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Antioxidant Activities of α-Tocopherol and Hydroxycarbazole against Lipid Peroxidation in Homogeneous Solution and in Liposomal Membranes#

Misato IWATSUKI, Etsuo NIKI,* Shinichiro KATO,† and Koji NISHIKORI†

Research Center for Advanced Science and Technology, The University of Tokyo,
4-6-1 Komaba, Meguro, Tokyo 153

†Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., Takasaki, Gunma 370-12

α-Tocopherol and carazostatin (1-heptyl-3-hydroxy-2-methyl-carbazole) acted as a potent antioxidant against lipid peroxidation. α-Tocopherol was more active than carazostatin in homogeneous solution, whereas, in the liposomal membranes, carazostatin exhibited a stronger antioxidant activity than α-tocopherol. This novel finding on such a reversal in relative antioxidant activity by the change in reaction media suggests the importance of mobility of an antioxidant at microenvironment in determining antioxidant activity.

Free radical-mediated autoxidations of hydrocarbons have been the subjects of extensive studies from both fundamental and practical viewpoints.[1] Recently, the oxidations of lipids have received renewed attention with increasing evidence which shows that the lipid peroxidation is one of the important primary events in the free radical-mediated oxidative damage of biological membranes and tissues. The aerobic organisms are protected against such an oxygen toxicity by an array of defense systems. Among others, α-tocopherol (vitamin E) is known as an important radical-scavenging antioxidant.[2] The chroman ring makes α-tocopherol quite reactive toward oxygen radicals.[2] However, we have found that the antioxidant activity of α-tocopherol is markedly reduced in the membranes due primarily to its reduced mobility.[3] It was found experimentally that the

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#This paper is dedicated to Professor Dr. Osamu Shimamura in the appreciation of his scientific contribution and encouragement.

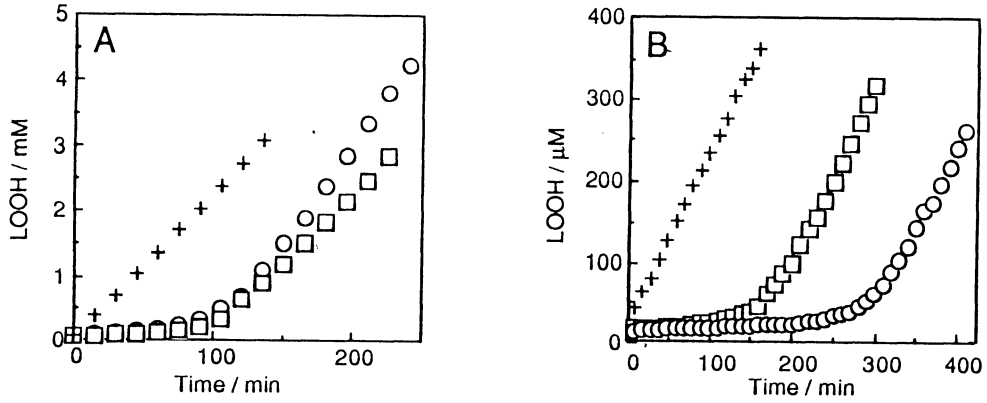

Fig. 1 Formations of lipid hydroperoxides (LOOH) in the oxidations of (A) 453 mM methyl linoleate in acetonitrile induced by 0.20 mM AMVN and (B) 5.1 mM soybean PC liposomes induced by 1.0 mM AMVN in 0.1 M NaCl/0.1 mM EDTA aqueous dispersions at 37 °C in air in the absence (+) and presence of 3 μM either α-tocopherol (□) or carazostatin (○).

efficiency of radical scavenging by α-tocopherol incorporated into the membrane became smaller as the radicals went deeper into the interior of the membranes.[3)]

Carazostatin, 1-heptyl-3-hydroxy-2-methylcarbazole, is a novel antioxidant which is isolated from a culture of *Streptomyces Chromofuscus*.[4)] Its structural characteristic is that it has a side chain whose length is half as long as that of α-tocopherol.

We wish to report here the case, for the first time to our knowledge, where the relative antioxidant activities are reversed depending on the medium, that is, α-tocopherol was a stronger antioxidant than carazostatin in homogeneous solution, whereas carazostatin was more active than α-tocopherol in the membranes.

The materials and methods are substantially the same as reported previously.[5)] Methyl linoleate was purified with silica-gel column and soybean phosphatidylcholine (PC) was purified with alumina and silica-gel columns before use. Natural (*2R, 4'R, 8'R*)-α-tocopherol was kindly provided from Eisai Co. Carazostatin was isolated and purified from a culture of *Streptomyces Chromofuscus*. The oxidations of methyl linoleate in acetonitrile and of soybean PC liposomes in aqueous dispersions were carried out at 37 °C in air. The formations of methyl linoleate and PC hydroperoxides and consumptions of antioxidants were measured with an HPLC as reported previously.[5)]

Figure 1 shows the examples of the oxidations of methyl linoleate in acetonitrile and also of soybean PC liposomes induced by 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN) in the absence and presence of either α-

