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Inhibition of Lipoxygenase Activity by the Extract of Various Processed Garlic
- Inhibitory Effect of Garlic Extracts on Soybean Lipoxygenase Activity -

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Abstract

The bioactivity of garlic extract was evaluated, based on the inhibition of soybean lipoxygenase. While the inhibition of lipoxygenase by the chloroform extract (I_{50} value after 10min preincubation, 55mg/ml) of garlic homogenate shows the property as irreversible inhibitors, the aqueous extract (I_{50} value, 65mg/ml) appeared to contain mainly reversible inhibitors. In the related study, diallyldislufide and dimethyldisulfide inhibited the enzyme with I_{50} value of 1.3mM and 18mM, respectively. These disulfides demonstrated both irreversible and reversible patterns of inhibition. In addition, synthetic alliin(allylcysteine sulfoxide) was found to inhibit the enzyme at high concentration (approximately 22% at 10mM), and its decomposition products showed the irreversible property in the inhibition, in contrast to S-ethyl cysteine sulfoxide which expressed no significant inhibition. Thus, it is suggested that the garlic macerate contains both irreversible and reversible sulfur inhibitors.

Key words : garlic extract, lipoxygenase, reversible inhibition, irreversible inhibition

INTRODUCTION

It is well known that *Allium* species produce a characteristic flavor when they are cut, crushed, or squeezed to juice. Their flavor is produced by sulfenic acids derived from the action of CS-lyase (alliinase) on S-alk(en)yl-L-cysteine sulfoxides[1].

Garlic extracts are reputed to offer protection against strokes, coronary thrombosis, atherosclerosis, and platelet aggregation[2-4]. Ariga et al.[5] have isolated a platelet aggregation inhibitor identified as methylallyltrisulfide from garlic. Recently, it was reported that garlic had a potent antithrombotic agent identified as 2-vinyl-4H-1,3-dithiin, 3,4-dihydro-3-vinyl-1,2-dithiin, diallyltrisulfide, and a compound named ajoene ; these compounds were nonenzymatically derived from allicin (CH_2=CHCH_2S(O)-SCH_2CH=CH_2, allyl-2-propenethiosulfinate)[6].

Recently, allicin, the unstable component, has been reported to irreversibly inhibit prostaglandin synthetase or 5-lipoxygenase responsible for the formation of inflammation mediator[7,8]. Further studies showed that ajoenes, vinyldithiin or others also exerted the inhibitory effect on platelet aggregation[6,9]. Futhermore, even dialkylsulfides were found to inhibit lipoxygenase[10]. Therefore, it was surmised that the inhibition of lipoxygenase by garlic extract may be due to the presence of stable and/or unstable inhibitors in garlic homogenate. In this respect, it was suggested that decomposition products of allicin or the other sulfur constituents in garlic may be responsible for various bioactivities of garlic. In this study, the bioactivity of garlic extract was based on the inhibition of lipoxygenase.

MATERIALS AND METHODS

Materials

α-Linoleic acid (99%), soybean lipoxygenase (Type IV), and Tween 20 were products of Sigma Chemical Company. Dimethyldisulfide and diallyldisulfide were from Fluka Chemical Company. Chloroform, DL-α-tocophrol (99%), L-ascorbic acid, boric

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acid were products of Junsei Chemical Company. Other reagents were of GR reade. Garlic oil(pure refined) was from Cho Dang Pharmaceutial Cooperation. Alliin was synthesized by the method of Stoll and Seebeck[11]. Garlic clove which was from Yeongi-Kun, was purchased at a local market on June 1992.

Assay of lipoxygenase activity

Lipoxygenase activity was determined at 234nm using a spectrophotometer (Milton Roy Spectronic 1201), as previously described[12]. One unit is expresed as the increase in A_{234} of 0.001 per min at pH 9.0 at 25°C when linoleic acid is the substrate in 3.0ml volume.

Preincubation of soybean lipoxygenase with garlic extracts or other sulfur inhibitors

Garlic extract (10~180mg/ml), sulfides (0.1~10 mM) or garlic oil (4~32μl/ml) was preincubated with 150, 300 units of soybean lipoxygenase in 15 ml of 0.1N borate buffer, pH 9.0 at 25°C. At the indicated time intervals, the aliquot (100μl) was taken, and used for the assay of the remaining activity of lipoxygenase. The inhibition degree was expressed as the relative percentage (%) of the inhibited activity of total activity (control). The amount of each extract to accomplish 50% inhibition was expressed as the weight (mg) of garlic per ml.

