東北大学

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報道機関　各位

東北大学生命科学研究科

銀過酸化物 Ag_2O_3 が持つ高い抗菌活性の発見

－東北大学科学者の卵養成講座の仙台二高生によるブレークスルー－

＜研究内容の概要＞

銀化合物や銀イオンが抗菌活性を有することは古くから知られており、医療器具をはじめ日常生活用品など様々な形で利用されています。今回、「東北大学科学者の卵」養成講座[注1)]において、仙台第二高等学校・化学部（顧問　渡辺尚教諭）に所属する安東沙綾さん、日置友智君、山田学倫君らは、硝酸銀の電気分解により銀樹（陰極側）を作製する過程で、陽極側に析出する金属光沢のある黒い結晶についての詳細な解析を行いました。最終的に X 線回折から銀過酸化物 Ag_2O_3 の結晶構造からなることを突き止めるとともに、汎用されている酸化銀 Ag_2O と比べて、より強力な抗菌活性（図 1）、高い酸化活性、電導性、さらに 10 倍以上の銀イオンを水に溶出する能力を有した Ag_2O_3 クラスレートであることを明らかにしました。この発見は、強力な抗菌作用を有する新たな銀酸化物として、様々な領域での広範囲な利活用が期待されます。本研究成果は、2011 年 11 月 23 日付けで米科学専門誌 Journal of Materials Science のオンライン版に掲載されました。[注2)]

[注1)] 本講座は独立行政法人科学技術振興機構「未来の科学者養成講座」委託事業。

[注2)] http://www.springerlink.com/content/831q480317723456/fulltext.pdf

【背景】

銀化合物や銀イオンが細菌やウイルス、真菌などに対する抗菌活性を有することは古くから知られており、特に、第一次大戦以降、スルファジアジン銀（通称　ゲーベンクリーム）が火傷などの薬として重用されてきました。その後、様々な抗生物質の発見により、これら銀化合物の利用は減少傾向にありましたが、近年、抗生物質耐性菌の新たな出現、さらに、銀化合物を含む様々な医療器具やナノパーティクル、日常生活用品における除菌・殺菌剤としての利活用など、再び脚光をあびています。

【研究のインパクト】

・Ag_2O_3 化合物は、国内外の試薬メーカーの市販品は無く、その特性についての報告はほとん

ど皆無であった。
・Ag_2O_3 は、これまでに $NaClO_4$ と $AgClO_4$ を用いた電気精錬法が Fisher ら(2007)により報告されてきたが、本法はより簡易で低コストなものである。
・粉末固体として、また、水に対する飽和水溶液（上澄み）としてのいずれにおいても、Ag_2O と比較して、より強力な抗菌活性を示す。
・酸化作用が強く、食紅などの脱色ができる。
・酸化物でありながら電気電導性を有する。

以上、大変ユニークな物性を持った酸化物であり、MRSA（メチシリン耐性黄色ブドウ球菌）などが形成するバイオフィルムに対しても有効となる可能性が示唆される。

【論文題目】
Ag_2O_3 clathrate is a novel and effective antimicrobial agent. Saaya Ando*, Tomosato Hioki*, Takamichi Yamada*, Naoshi Watanabe+, Atsushi Higashitani+ (*These author contributed equally; +Corresponding authors)
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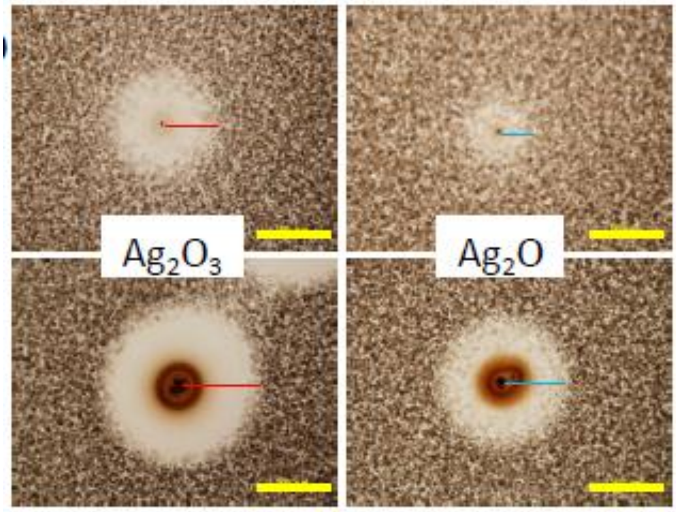
図１　Ag_2O_3 にみられる強い抗菌作用
酸化銀 Ag_2O と比較してより強い大腸菌に対する増殖阻止効果を示す。



