchange capacity (CEC) of the montmorillonite was 1.15 meq/g as reported by the supplier. The basal spacing of the montmorillonite with one-water layer was 1.21 nm. This montmorillonite was used without any further purification. The chemical grade, 6-benzylaminopurine (6-BAP, $C_{12}H_{11}N_5$, Wako Pure Chemical Ind. Ltd.) and silver nitrate ($AgNO_3$, Wako Pure Chemical Ind. Ltd.) were used as received.

2.2. Synthesis

Montmorillonite (10.67 g) was dispersed in water (*ca.* 2 mass%) and stirred at room temperature. Silver nitrate (2.50 g) was dissolved in deionized water in an amount of 1.2 CEC and 6-BAP (6.63 g) was dissolved in ethanol in an amount of 2.4 CEC. The two solutions were mixed at room temperature forming a white suspension of the silver-6-BAP chelate complex, $Ag^+(6\text{-BAP})_2NO_3^-$. The aged white sol was added to the montmorillonite sol and kept at 40 °C for 48 h with stirring. The intercalated montmorillonite (abbreviation: Ag(BAP)

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Mont) was repeatedly washed with deionized water to remove free 6-BAP, followed by air-drying at 40 °C. Ag(BAP)Mont was calcined at 200, 250, 300, 400 and 500 °C under an air stream with a heating rate of 10 °C/min and during 1 h.

2.3. Analyses

X-ray diffraction (XRD) patterns were obtained at 30 mA, 40 kV with a Rigaku RINT-2100 powder diffractometer equipped with Ni-filtered Cu-Kα radiation. The scanning step was 0.02 °, and the step time was 2° /min, TG-DTA were conducted using a Shimadzu DTG-50H thermogravimeter at a heating rate of 5 °C/min under air. Infrared spectra of the KBr disks were recorded on a Perkin-Elmer Spectrum-2000 FT-IR spectrometer. Carbon and nitrogen contents were determined by a Yanako CHN-corder MT-3 using a heat–conductivity detection with helium and Cu and W particles as combustion accelerator. The release studies were conducted as follows: 150 mg of intercalated montmorillonite was dispersed in 30 ml of deionized water and shaken at ambient temperature by using a rotary shaker (60 rpm). After specific intervals, 25 ml of the supernatant was taken out and separated by a centrifugation (3000 rpm, 10 min.). The same volume of deionized water was added to the sample retaining the total volume of 30 ml. The silver concentration in the supernatant was determined by an inductively coupled argon plasma emission spectrometer. Antibacterial and antifungal tests were carried out by minimum inhibitory concentration (MIC) tests. In the antibacterial tests, *Escherichia coli* (NBRC3972) and *Staphylococcus aureus* (NBRC12732) were used. *Aspergillus niger* (NBRC6341), *Penicillium citrinum* (NBRC6352), *Aureobasidium pullulans* (NBRC6353) and *Rhizopus oryzae* (NBRC31005) were employed for the antifungal tests. Ag(BAP)Mont was subjected to the MIC tests as follows. Bacterial strains were incubated at 37 °C overnight on nutrient agar (Eiken chemical, Tokyo, Japan). The suspensions were adjusted to approximately 10^4 CFU/ml in sterile normal saline solution. Ag(BAP)Mont was suspended in 5 mass% dimethyl sulfoxide solutions and diluted to the required concentration by serial 2-fold dilution in sterile Mueller Hinton Broth (MHB, BD, Sparks, MD). Each inoculum of 0.1 ml bacterial suspensions was applied to these serial MHB tubes. The minimum concentration without bacterial growth was considered to be the MIC after incubation at 36 ± 1 °C for 19 ± 1 h. Fungal strains were incubated at 25 °C for 7 to 10 days on potato dextrose agar slant. The fungal colonies were covered with approximately 1 ml of sterile 0.85 mass% saline solution, and the suspensions were withdrawn and transferred to a sterile tube. Inoculum density of the spore suspensions were adjusted to 10^4 CFU/ml using a hemacytometer. Ag(BAP)Mont suspended in 5 mass% dimethyl sulfoxide solutions were diluted to two times the strength of the final test concentration by following the standard additive twofold drug dilution schema with RPMI1640 medium (Nissui, Japan) used as the diluent. These suspensions were pipetted in 0.1 ml volumes into microdilution tray's wells. Each well was inoculated by adding 0.1 ml volume of the spore suspension. The microdilution tray was incubated at 25 °C for 4 days. After incubation, the minimum growth inhibitory concentration without fungal growth was considered to be MIC.