Preparation of water or ethanol extract of garlic

Fresh or boiled (7.5, 15, 30min) garlic clove (10g) was macerated for 3min with homogenizer and extracted with distilled water (15ml) or ethanol(15ml) for 5min. The extract was centrifuged (3,000rpm) for 30min, and then the supernatant was used.

Preparation of chloroform extract of garlic

Macerated fresh or boiled garlic clove (10g) was extracted with chloroform (15ml), and filtrated, and moisture was removed from the filtrate with anhydrous sodium sulfate. Chloroform was evaporated by flushing with N_2 gas. The residue was dissolved in ethanol (1ml) and then used for the test of the inhibitory effect.

Preparation of sulfur containing compounds

Diallyldisulfide, dimethydisulfide or diallylsulfide were dissolved in ethanol, and then used. Alliin (allylcysteine sulfoxide) and ethylcysteine sulfoxide were synthesized according to Stoll and Seebeck[11], and the white bundled crystal was dissolved in distilled water and then used.

RESULTS AND DISCUSSION

As shown in Fig. 1, the lipoxygenase activity, represented by a time-dependent and linear increase of absorbance at 234nm, was lowered by the addition of garlic extract (n=3, $p<0.05$). The inhibitory effect of garlic extract seems to be mainly of reversible property, since the linearity of the slope was observed during a short time (<5min) assay condition. However, the slight deviation from the lineary after 5min suggests that the enzyme activity may be inactivated slowly by the irreversible component from the extract.

In the following experiment, various extracts such as water, ethanol or chloroform extract were prepared, and investigated for the inhibition of lipoxygenase. Fig. 2A shows that the inhibition of lipoxygenase by aqueous extract demonstrates a concentration-dependent inhibition. After 10min preincubation, the inhibitory potency increased a little bit (I_{50} value, 65mg/ml). Thus, the inhibition by the aqueous extract appears to be instantaneous, sug-

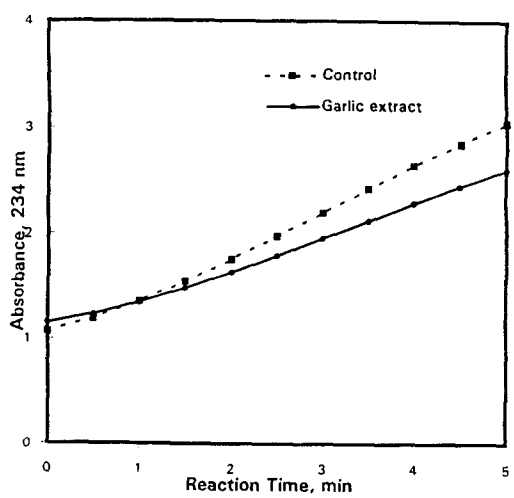
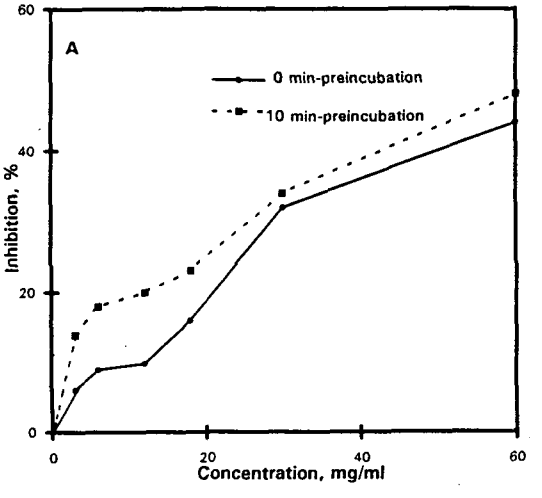


Fig. 1. Inhibition of lipoxygenase by garlic extract.

gesting that the water extract contain largely the reversible inhibitors. Also, the same pattern of inhibition was observed with the ethanolic extract (Fig. 2B). After 10min preincubation, the I_{50} value decreased from 65mg/ml to 46mg/ml. In comparison, the ethanolic extract showed a more inhibitory potency after 10min preincubation, compared with the aqueous extract. These results indicate that the water-unstable inhibitor is being produced during preincuba tion.

