Note

Sulforaphane shows growth inhibitory effects against *Escherichia coli* and *Bacillus subtilis* via reactive oxygen species production

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要旨：ブロッコリー等のアブラナ科野菜に微量に含有されるイソチオシアネートの一種であるスルフォラファンは、細菌類、アブラムシ、ダニやネマトーダ等に対する毒性を有し、これらの植物において生体防御に重要な役割を担っていると考えられている。一方、スルフォラファンは生活習慣病の予防に寄与する食品成分として注目を集めており、これを高濃度で含有するブロッコリースプラウト（ブロッコリーの新芽）に対する関心も高まっている。スルフォラファンの抗菌効果については幾つかの報告があるが、その作用機作には不明の点が多く残されている。本論文では、代表的なグラム陰性細菌である大腸菌（*Escherichia coli*）及び代表的なグラム陽性細菌である枯草菌（*Bacillus subtilis*）を用いてこれらの細菌の増殖に対するスルフォラファンの影響を調べた。その結果、ヒト細胞に比べると高い濃度（280 μM）で、スルフォラファンはこれらの細菌に対する増殖抑制効果を示した。また、この増殖抑制効果は活性酸素スカベンジャーである N-アセチルシステインの添加で顕著に緩和された。この結果は、スルフォラファンの細菌に対する増殖抑制効果には活性酸素が関与する可能性を強く示唆している。

キーワード：スルフォラファン、大腸菌、枯草菌、細胞増殖、N-アセチルシステイン

Summary

Sulforaphane is a typical phytochemical belonging to isothiocyanate derivatives from cruciferous vegetables such as broccoli and its sprout. As is well known, anti-cancer effects of sulforaphane are receiving increased attention throughout the world, now. On the other hand, sulforaphane protects higher plants from various invaders such as aphids, ticks, bacteria or nematodes. Although several findings on the anti-bacteria effects of sulforaphane have been reported, elucidation of the mechanisms of its anti-bacteria effects still remains poor. In this paper, we examined the effects of sulforaphane on growth of *Escherichia coli* (a typical gram-negative bacterium) and *Bacillus subtilis* (a typical gram-positive bacterium). High concentration of sulforaphane (280 μM) showed gradual growth inhibition against these two bacteria. Interestingly, the growth inhibition was attenuated by N-acetyl-L-cysteine, a scavenger of reactive oxygen species (ROS). Our data suggested that sulforaphane causes growth inhibition against bacteria via generating ROS.

Key words: sulforaphane, *Escherichia coli*, *Bacillus subtilis*, cell growth, N-acetyl-L-cysteine

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Introduction

Higher plants produce various chemical compounds with many biological activities called phytochemicals. Among them, sulforaphane [1-isothiocyanato-4-(methyl-sulfinyl) butane] is an isothiocyanate compound derived from cruciferous vegetables, i.e. broccoli and its sprouts. It is released by hydrolysis of sulforaphane glucosinolate catalyzed by certain glucosidases[1]. As is well known, sulforaphane protects higher plants from infectious diseases caused by various bio-invaders including bacteria[2]. While sulforaphane reveals anti-bacterial activity, there is little information on the detailed mechanisms of its anti-bacterial activity. Nowicki *et al*. reported that sulforaphane is effective against various bacteria belonging to gram positive or gram negative bacteria[3,4]. They revealed that isothiocyanates may have more than one target in the bacterial cells, and the mechanism of their antibacterial activity is species-specific. On the other hand, Wu *et al*. indicated that sulforaphane can affect cell membrane permeability, material and energy metabolism and inhibit the synthesis of nucleic acids and proteins[5]. However, the detailed mechanisms of anti-bacterial activity of sulforaphane remain to be dissolved still now.

In this paper, we investigated the effects of sulforaphane on growth of *Escherichia coli* (a typical gram-negative bacterium) and *Bacillus subtilis* (a typical gram-positive bacterium) in liquid culture. In addition, we studied the influences of N-acetyl-L-cysteine (NAC), a reactive oxygen species (ROS) scavenger, on the inhibitory effects of sulforaphane against bacterial growth. Here, we revealed that sulforaphane shows the NAC-sensitive growth inhibitory effects against *E. coli* and *B. subtilis*.

