Original Article

Mechanism for the antibacterial activity of a mixture of 3,4,5-trihydroxy benzoic acid and magnesium oxide

Hiroyuki Noda (Graduate School of Science and Engineering, Yamagata University, hironoda@yz.yamagata-u.ac.jp)
Yoko Miyamoto (Institute for Life Support Technology, Yamagata Promotional Organization for Industrial Technology)

Abstract

In this paper, the mechanism for the antibacterial activity of a mixture of 3,4,5-trihydroxy benzoic acid (GA) and magnesium oxide (MgO) for *Escherichia coli* (JCM1649, *E coli*) is investigated. Antibacterial activity was assayed by the liquid dilution method against *E coli*. *E coli* in the logarithmic phase and the culture medium with pepton, yeast extract, NaCl, glucose, and $MgSO_4$ $7H_2O$ were used in all experiments. The logarithmic phase culture of *E coli* ($1x10^5$ CFU cm^{-3}) was added to the culture medium with GA and/or MgO. A Clark-type oxygen electrode with 1.1 cm^{-3} volume cell was used for the measurements of the oxygen consumption profile of a mixture of GA and MgO. The concentration of hydrogen peroxide (H_2O_2) in the mixture was estimated by using porphyrinato-titanium complex. Efficient antibacterial activity was observed on a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO. The addition of 110 units cm^{-3} catalase, which is an enzyme of H_2O_2 decomposition, in a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO led to the decrease of the antibacterial activity as same as the case of 2 mg cm^{-3} MgO. Moreover, the production of H_2O_2 in the mixture was confirmed by the oxygen consumption profile under the addition of 500 units cm^{-3} catalase. The accumulation of 3-4 mmol dm^{-3} H_2O_2 was confirmed in a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO. The increase in the concentration of H_2O_2 from 3 mmol dm^{-3} to 4 mmol dm^{-3} led to a drastic increase in the antibacterial activity. Based on the results of this study, a possible mechanism for the antibacterial activity of a mixture of GA and MgO is discussed, concerning a synergy effect on the antibacterial action of MgO and that of H_2O_2.

Key words

3,4,5-trihydroxy benzoic acid, MgO, H_2O_2, antibacterial activity, synergy effect

1. Introduction

Some antibacterial and antiviral properties of 3,4,5-trihydroxy benzoic acid (GA) have been reported against oral bacteria (Kang et al., 2008), *Staphylococcus aureus* (Akiyama et al., 2001), and herpes simplex virus (Kane et al., 1988; Kratz, 2008). The antibacterial activity of GA against cariogenic bacteria was about three orders of magnitude lower than that of chlorhexidine as a sterilizer (Kang et al., 2008). Therefore, GA may be too weak to use as a sterilizer.

It is reported, recently, that some heterogeneous catalysts adsorbed on magnesium oxide (MgO) powder had higher reactivity in chemical reactions than homogeneous systems (Noda et al., 1994; 1999; 2004). In the case of the above systems, a catalytic capability of MgO, such as surface basicity, plays a key role on their higher reactivity. Furthermore, a synergy effect on the antibacterial activity against *Escherichia coli* (*E. coli*) had been observed for GA, epigallocatechin, epigallocatechin gallate, and some plants powder containing polyphenols such as green tea, *rosmarinus officinalis* L. and *Syzygium aromaticum* (L.) Merrill & Perry with MgO (Noda et al., 2008, Kurita et al., 2012; 2013). The cause of these synergy effects was considered to be related to the production of hydrogen peroxide (H_2O_2) by the reaction between polyphenol and MgO in the presence of dissolved oxygen. However, the mechanism for the antibacterial mechanism on above systems has not yet been cleared.

During this study using a mixture of GA and MgO to clarify the mechanism of its antibacterial activity for *E. coli*, it was found that the production of H_2O_2 plays a key role on its antibacterial activity. The details of the experimental result will be described and a possible mechanism for a synergy effect on the antibacterial action of MgO and H_2O_2 produced in the mixture will be discussed.

2. Materials and methods

2.1 Materials

Magnesium oxide (99.9 %, 0.01 μm), 3,4,5-trihydroxy benzoic acid (GA) monohydrate, 30 % H_2O_2 solution, and catalase were purchased from Wako pure chemical Ind. Ltd. Porphyrinato-titanium complex was purchased from Tokyo Chemical Industry Co., Ltd.