Meanwhile, the chloroform extract (Fig. 3) expressed a greater enhancement of inhibitory potency after preincubation. The I_{50} value decreased remarkably from 300mg/ml to 55mg/ml afer 10min incubation. Moreover, the enhanced inhibitory effect appeared to be time-dependent. These results suggest that some inhibitory components are more stable in chloroform than ethanol-water mixture. This might be due to the presence of allicin or its conversion products such as ajoene, vinyldithiin or diallyltrisulfide as had been reported previously[8]. The above suggestion is further supported by another observation (Fig. 4) that the aqueous extract, ethanol extract or chloroform extract of heat-treated sample exhibited no significant difference in the pattern of inhibition. As might be expected, the heat-treatment decreased the inhibitory potency by 25 to 30%. Thus, since some of the inhibitory component was decomposed during heat-treatment, it is suggested that a larger part of the inhibitory effect is expressed by the heat-stable components. Therefore, it is assumed that the irreversible inhibition may be ascribed to the unstable components. Based on these observations, it is suggested that the garlic extract contains both reversible inhibitors and irreversible inhibitors.

In the attempt to further clarify the different inhibitory effect of various extracts, various sulfur compou-



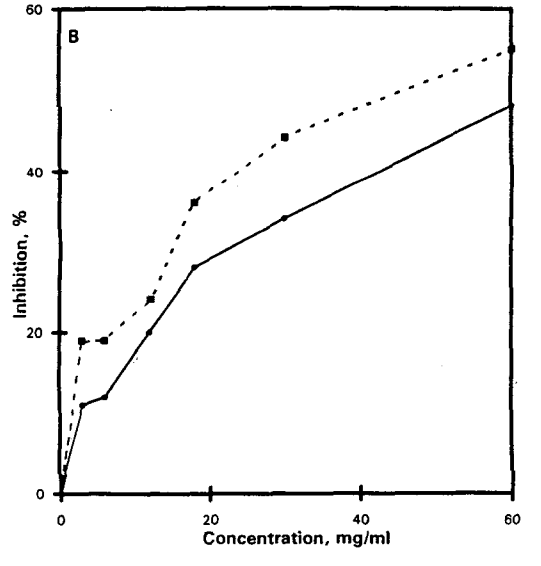


Fig. 2. Inhibition of lipoxygenase by water(A) and ethanol(B) extract of garlic.

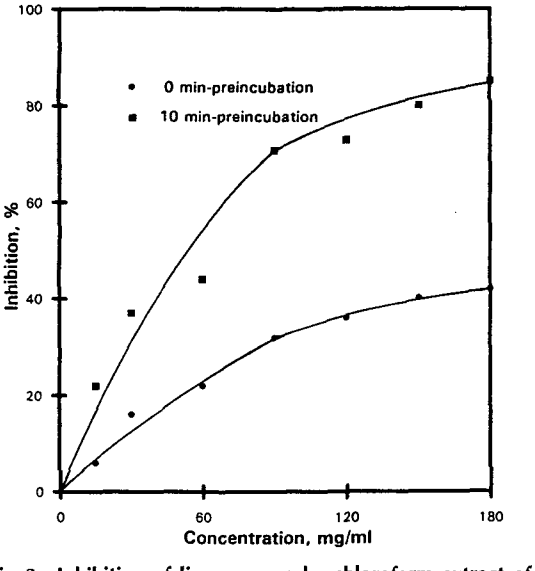


Fig. 3. Inhibition of lipoxygenase by chloroform extract of garlic.

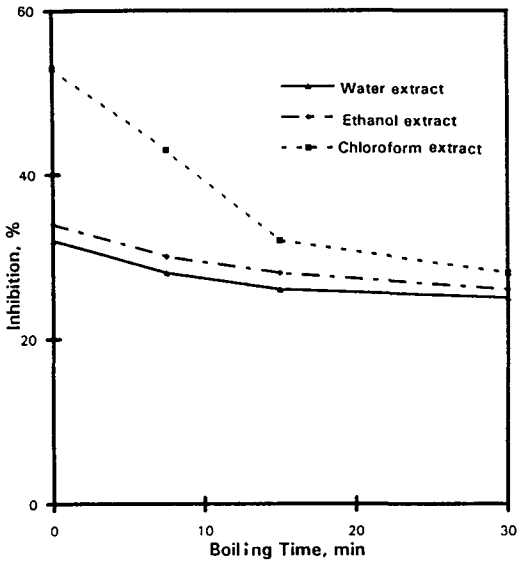


Fig. 4. Effect of heating time on the inhibitory action of garlic extract.

