Effect of Tannic Acid and α-Tocopherol as an Antioxidant on Methyl Linoleate Autoxidation and Inhibitor of Lipoxygenase in Phospholipid

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Methyl linoleate 의 자동산화와 인지질에 작용하는 lipoxygenase의 억제제로서 탄닌산과 알파토코페롤의 영향

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요 약

Methyl linoleate의 자동산화와 인지질에 작용하는 lipoxygenase의 활성에 미치는 탄닌산과 알파토코페롤의 영향을 HPLC로 조사하였다. 반응혼합물은 methyl linoleate 70mM, 라디칼 생성제 AMVN와 탄닌산 및 알파토코페롤 각각 0.7mM를 함유하였다.

Hydroperxide 생성량을 측정하여, 탄닌산과 알파토코페롤이 상당히 좋은 항상화제임을 알았고, 인지질 1 μM에 탄닌산과 알파토코페롤을 각각 1μM 함유한 혼합물에 효소를 첨가하여 활성 억제효과를 측정하여, 탄닌산과 알파토코페롤이 좋은 효소 활성 억제작용을 하고 있음을 알았다.

Introduction

Lipid oxidation in food has much corncern in recent years. It is clear understood that nutritional damage, disorderat in toxic substance, producing and variable flavor in edible oils come from the autoxidation of polyunsaturated fatty acids and lipoxygenase activity.[1~7]

The superoxide anion radical, hydrogen peroxide, the hydroxyl radical, and singlet oxygen molecule as an oxygen generation in food has been studied.[8~11] Carotenoid pigments are widely distributed in plant-kingdom, and β-carotene is able to inactive electronically excited molecules. The excited compound, singlet oxygen molecules, is generated by several ways, such as the lipid peroxidation and enzymetically. The antioxidants for lipid peroxidation and some inhibitor of lipoxygenase decreased the rate of formation of linoleate hydroperoxides.[12~14]

The quenching of $^1O_2$ by tocopherol depend on a free hydroxyl group in the chromane ring. Tocopherols are known to act as antioxidants by donating a hydrogen atom to chain-propagating free radicals.[15~17] Tannin(mostly tannic acid) extracted from variable plant, is reacted on lipid

oxidation as an antioxidant.[18] There fore, the tannic acid as an antioxidant on methyl linoleate autoxidation and inhibition of lipoxygenase has been studied.

Materials and Methods

Materials

Methyl linoleate, α-tocopherol, tannic acid, lipoxygenase, and phospholipid were provided by Sigma Chemical Co.(St. Louis, Missouri, USA.). Mimyristyl phosphad ylcholine(DMPC) and radical initiator, 2, 2-azobis(2, 4-dimethyl-valeronitril)(AMVN) were obtained from Wako Pure Chemical Industries,(Osaka, Japan).

Methods

In Experiment 1. Tannic acid and α-tocopherol 0.1μM each were added to a mixture of hexane/isopropanol(1 : 1, v/v, 1m*l*) containing methyl linoleate 100μM. Oxidation was initiated by adding a hexane solution of AMVN(10μM in 0.1m*l*) and the reaction mixture was incubated with continuous shaking under air in the dark at 37℃. At proper time intervals, aliquots of the reaction mixture 10μ*l* took out and injected into the HPLC column.

In Experiment 2. The reaction mixture of DMPC solution(9μM) and phosphatidylcholine (PC) solution (1μM) put into screw capped test tube, and then the solvent was removed by nitrogen gas. After that, added 0.9m*l* 0.01M Tris-HCl buffer(pH 7.4) and inhibitors 0.1μM each, and mixed vigorously. The enzyme solution(enzyme 10mg/m*l*) added, and incubated the mixture at 37℃ with continuous shaking. In order to reaction stopped, chloroform 0.1m*l* and methanol 0.1m*l* were added.

The reaction mixture was shaked well and centrifuge at 3500rpm for 5minutes, then lower portion 50μ*l* took out, and evaporate solvent by nitrogen gas. The residue was dissolved in hexane and injected into the HPLC column.

HPLC. HPLC operation conditions were shimadzu LC-4A SPD-2A model, Zorbax SIL(4.6 mm×250mm) and YMC-C8(6mm×150mm) column used, and the flow rate was maintained at 2.0m*l*/min. The elutriated solution was the mixture of acetonitril/isopropanol(3 : 1, v/v). Methyl linoleate hydroperoxide were determined in the same condition previously described.[12]

Results and Discussion

Fig. 1 shows the results of autoxidation of methyl linoleate in a period of 6 hours. The amount of hydroperoxides before autoxidation (initial level) was estimated to be. 0.08% of methyl linoleate. The slope of the reaction