2.2 Evaluation of antibacterial activity

Antibacterial activity was assayed by the liquid dilution method against Escherichia coli (JCM1649, *E coli*). *E coli* in the logarithmic phase and the culture medium with pepton (1 g dm^{-3}), yeast extract (0.2 g dm^{-3}), NaCl (0.5 g dm^{-3}), glucose (0.1 g dm^{-3}), and $MgSO_4$ $7H_2O$ (0.1 g dm^{-3}) were used in all experiments. The logarithmic phase culture of *E coli* ($1x10^5$ CFU cm^{-3}) was added to the culture medium with GA and/or MgO. After incubation under aerobic condition at 310 K for

3 or 5 h, the levels of *E coli* growth were measured by using a plate method with 1/10 nutrient ager.

2.3 Measurements of the concentration of oxygen and H_2O_2

A Clark-type oxygen electrode with 1.1 cm^{-3} volume cell (Central Kagaku Co.) was used for the measurement of the oxygen consumption profile of a mixture of GA and MgO. The oxygen consumption profile was recorded after the addition of the suspension of MgO in the mixed solution of GA and 50 mmol dm^{-3} phosphate buffer (pH7.4) at room temperature. The production of H_2O_2 was confirmed by the oxygen consumption profile observed under the addition of 500 units cm^{-3} catalase.

The concentration of H_2O_2 in the reaction mixture was estimated by using porphyrinato-titanium complex (Matsubara et al., 1992).

3. Results

Figure 1 shows the time dependence for the numbers of survival cells under various conditions. Efficient antibacterial activity was observed on a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO (closed circle). Bacteriostasis was observed on the culture medium with 2 mg cm^{-3} GA (closed square). Weak antibacterial activity was observed on the culture medium with 2 mg cm^{-3} MgO (closed triangle). The antibacterial activity of a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO decreased by the addition of 110 units cm^{-3} catalase, which is an enzyme of H_2O_2 decomposition (cross). These results, therefore, suggest that H_2O_2 plays a key role on the antibacterial activity of a mixture of GA and MgO.

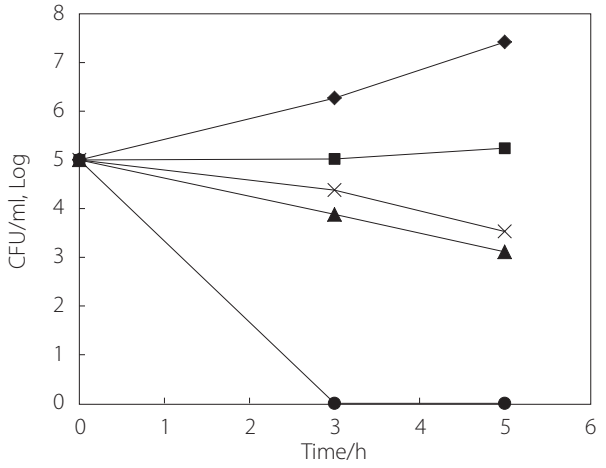

Figure 1: Time dependence for the numbers of survival cells under various conditions

Note: Closed diamond, control; closed square, 2 mg cm^{-3} GA; cross, a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO with 110 units cm^{-3} catalase; closed triangle, 2 mg cm^{-3} MgO; closed circle, a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO.

In order to clarify the mechanism for the antibacterial activity of our system in more detail, we conducted an estimation for the production of H_2O_2 and the evaluation of its antibacterial activity. Figure 2 shows the time dependence for the concentration of oxygen in a mixture of 0.1 mmol dm^{-3} GA and 4.5 mg cm^{-3} MgO. The addition of MgO led to the decrease of the concentration of oxygen. Moreover, the addition of catalase led to the production of oxygen ($H_2O_2 \rightarrow 1/2O_2 + H_2O$). This indicates the presence of H_2O_2 in the system. Figure 3 shows the time dependence for the production of H_2O_2 in the mixture. The accumulation of 3-4 mmol dm^{-3} H_2O_2 were confirmed in the mixture. Figure 4 shows the time dependence for the numbers of survival cells under various H_2O_2 concentrations. The increase in the concentration of H_2O_2 from 3 mmol dm^{-3} to 4 mmol dm^{-3} led to a drastic increase in the antibacterial activity.