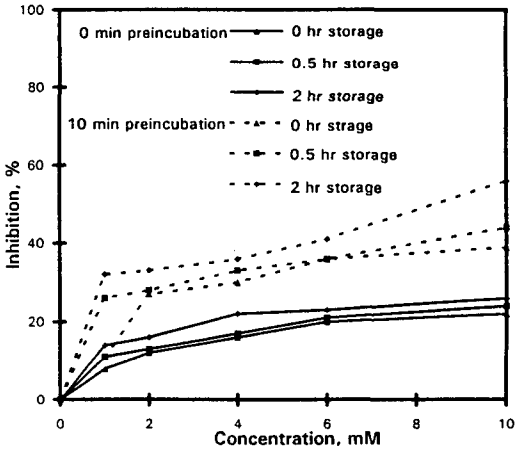


Fig. 5. Concentration- and time-dependent inhibition of lipoxygenase by alliin.

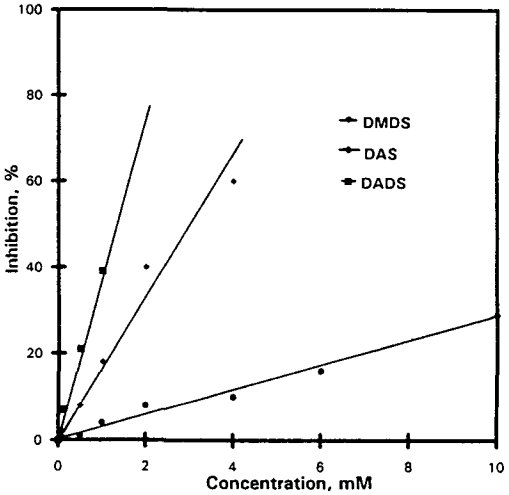


Fig. 6. Effect of various sulfides on lipoxygenase activity.

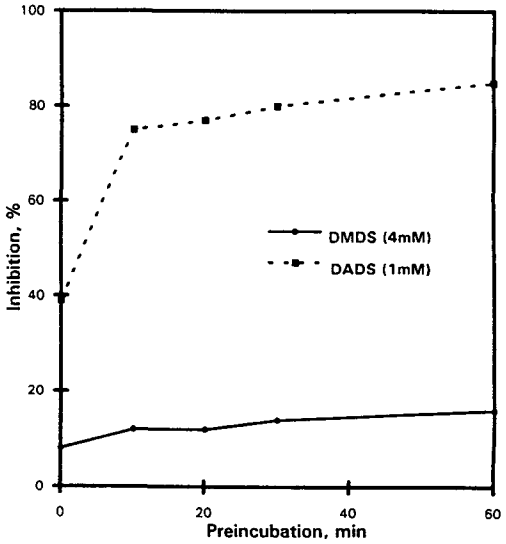


Fig. 7. Time-dependent inhibition lipoxygenase by various sulfides.

nds were incubated with lipoxygenase, and the remaining activity was measured. Fig. 5 shows that alliin (1~10mM), an endogeneous precursor substrate for the formation of allicin, inhibited lipoxygenase in the concentration-dependent manner (22% inhibition at 10mM). It is interesting to find that alliin itself appeared to express both irreversible and reversible inhibition. In further studies, it was observed that alliin stored for 2hr in the aqueous system expressed a higher inhibitory effect, which seems to possess the irreversible property. Although the irreversible component was not further elucidated in this experiment, it is possible to surmise that alliin is decomposed slowly in the aqueous system to produce an irreversible decomposition product. Meanwhile, the reversible inhibition by alliin might be due to the competition between alliin itself and substrate toward the active site of lipoxygenase. This assumption might be supported by the earlier report that alkyl sulfoxide can inhibit 15-lipoxygenase competitively[12].

In the subsequent experiment, alkyldisulfides or mono-sulfide were tested for the inhibition of lipoxygenase. Fig. 6 indicates that dimethyldisulfide or diallyldisulfide inhibits lipoxygenase in a concentr at-