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Ag_2O_3 clathrate is a novel and effective antimicrobial agent

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Abstract Silver compounds and silver ions are used extensively in medical devices because of their wide-spectrum antimicrobial activity. In particular, nanoparticles of silver and silver (I) oxide show great promise for widespread usage in medical polymers and nanodrugs. Here, we demonstrate that a crystalline powder and a saturated aqueous solution of silver (III) oxide clathrate show much stronger antimicrobial activities and oxidative activities than silver (I) oxide.

Introduction

Silver compounds and silver ions exhibit antimicrobial properties [1–5]. They have a toxic effect on bacteria, viruses, and fungi, which is typical of heavy metals such as mercury, cadmium, and lead. However, in humans, they do not show the high levels of toxicity that are usually associated with other heavy metals. Since World War I, silver compounds have been used to prevent infection. For example, silver sulfadiazine cream has broad antimicrobial activity and is commonly used for burn wounds [2, 3].

Saaya Ando, Tomosato Hioki, and Takamichi Yamada contributed equally to this study.

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Although the use of silver compounds reduced after the introduction of antibiotics, the evolution of antibiotic-tolerant bacteria, such as multi-drug resistant bacteria, has prompted a need to re-evaluate treatment strategies. In addition to the development of new medical devices, there is widespread use of silver alloy-coated urinary catheters, endotracheal breathing tubes and glass products coated with silver, silver compounds or silver-containing nanoparticles and glasses [6–10].

There are three oxidative forms of silver oxide: silver (I), Ag_2O; silver (II or I, III), AgO; and silver (III), $Ag_2O_3 \cdot Ag_2O$ (I) is the most common oxide and it is used in the production of certain medical devices. AgO is part of the manufacture of silver oxide-zinc alkaline batteries and is formulated as $Ag_2O \cdot Ag_2O_3$. It has been reported that Ag_2O_3 can be isolated by electrolysis of $NaClO_4$ and $AgClO_4$ [11]. Although $AgClO_4$ is a useful source of Ag^+ ions, the presence of perchlorate represents human health and explosion risks. Pure Ag_2O_3 may be difficult to obtain commercially and industrially, and its therapeutic characteristics remain poorly understood.

In this study, we have identified a silver crystal which was produced by anodic oxidation of silver salts in aqueous solutions including $AgNO_3$. We also performed to analyze the crystal with X-ray diffraction (XRD), and evaluate its antimicrobial activity.

Materials and methods

Preparation and identification of Ag_2O_3 clathrate

To obtain Ag_2O_3 clathrate, electrolysis of 100 mL of 1 M $AgNO_3$ were carried out in 100-mL beaker with platinum electrodes, one of which was installed into a 35-mm film

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plastic case with two slits (40 × 5 mm^2) as anode. Ag_2O_3 clathrate and pure silver were deposited on the anode and cathode, respectively. The plastic case prevents electrical short circuit caused by connection of either product on the electrodes. Approximately, 380 mg Ag_2O_3 clathrate was obtained in the plastic case of the electrolysis at 5 V dc for 15 min. XRD analysis of the Ag_2O_3 clathrate was performed with MO3XHF22 (MAC Science: X-rays Cu Kα: $\lambda = 1.5406$ Å, 40 kV, 30 mA, Ni filter and scan area: $10° \leqq 2\theta \leqq 70°$, $2\theta = 0.02$).

Measurements of antimicrobial activity

Escherichia coli K-12 wild-type W3110 strain was used for measurement of antimicrobial activity of silver compounds. To suppress the precipitation of AgCl, we used a bacterial culture broth LB excluding NaCl for the bioassay. Radius of inhibitory zone of bacteria growth and area of compounds were measured by a stereomicroscopy with digital camera (Olympus) and Image J software (National Institute of Health, USA).

Results and discussion

In an earlier report, Ag_2O_3 crystal could be produced by anodic oxidation of silver salts in aqueous solutions including $AgNO_3$ [12]. The crystal shows a cubic face centered oxide phase of the "ideal and stable composition" but it also contains Ag^{3+} and Ag^+ ions in various proportions, as Ag_2O_3 clathrate [13].