3. Results

The basal spacing of 1.73 nm of Ag(BAP)Mont as-prepared decreased gradually between 200 and 400 °C to reach to 1.55 nm and to 1.29 nm after heating to 500 °C. At 500 °C, the reflection of metallic silver was observed (Fig. 1). In the FT-IR spectrum of montmorillonite, characteristic broad absorption bands appeared at 3624, 3434 cm^{-1} (assigned O–H stretching vibration), 1639 cm^{-1} (assigned O–H bending vibration), 1038 cm^{-1} (assigned Si–O stretching vibration), 914 cm^{-1} (assigned Al_2O–H bending vibration) 847 cm^{-1} (assigned AlMgO–H bending vibration) and 520, 467 cm^{-1}

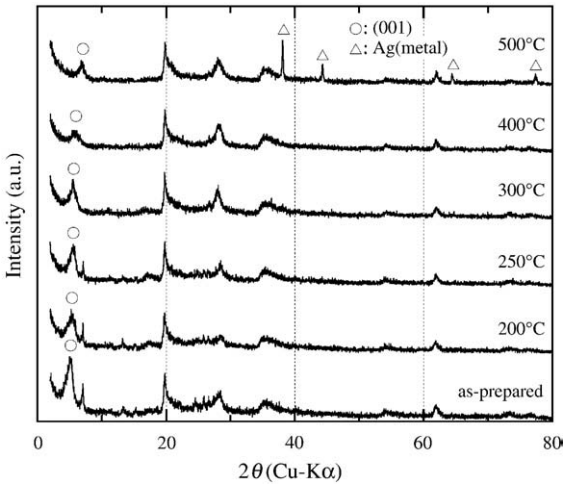

Fig. 1. XRD patterns of Ag(BAP)Mont before and after heating.

(assigned Si–O–Al bending vibration) (white arrows in Fig. 2). Some absorption bands of Ag(BAP)Mont resulted from 6-BAP, namely at 1541, 1450, 1400 and 1349 cm^{-1} (assigned C C, C N stretching vibration), 725 and 698 cm^{-1} (assigned aromatic C–H bending vibration) (black arrows). In Fig. 3, the relatively sharp endothermic peak of 6-BAP was observed at 230 °C, due to melting. Exothermic peaks at 356 °C and 520 °C were attributed to the thermal decomposition. The TG curve in Fig. 4 showed three-steps. The first mass loss and endothermic peak were due to free water evaporation. A small endothermic peak at 260 °C was attributed to the melting of interlayer 6-BAP. The second and third mass losses and exothermic peaks at 370 and 503 °C were due to the thermal decomposition. The final mass losses for 6-BAP and Ag(BAP)Mont at 1050 °C were 100% and 34%, respectively. Elemental contents and basal spacings for Ag(BAP)Mont before and after heating were indicated in Fig. 5. The original Ag(BAP)Mont before heating showed 15.5 mass% carbon and 7.50 mass% nitrogen. These contents decreased and reached 4.21 mass% carbon and 2.51 mass% nitrogen at 500 °C. A similar behavior was also observed the basal spacing. In the leaching tests in deionized water (Fig. 6) pronounced release during 7 days was followed by very small release rates.

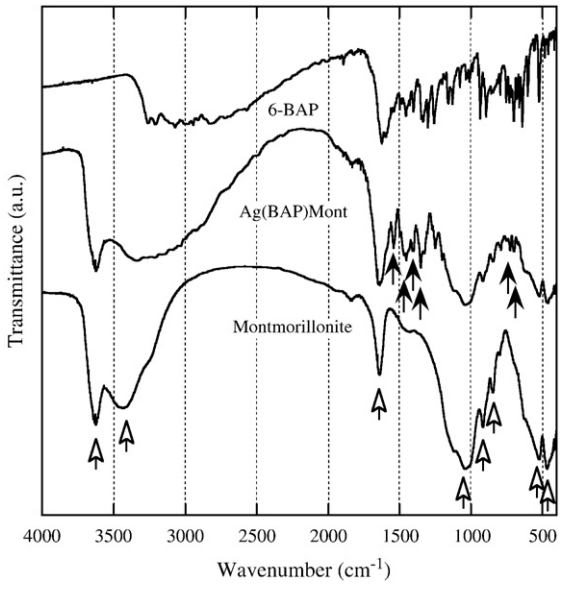

Fig. 2. FT-IR spectra of the samples.