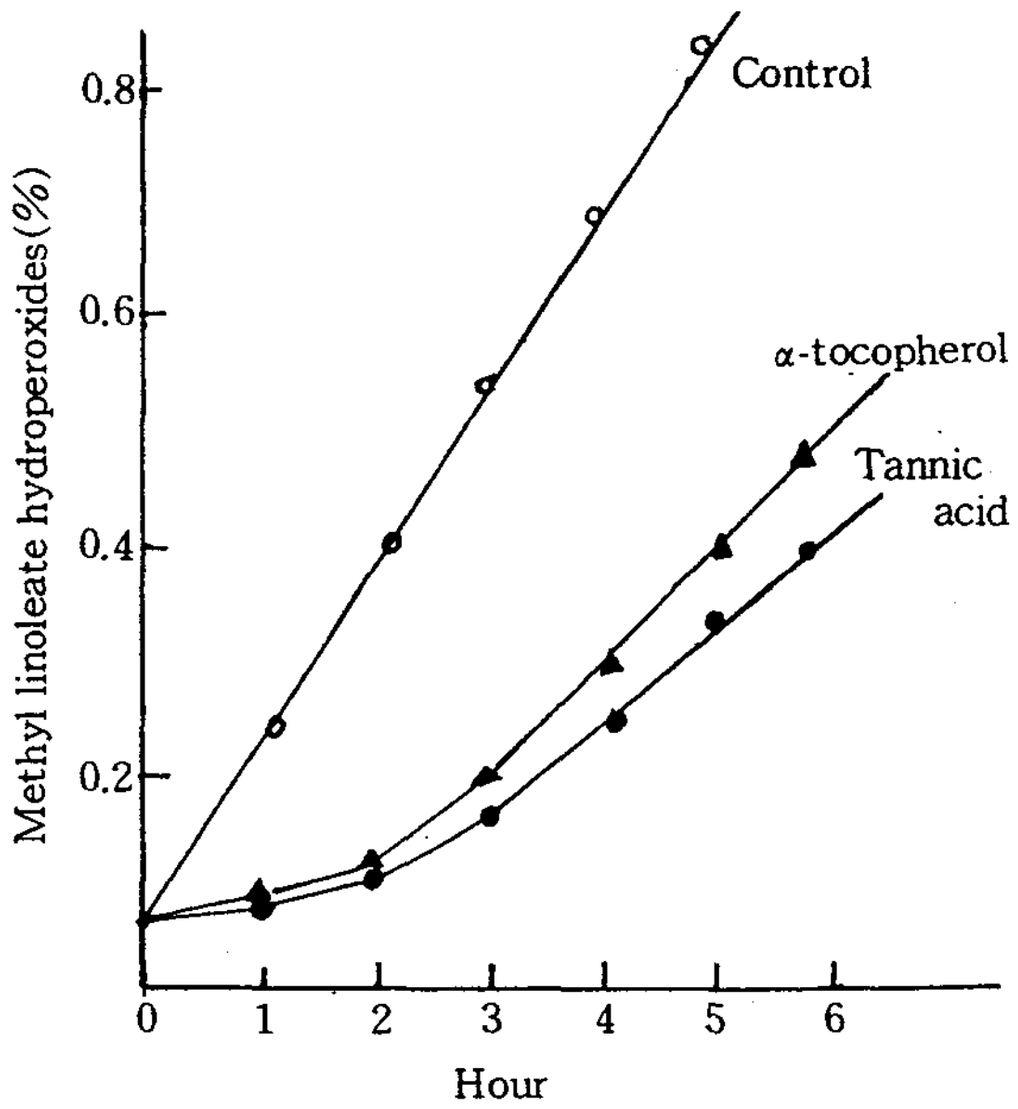

Fig. 1. Effect of tannic acid and α-tocopherol on the rate of formation of methyl linoleate hydroperoxide. Reaction system consisted of methyl linoleate(70mM), AMVN(0.7 mM) tocol(0.1μm) and tannic acid(0.1 μm) in a mixture of hexane/isopropanol(1 : 1, v/v, 1.3m*l*)

curve of methyl linoleate was 1.6. In the absence of antioxidant, methyl linoleate hydroperoxide accumulated lineary at the rate of 2.0 μM/min.