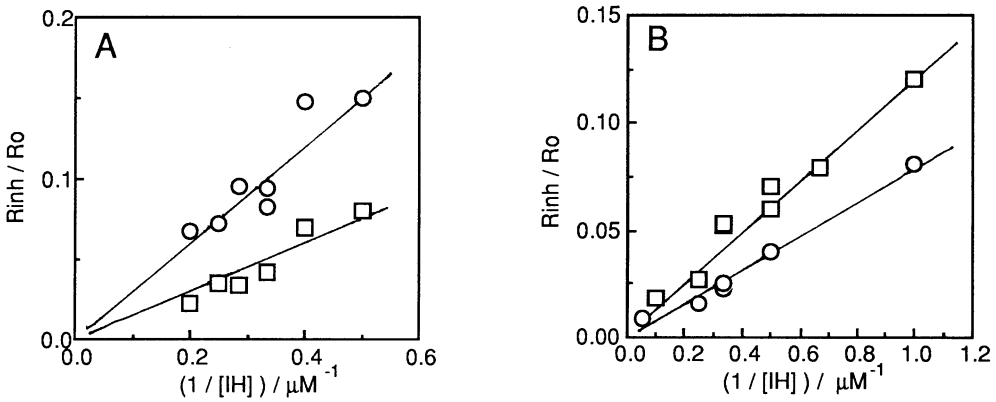

Fig. 2 Plots of the ratio of the rate of inhibited oxidation to that of non-inhibited oxidation (Rinh/Ro) as a function of a reciprocal of antioxidant concentration (1/[IH]) in the oxidation of (A) 453 mM methyl linoleate induced by 0.20 mM AMVN in acetonitrile and (B) 5.1 mM soybean PC liposomes induced by 1.0 mM AMVN at 37 °C in air. □ : α-Tocopherol; ○ : Carazostatin

tocopherol or carazostatin. In the absence of antioxidant, methyl linoleate was oxidized at a constant rate without any induction period, while α-tocopherol and carazostatin suppressed the oxidation markedly and gave a clear induction period. α-Tocopherol and carazostatin were consumed linearly with time and when they were depleted, the induction period was over and a fast oxidation took place. Similar results were observed in the oxidations of soybean PC liposomes (Fig. 1B). Interestingly, α-tocopherol suppressed the oxidation of methyl linoleate more effectively than carazostatin, whereas the reverse was the case for PC liposomal oxidations.

Figure 2 shows the plot of the ratio of the rate of inhibited oxidation (R_{inh}) to the rate of oxidation in the absence of antioxidant (R_0) against a reciprocal of antioxidant concentration. This ratio gives the efficiency of inhibition of oxidation by an antioxidant. Figure 2A shows that α-tocopherol was a more potent antioxidant than carazostatin against the oxidation of methyl linoleate in acetonitrile solution, but α-tocopherol was less active than carazostatin in the oxidation of PC liposomal membranes (Fig. 2B).

Figure 3 shows the rates of consumption of α-tocopherol and carazostatin during the oxidations of methyl linoleate and soybean PC liposomes induced by AMVN and inhibited by both α-tocopherol and carazostatin which were added into acetonitrile solution or soybean PC liposomal membranes simultaneously. In the homogeneous solution, only α-tocopherol was consumed while carazostatin was spared quite efficiently at the initial stage, and carazostatin decreased after most of α-tocopherol was consumed. When both antioxidants were depleted, a fast oxidation took place. This is probably because α-tocopherol scavenged peroxyl radicals faster than carazostatin in the homogeneous solution and furthermore, α-tocopherol regenerated carazostatin by reducing carazostatin radical which was formed when carazostatin scavenged peroxyl radical. On the contrary,

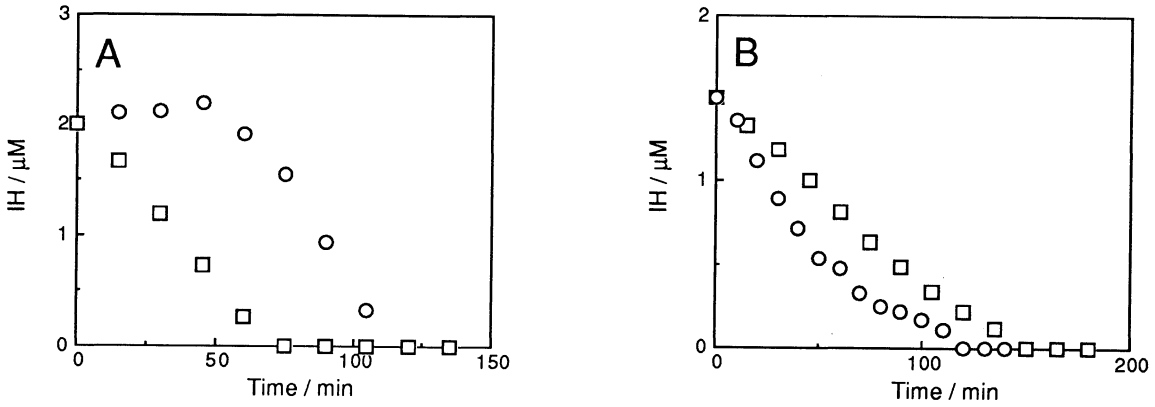

Fig. 3 The consumption of α-tocopherol (□) and carazostatin (○) in the oxidations of (A) 453 mM methyl linoleate initiated by 0.20 mM AMVN in acetonitrile and (B) 5.1 mM soybean PC liposomes initiated by 1.0 mM AMVN at 37 °C. The two antioxidants were present simultaneously in the media.

both α-tocopherol and carazostatin were consumed from the beginning in the oxidation of soybean PC liposomes. Interestingly, carazostatin was consumed faster than α-tocopherol.

The above results clearly show that both α-tocopherol and carazostatin act as a potent radical-scavenging antioxidant and their relative antioxidant activities are reversed depending on the medium. Such a phenomenon has not been observed before. These results are interpreted by a higher chemical reactivity toward peroxyl radical but a smaller mobility in the liposomal membranes of α-tocopherol than carazostatin. These results suggest that, as emphasized before,[3] the antioxidant activity in the membranes, and probably in the particles such as low density lipoproteins, is determined not only by an inherent chemical reactivity of the antioxidant toward oxygen radicals but also by physical factors such as concentration and mobility at the microenvironment.

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