Materials and Methods

1. Materials

Sulforaphane (Cayman Chemical, MI, USA) and NAC (Tokyo Chemical Industry, Tokyo, Japan) were obtained.

2. Bacteria and treatment with sulforaphane

E. coli DH5α was purchased from Nippon Gene Co. (Tokyo, Japan). *B. subtilis* (NBRC 3009) was provided by the NITE Biological Resource Center, Japan. These bacteria were inoculated in 1 ml of LB medium were incubated in the absence or presence (140 or 280 μM) of sulforaphane at 37°C. The growth of bacteria was evaluated by measuring optical density at 600 nm using CO8000 Biowave turbidity meter (Biochrom Ltd., Cambridge, UK).

3. Statistical analysis

Data are presented as averages of three separate and independent experiments. Error bars indicate standard deviation. Statistical differences were calculated with Student's *t* test.

Results and Discussion

To study the growth inhibition caused by sulforaphane against bacteria, we examined the effects of commercially available sulforaphane on growth of *E. coli* and *B. subtilis*.

First, as shown in Fig. 1, the growth rates of both *E. coli* and *B. subtilis* were remarkably decreased in the presence of 280 μM sulforaphane. In the presence of 140 μM sulforaphane, the growth of *B. subtilis* was moderately prevented upto 100 min while that of *E. coli* were clearly inhibited upto 120 min. In contrast, the proliferation of human leukemia U937 cells would be inhibited by low concentration sulforaphane (~ 5 μM)[6, 7]. These results suggested that these two bacteria show certain resistance against sulforaphane but their resistance against sulforaphane is very higher than human cells. Choi *et al*. reported that the sulforaphane-induced U937 cell death is responsible for the generation of intracellular reactive oxygen species (ROS)[6]. Moreover, addition of NAC dramatically protected U937 cells from apoptosis via inhibition of the sulforaphane-induced ROS generation[6].

Therefore, to know whether ROS are involved in the sulforaphane-mediated growth inhibition,

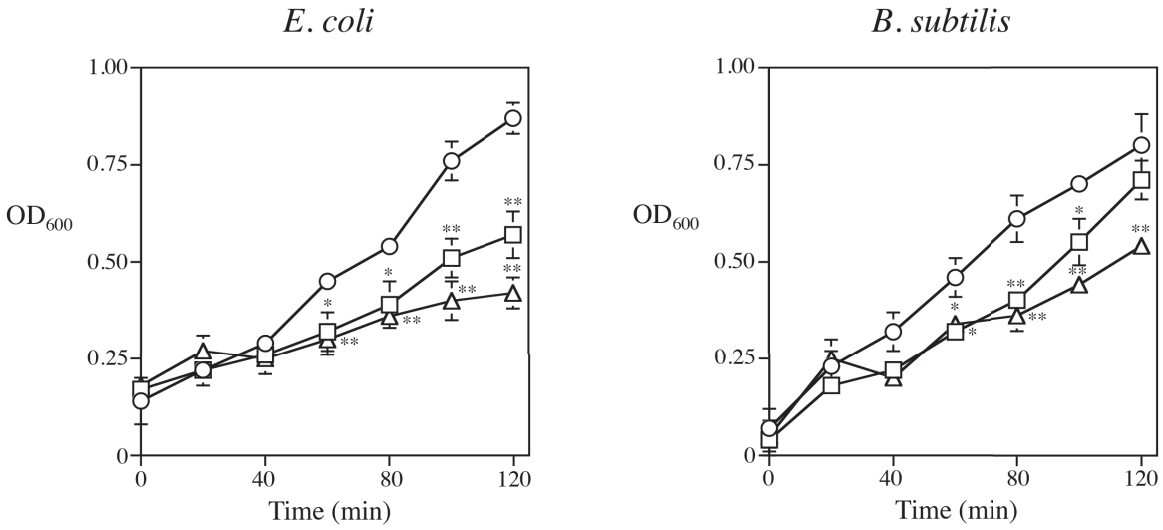

Fig. 1. Influences of sulforaphane on growth of bacteria (*E. coli* and *B. subtilis*). Bacteria were incubated in LB medium without (open circles) or with 140 (open squares) or 280 μM (open triangles) sulforaphane for upto 120 min. The growth of bacteria was evaluated by measuring optical density at 600 nm using CO8000 Biowave turbidity meter (Biochrom Ltd., Cambridge, UK). Data represent the averages of three separate experiments. Error bars indicate standard deviation. Statistical differences at each time point were calculated using Student's *t* test. *, $p < 0.05$; **, $p < 0.01$ compared with the data for without sulforaphane.