Methyl linoleate containing 0.1μM α-tocopherol and 0.1μM tannic acid as an antioxidant showed a linear increased in total hydroperoxide with incubation times after 2 hours passed. The slope of the reaction curve of methyl linoleate containing α-tocopherol and tannic acid was 0.69 and 0.97, respectivily. During the first 2 hours, the rate of hydroperoxides formation was verly slowly increased. The unimolecular decomposition of this initiator induces a free radical chain oxidation of methyl linoleate through peroxyl radicals as an intermediates resulting in the formation of hydroperoxides. The inhibition of the hydroperoxides formation by some antioxidant depends upon their lipid peroxyl rad ical-trapping activity.[8] The percentages of methyl linoleate hydroperoxides after 2 hours were determined to be follows : 0.12%(2hrs), 0.20% (4hrs) for α-tocopherol and 0.1%(2hrs), 0.13% (4hrs) for tannic acid. In this reaction system, the concentration of antioxidants decreased with as much as methyl linoleate hydroperoxides formation. The antioxidant such as α-tocopherol and tannic acid which has the hydroxy (−OH) group and conjugated polyene system of the carotenoid react differently on lipid hydroperoxides formation as an inhibitor.[14, 20]

Tannic acid(TaNH) reacts with lipid peroxyl radical(LOO ·) in a solution as follows :

$$TaNH + LOO\cdot \rightarrow LOOH + TaN^-$$
$$TaN^- + LH \rightarrow TaNH + L\cdot$$
$$Tan^- + LOO\cdot \rightarrow \text{Inactive material products}$$

Even though tannic acid has several hydroxy radicals, it reacts as three hydrogen atoms donation in solution.

In order to figure out the inhibition activity of tannic acid and α-tocopherol for lipoxygenase, did like experiment 2. Fig. 2 shows tannic acid and α-tocopherol reacts on lipid hydroperoxides formation as an inhibitor. The inhibition activity of tannic acid for lipoxygenase was stronger than α-tocopherol untill 2 hours react, thereafter the activity of inhibition for α-tocopherol was constant concentration, but in case of tannic acid the slope of the reaction curve was 0.6. It is assumed that three hydrogen atom donor is better inhibitor for lipoxygenase than monohydrogen atom donation.

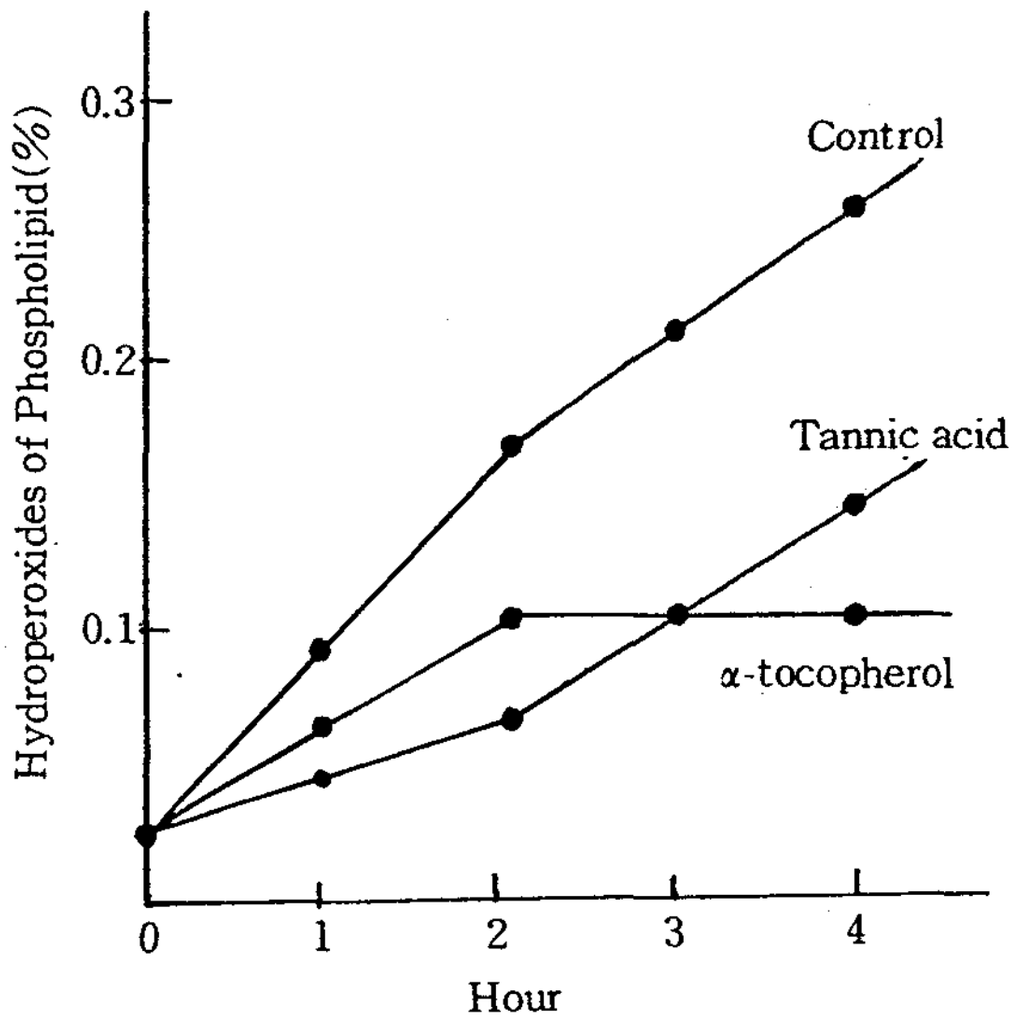

Fig. 2. Effect of tannic acid and α-tocopherol on the rate of hydroperoxides formation by lipo-oxygenase. Phospholipid(Phosphaditdylcholine), α-tocopherol 1 μM, tannic acid μM.