To confirm crystallizing Ag_2O_3 clathrate, electrolysis of the $AgNO_3$ were performed, and resulted in the deposition of silver oxide and pure silver on the anode and cathode, respectively. The silver compound on the anode formed shiny black, needle-like crystals (Fig. 1). After washing the crystals several times with distilled water, XRD analysis indicated the presence of a single compound, which was identified as silver (III) oxide, i.e., Ag_2O_3 (Fig. 2). In addition, AgCl precipitation reaction with reactions of excess Cl^- ions was used to estimate the concentration of Ag^+ ions in the saturated solutions. The Ag^+ ion concentration in the saturated water solution was 7.26 mM and much higher than that of Ag_2O solution (0.40 mM) (Fig. 3). These results well confirmed that the shiny black compound is Ag_2O_3 clathrate.

To study its antimicrobial activity, 100 mg of Ag_2O_3 clathrate powder was spread onto an agar plate. An untreated control plate and silver-treated plate were then exposed to the environment, whereby the lids were removed and the plates left open to the air at room temperature. After more than a week, it was clear that the Ag_2O_3 clathrate had completely inhibited the growth of

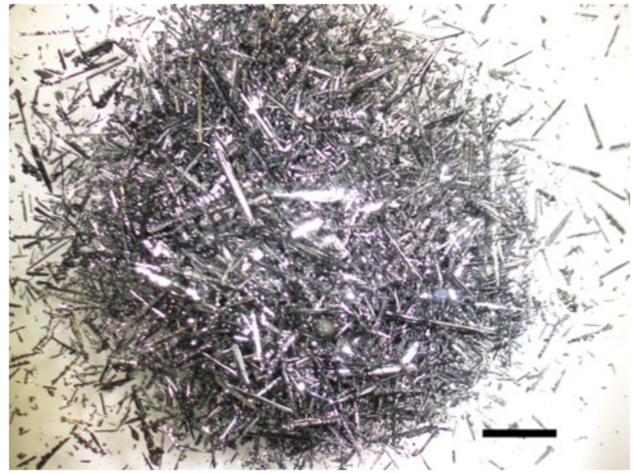

Fig. 1 Electrolysis of silver nitrate generates shiny black crystals of silver (III) oxide (Ag_2O_3) clathrate. Platinum electrodes were submerged in 1-M silver nitrate solution and electrolysis was performed using 5 V for 15–30 min. Approximately, 2.8% of the silver was recovered around the anode. *Scale bar* represents 5 mm

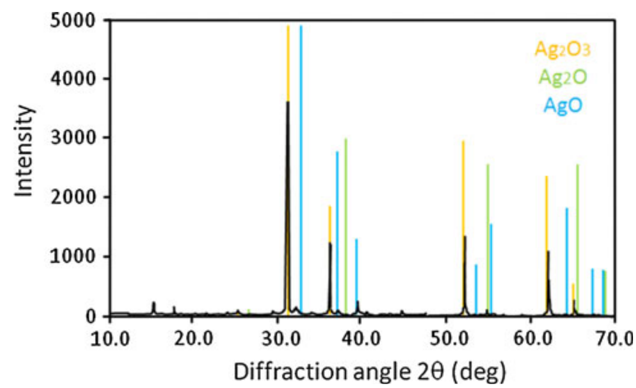

Fig. 2 X-ray diffraction pattern of the electrolytically deposited silver compound. Material deposited at the anode (*black*) showed major peaks identical to those for silver (III) oxide, Ag_2O_3 (*orange*). No major peaks corresponding to AgO (*blue*) or Ag_2O (*green*) were observed

bacteria, fungi and myxomycetes, which were present on the untreated control plate (Fig. 4a). In addition, the presence of a small amount of Ag_2O_3 clathrate solid inhibited the growth of *E. coli* K-12 much more effectively than an equivalent amount of Ag_2O (Fig. 4b, c). A saturated solution of Ag_2O_3 clathrate was prepared in Milli-Q water and 1 μL of the Ag_2O_3 solution was applied to a lawn of *E. coli* cells. A cleared area was evident for 24 h of incubation. Moreover, the saturated solution could completely sterilize 3 × 10^6 *E. coli* cells/mL within 3 h. The antimicrobial activity of the Ag_2O_3 clathrate solution appeared to be approximately 10-fold greater than that of the Ag_2O solution (Figs. 3, 4d).