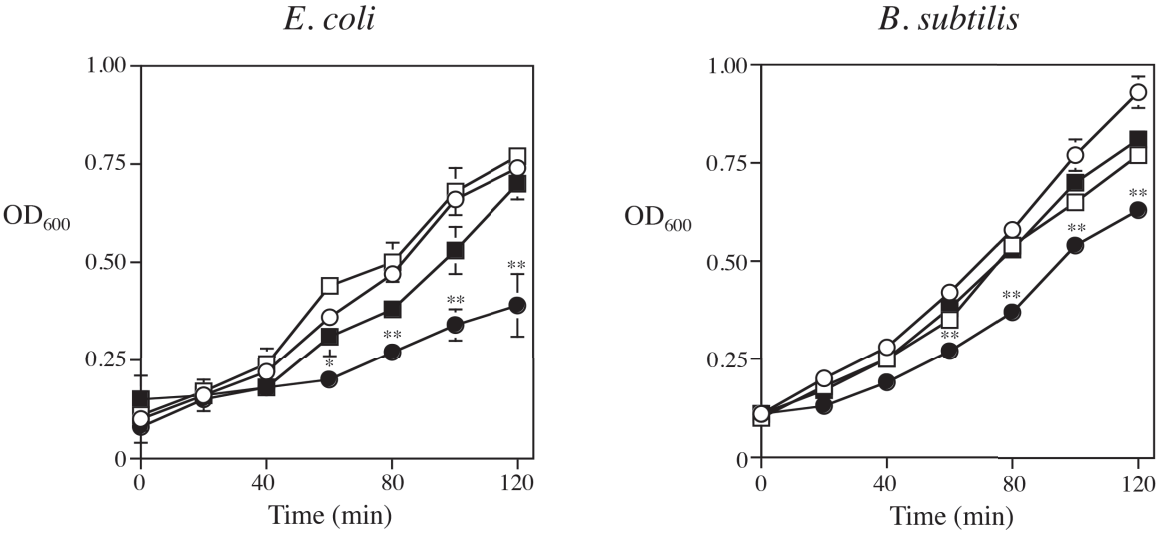

Fig. 2. Influences of NAC on growth of bacteria in the presence of sulforaphane. Bacteria were incubated in LB medium without (open symbols) or with 280 μM (filled symbols) sulforaphane in the absence (circles) or presence (squares) of 5 mM NAC for upto 120 min. The growth of bacteria was evaluated by measuring optical density at 600 nm using CO8000 Biowave turbidity meter (Biochrom Ltd., Cambridge, UK). Data represent the averages of three separate experiments. Error bars indicate standard deviation. Statistical differences between "sulforaphane only" (●) and "sulforaphane plus NAC" (■) at each time point were calculated with Student's *t*-test. *, $p < 0.05$; **, $p < 0.01$.

we added NAC to the bacterial culture in the presence of sulforaphane. As expected, the growth rates of both *E. coli* and *B. subtilis* were remarkably recovered by addition of 5 mM NAC in the presence of 280 μM sulforaphane (Fig. 2). These results revealed that sulforaphane-mediated growth inhibition would be brought about by ROS not only in human cells but also in bacteria. As is well known, the mitochondrial electron transport system is one of the major cellular generators of ROS[8]. The reason why two bacteria used in our study show stronger resistance as compared with U937 cells against sulforaphane may be due to the difference of the electron transport system between bacteria and human being.

Our findings in this study revealed that sulforaphane shows the growth inhibitory effects against *E. coli* (a typical gram-negative bacterium) and *B. subtilis* (a typical gram-positive bacterium). It is expected that sulforaphane with antibacterial activity will become an important reagent for development of treatment for various types of bacterial infections in combination with other drugs.

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