Abstract

In order to investigate the effect of tannic acid and α-tocopherol on methyl linoleate autoxidation and on inhibition activity of lipoxygenase in phospholipid, the rate of formation of methyl linoleate hydroperoxide was measured by HPLC. The reaction mixture contained me-

thyl linoleate 70mM, radical initiator AMVN 0.7mM, tannic acid and α-tocopherol 0.7mM, each, in a mixture of hexane/isopropanol(1 : 1, v/v). Under this reaction condition, tannic acid was good enough antioxidant. The tannic acid and α-tocopherol for the inhibition activivity of lipoxygenase were measured at the reaction conditions ; phospholipid 1μM, tannic acid and α-tocopherol were reacted as an inhibitor of lipoxygenase in phospholipid, especialy in phosphatidy lcholine.

Key words : Methyl linoleate, lipoxygenase, tannic acid, hydrogen donating, autoxidation

References

1. Crampton, EW, Common RH, Pritchard ET, and Farmer FA : Studies to determine the nature of the damage to the nutritive value of some vegetable oils from heat treatment. *J. Nutrition*, 60, 13(1956)
2. Dahl LK, Hill EG, and Holman RT : The thiobarbituric acid reaction and the autoxidation of polyunsaturated fatty acid methylesters. *Arch. Biochem. Biophys*, 98, 253(1962)
3. Labuza TP, : CRC Critical Rev., *Food Technol.*, 2, 355(1971)
4. Sherwin ER, : Autoxidation and antioxidants for food fats and oils. *J. Am. Oil Chem. Soc.*, 49, 468(1972)
5. Frankel EN, : *Autoxidation in Food and Biology*, Simic MG, and Kare M.(eds), Plenum Press, New York, 141(1980)
6. Porter NA, Weber BA, Weenen H. and Khan JA : Autoxidation of polyunsaturated lipids. *J. Am. Chem. Soc.*, 102, 5597(1980)
7. Wong YJ, Miller LA, and Addis PB : Effect of heat inactivation of lipoxygenase on lipid oxidation in lake Herring. *J. Am. Oil Chem. Soc.*, 68, 752(1991)
8. Rawis HR, and Van Santen PJ : A possible role for siglet oxygen in the initiation of fatty acid autoxidation. *J. Am. Oil Chem. Soc.*, 47, 121(1970)
9. Pryor WA, : Free radical reaction and their importance in biochemical system. *Fed. Proc.*, 32, 1852(1973)
10. Tappel AT, : *Free Radical in Biology*, Pryor. WA.(ed), Academic Press, New York, 1 (1980)
11. Tien M, Swinben BA, and Aust SD, : Superoxide dependent lipid peroxidation. *Fed. Proc.*, 40, 179(1981)
12. Terao J, and Matsushita S, : The peroxidizing effect of α-tocopherol on autoxidation of methy linoleate in bulk phasa. *Lipids*, 21, 255(1986)
13. Terao J, : Antioxidant activity of β-carotene related carotenoids in solution, *Lipids*, 24, 659(1989)
14. Mascio PD, and Murphy ME, : Antioxidant defense system : the rate of carotenoids, tocopherol and thiols. *Am. J. Clin. Nutr.*, 53, 1948(1991)
15. Foote Cs, and Denny RW, : β-carotene as a singlet oxygen quencher. *J. Am. Chem. Soc.*, 90, 6233(1968)
16. Burton GW, and Ingold KU, : Vitamin E, the only lipid-soluble chainbreaking antioxidant in human plasma. *J. Am. Chem. Soc.*, 103, 6472(1981)
17. Esterbauer H. : *Free Radicals, Lipid peroxidation and cancer*, McBrien, D. C. H. and Slater TF.(eds), Acadmic Press, New York, 100(1980)
18. Cho JS. and Shin DH. : Antioxidative activity of various extracts of Quercisemen to linoleic acid. *J. Korean Oil Chem. Soc.*, 8, 79 (1991)
19. Kwon TW, Synder HE, and Brown HD. : Oxidation stability of soybean oil different stages of refining. *J. Am. Oil Chem. Soc.*, 61,

184(1984)
20. Niki E, Saito T, Kawakami A, and Kamiya Y. : Inhibition of oxidation of methyl linoleate in solution by vitamin E. *J. Bio. Chem.*, 259, 4177(1984)