Although scientists do not fully understand how silver compounds function against microorganisms, one hypothesis suggests that the antimicrobial activity is due to the

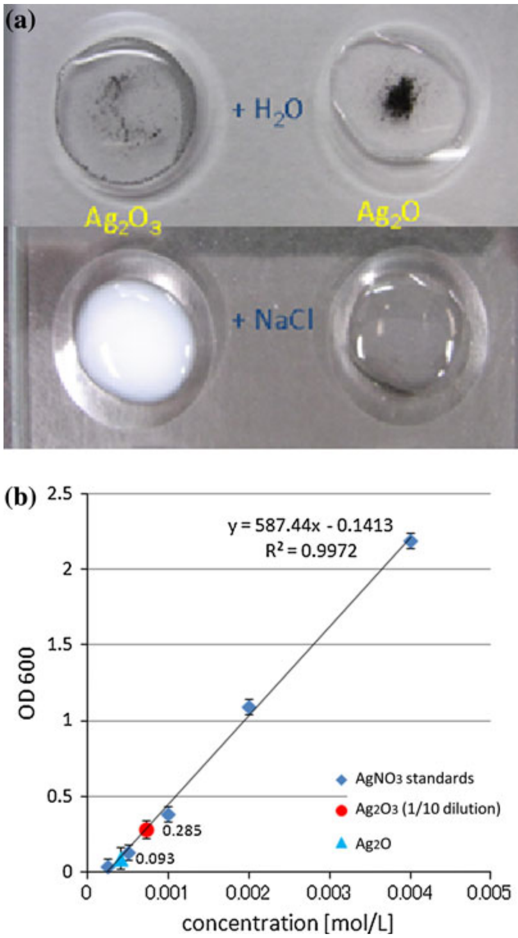

Fig. 3 Solubility of Ag_2O_3 clathrate in Milli-Q water. **a** Excess NaCl solution was added to the supernatants of Ag_2O and Ag_2O_3 clathrate saturated in Milli-Q water. *White* precipitates, AgCl appeared for the Ag_2O_3 clathrate solution (*bottom*). **b** Precipitation of silver oxide solutions. Calibration curve of the precipitation of $1\times$ Ag_2O solution and a 1/10 dilution of Ag_2O_3 solution were plotted with $AgNO_3$ and excess NaCl standards. The measurement was triplicated and data represents the mean $\pm$ standard error (SE)

chemical properties of its ionized form, i.e., Ag^+. In bacteria, it appears that the ions form strong molecular bonds with substances used for respiration and, in particular, with molecules containing sulfur, nitrogen and oxygen [4]. Ag_2O_3 clathrate also exhibits strong oxidizing activity, since it can bleach red food coloring (Fig. 5) and react strongly to ammonium hydroxide, producing oxygen bubbles. Ag_2O_3 clathrate crystals are stable and show no alteration to electronic conductivity after 30 h UV (254 nm) irradiation. Therefore, Ag_2O_3 clathrate shows better solubility in pure water than Ag_2O, leading to a greater availability of Ag^+ ions, and its oxidizing activities may also play a role in the strong antimicrobial activity observed.

In conclusion, these results clearly indicate that Ag_2O_3 clathrate may serve as a useful and potent antimicrobial