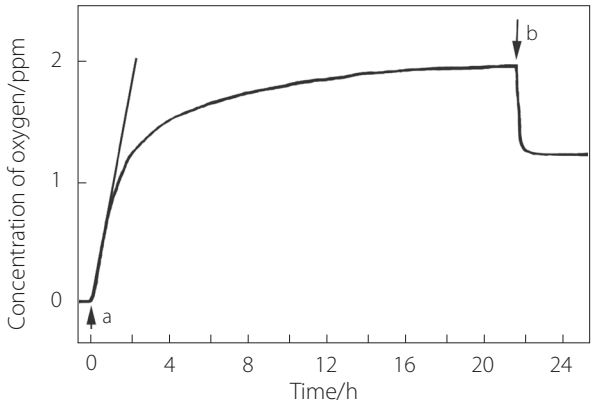

Figure 2: Time dependence for the concentration of oxygen in a mixture of 0.1 mmol dm^{-3} GA and 4.5 mg cm^{-3} MgO

Note: a, addition of MgO; b, addition of 500 units cm^{-3} catalase.

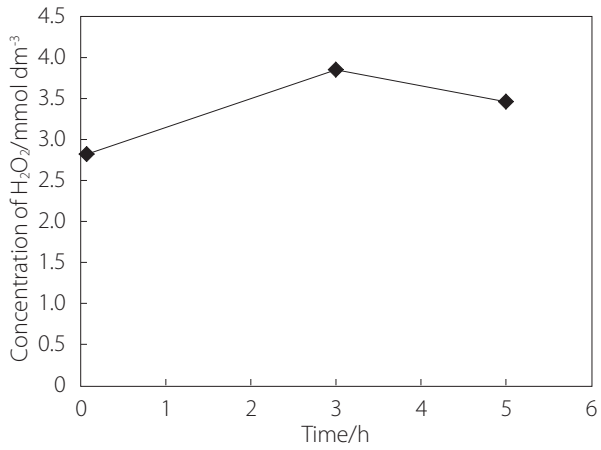

Figure 3: Time dependence for the concentration of hydrogen peroxide in a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO

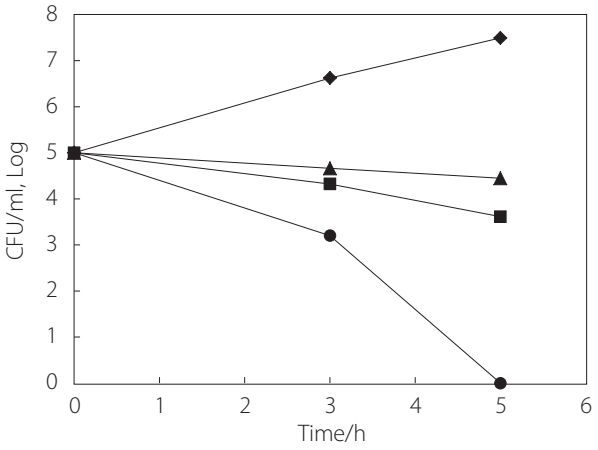

Figure 4: Time dependence for the numbers of survival cells under various conditions

Note: Closed diamond, control; closed square, 1 mmol dm^{-3} H_2O_2; closed triangle, 3 mmol dm^{-3} H_2O_2; closed circle, 4 mmol dm^{-3} H_2O_2.

4. Discussion

On the basis of the results obtained in the present study, a possible mechanism for the antibacterial activity of a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO is presented here. The results shown in Figure 1 indicate that a mixture of 2 mg cm^{-3} MgO and 2 mg cm^{-3} GA with catalase (cross) has the same antibacterial activity of 2 mg cm^{-3} MgO (closed triangle). The results shown in Figure 2 indicate that a mixture of GA and MgO causes the oxygen consumption and the production of oxygen under the addition of catalase. The accumulation of 3-4 mmol dm^{-3} H_2O_2 was confirmed in a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO, as shown in Figure 3. Moreover, the antibacterial activity of 4 mmol dm^{-3} H_2O_2 was lower than that of a mixture of 2 mg cm^{-3} GA and 2 mg cm^{-3} MgO accumulating 3-4 mmol dm^{-3} H_2O_2, as shown in Figure 4. Therefore, efficient antibacterial activity observed in this study is considered to be caused by a synergy effect on the antibacterial action of MgO and H_2O_2, as shown in Figure 5.

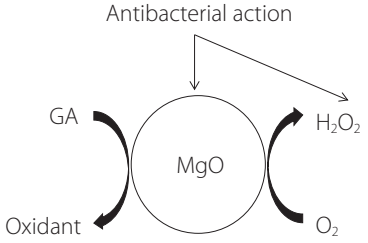

Figure 5: Schematic illustration of antibacterial action of a mixture of GA and MgO in the presence of oxygen

In conclusion, it has been demonstrated that the mechanism for the antibacterial activity of a mixture of GA and MgO can be explained based on a synergy effect on the antibacterial action of MgO and H_2O_2. The observed results of this report represent a useful approach to understanding the mechanism for the antibacterial activity of the mixture of polyphenol and MgO.

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