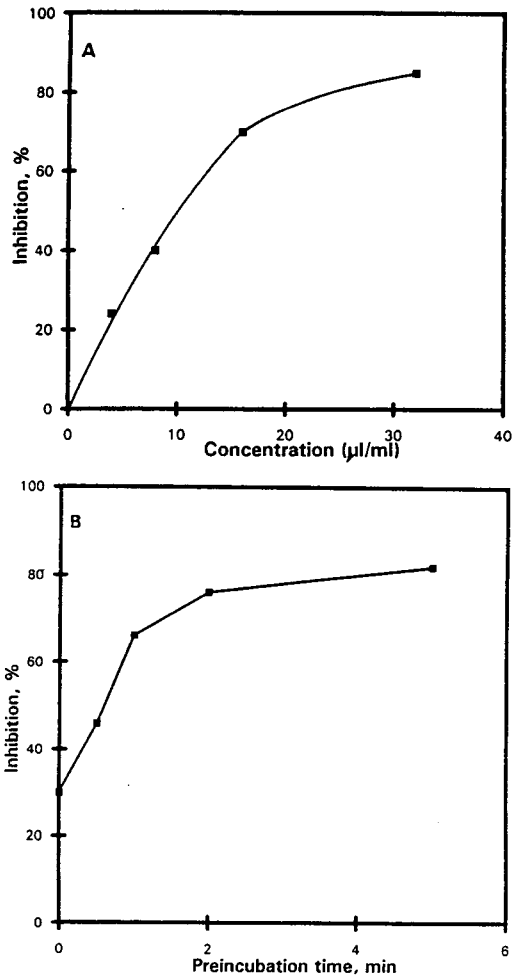


Fig. 8. Concentration-dependent inhibition of lipoxygenase by garlic oil.

ion-dependent manner. Compared to dimethyldisulfide (I_{50} value of 18mM), diallyldisulfide expressed a greater inhibitory effect with the I_{50} value of 1.3mM in instantaneous incubation. Meanwhile, diallylmonosulfide was more potent than dimethyldisulfide, suggesting that diallyl group may be more contributory than dimethyl group for the enhancement of the binding affinity. In the related study(Fig. 7), diallydisulfide was found to exhibit the irreversible inhibition, which might be explained by the assumption that disulfides can modify the thiol group of cysteine residue in the active site of lipoxygenase. Consistent with the above result, the irreversible property of diallyldisulfide was greater than that of dimethyldisulfide. Thus, it is suggested that diallyldisulfide, which is relatively stable, may be a more effective inactivator of lipoxygenase. Although the reason why inhibition of lipoxygenase by diallyldisulfide is limited to approximately 85% inhibition is not understood, it might be explained by the assumption that the modification of cysteine residue may not lead to the complete loss of lipoxygenase activity.

Since a synthetic specimen of allicin was not available, the garlic oil, which is known to contain allicin, disulfides etc., was used for the irreversible inhibition of lipoxygenase. As shown in Fig. 8A, garlic oil inhibited the lipoxygenase activity in a concentration-dependent manner with I_{50} value of 9μl/ml. Fig. 8B demonstrates that the inhibition by garlic oil is largely irreversible, although it contained components showing the reversible inhibition. Thus, the garlic oil also possessed both irreversible and reversible inhibitors. Moreover, the irreversible inhibitors present in the garlic oil may be the mixture of allicin or its decomposition products such as polysulfides. Therefore, the inhibition of lipoxygenase by garlic extract may be due to the presence of not only allicin but also its decomposition products. Since the irreversible sulfur inhibitors are decomposed easily in the aqueous system, the inhibitory effect of the decomposition products rather than allicin may be more real in *in vivo*.

Although the role of allicin or unstable intermediate products have been emphasized as bioactive compounds, our study suggests that the stable and reversible inhibitors present in garlic extract are to be added to bioactive components.

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마늘의 가공 조리방법에 따른 Lipoxygenase 활성도 저해효과
-마늘 추출액이 Lipoxygenase 활성도 저해에 미치는 영향-

김미리† · 모은경 · 김성희 · 석대은*
충남대학교 식품영양학과
*대전기계창

요 약

동물체내에서 천식, 염증, 혈소판 응고 등에 관련된 매개체를 생성시키는 효소인 lipoxygenase 작용기전과 유사한 대두 lipoxygenase (Type IV)를 사용하여 생마늘의 수용액, 에탄올, 클로로포름 추출 분획에 의한 lipoxygenase 저해 정도를 측정하였다. 효소를 클로로포름 추출 분획과 10분 preincubation시킨 후의 효소 저해 양상은 비가역적 저해(I_{50}값, 55mg/ml) 이었으며, 수용액 추출 분획의 경우는 주로 가역적 저해 양상(I_{50}값, 65mg/ml)을 나타내었다. 한편, diallyldisulfide와 dimethyldisulfide의 I_{50}값은 각각 1.3mM, 18mM 이었으며 이들은 가역적, 비가역적 저해 현상을 모두 나타내었다. 합성품 alliin은 비교적 높은 농도(10mM 농도에서 22% 저해)에서 저해하였으며, alliin의 분해산물은 비가역적 저해 양상을 나타낸 반면에, S-ethylcysteine sulfoxide는 효소를 거의 저해하지 않았다. 따라서 다진 마늘 속에는 가역적 저해제가 주로 함유되어 있고, 소량(25~30%)의 비가역적 저해제가 함유되어 있는 것으로 사료되었다.