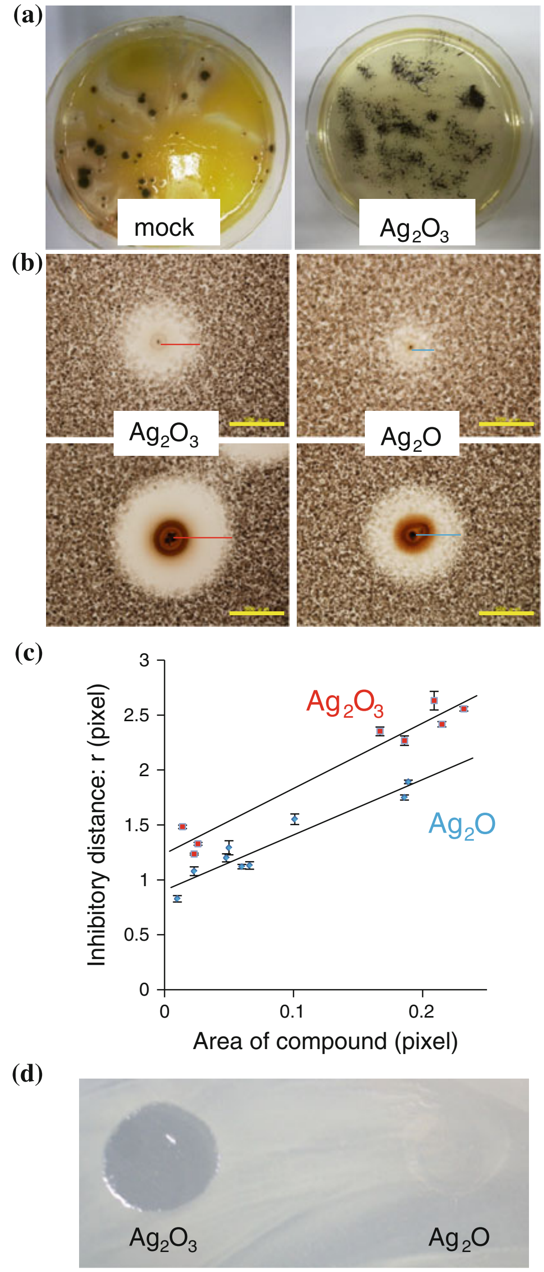

Fig. 4 Strong antimicrobial activity of Ag_2O_3 clathrate. **a** Inhibitory effect of Ag_2O_3 clathrate powder on the growth of environmental microorganisms. Agar plates were prepared with and without 100 mg Ag_2O_3 clathrate powder spread on the agar surface. The lids were removed and then the plates were incubated for more than 1 week at room temperature. **b–d** Effect of Ag_2O and Ag_2O_3 clathrate on the growth of *Escherichia coli* K-12. **b** Powder (*upper* particle area ca. 30 µm in diameter; *bottom* particle area ca. 150 µm in diameter) was applied to 3 mL of soft agar containing 10^7 bacteria. *Scale bars* represent 50 µm. **c** Radius of inhibitory zone of bacteria growth and area of Ag_2O and Ag_2O_3 clathrate compounds were measured and plotted. **d** Liquid (1 µL Ag oxide-saturated MilliQ water) was deposited on plates spread with 10^7 bacteria. Plates (9 cm in diameter) contained LB agar lacking NaCl. Plates with *E. coli* were cultured at 37 °C for 24 h

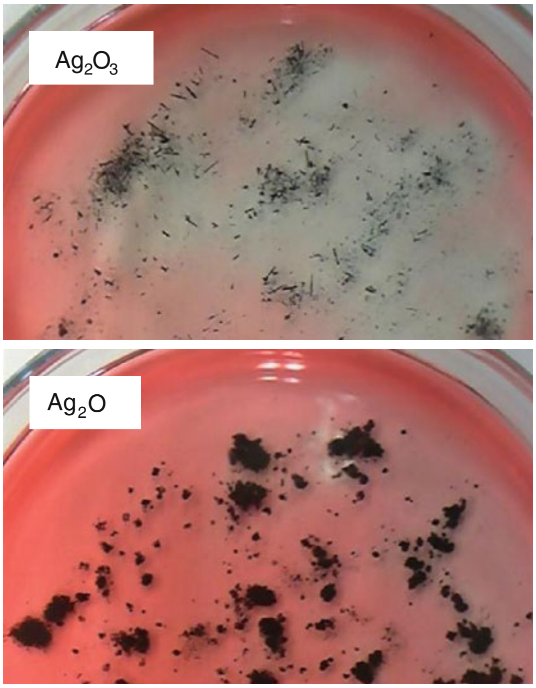

Fig. 5 Strong oxidative activity of Ag_2O_3 clathrate. Ag_2O_3 clathrate (*upper*) and Ag_2O powder (*bottom*) spread into aqua solution with *red* food coloring. Ag_2O_3 clathrate can efficiently bleach *red* food coloring

agent. It can be used as both a solid and in a saturated aqueous solution. Since the antimicrobial activity of Ag_2O_3 clathrate is greater than that of Ag_2O, it may be expected to replace the use of Ag_2O in certain medical equipment. It may be particularly suited to applications that require the reduction of biofilm formation by methicillin-resistant *Staphylococcus aureus* [6]. In addition, the procedure described for the isolation of Ag_2O_3 clathrate crystals is simple and inexpensive, which should enable its use to become widespread.

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