Effects of Lipophilic Derivatives of L-Ascorbic Acid and Dehydro-L-Ascorbic Acid on the Peroxidation of Linoleic Acid in Neutral Phosphate Buffer Containing Alcohol

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(Received February 19, 1986)

Summary 6-*O*-Palmitoyl-AsA (AP) and -DHA (DHAP) suppressed LA peroxidation considerably in both 10% and 20% EtOH solutions. The duration of the suppression of LA peroxidation was longer with AP than with DHAP. But after the initial suppression of LA peroxidation, both derivatives showed an accelerating effect. 6-*O*-Acetyl-AsA (Ac-AsA) and -DHA (Ac-DHA) accelerated LA peroxidation from the start of the reaction in 10% EtOH, but suppressed it notably in 20% EtOH. 4-Phenyl-2,3-dihydroxy-2-buten-4-olide (PDHB) and 4-phenyl-2,3-dioxo-4-butenolide (PDOB) accelerated LA peroxidation in 10% EtOH. With 20% EtOH solution, PDHB suppressed LA peroxidation notably, as did AP, but PDOB showed only a short duration (about 1 h) of suppression. These results suggest the complexity of LA peroxidation catalyzed by lipophilic AsA or DHA in aqueous solution containing alcohol.

Key Words peroxidation of linoleic acid, lipophilic catalysts, ascorbic acid, dehydroascorbic acid, palmitoyl ascorbate, acyl ascorbate, 4-phenyl-2,3-dihydroxy-2-butenolide

AsA is widely added to food or food ingredients as a non-nutrient agent to function as a preservative or oxygen acceptor, because of the oxidation-reduction reaction of the ene-diol group of AsA. However, as AsA is easily degraded to DHA in the presence of moisture (1), the role of AsA or DHA on the lipid peroxidation is difficult to understand clearly. We have reported in our previous paper (2) that the effects of acceleration and suppression on the linoleic acid (LA) peroxidation catalyzed by AsA and DHA were related to the state of dispersion of the lipid used.

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Since palmitoyl AsA is rather lipophilic compared with AsA, somewhat different results on LA peroxidation are to be expected. In fact, the palmitoyl ester of AsA has been used as a lipophilic antioxidant in food (3–7). Therefore, we tested the effects of 6-*O*-palmitoyl-AsA (AP) or -DHA (DHAP) on the peroxidation in neutral buffer containing 10% and 20% EtOH, in which AsA has the opposite effects of acceleration or suppression on LA peroxidation with different alcohol contents. In this paper, we also describe 6-*O*-acetyl-AsA (Ac-AsA), -DHA (Ac-DHA), 4-phenyl-2,3-dihydroxy-2-buten-4-olide (PDHB), and 4-phenyl-2,3-dioxo-4-butenolide (PDOB) as lipophilic catalysts for LA peroxidation.

MATERIALS AND METHODS

Materials. LA was purchased from Sigma Chemical Co. and diluted with freshly distilled EtOH to 100 mM under bubbling with N₂ gas. The solution was stored at –20°C until use. AP was obtained from Wako Pure Chemical Industries, Ltd. DHAP was prepared from AP by the method reported previously (8, 9). Highly purified water and distilled EtOH were used to remove any contaminant metals. All the buffer solutions used were passed through a short Chelex 100 column (10). Ac-AsA was prepared from AsA according to a method reported previously (11), and Ac-DHA was prepared from Ac-AsA as mentioned above. Other reagents were of analytical grade.

Analytical methods. To test LA peroxidation, the reaction conditions were the same as reported previously (8, 9). The reaction mixture for 10 mM LA and 20% EtOH solution was prepared by mixing 0.5 ml of 100 mM LA in EtOH, 0.5 ml of EtOH, 4 ml of phosphate buffer (0.1 M, pH 6.8), and 0.1 ml of the material to be tested (180 μ M). The reaction mixture was adjusted to pH 7 and incubated at 37°C with shaking. To vary the EtOH content, the pH of the buffer solution was first adjusted to 6.9 for 10% EtOH and to 6.8 for 20% EtOH, so that the final pH was 7.0. Consequently, the molarity of the buffer was 90 mM for 10% EtOH and 80 mM for 20% EtOH. A sample (0.3 ml) of the reaction mixture was withdrawn at specified times and 3 ml of 60% EtOH aq. solution containing EDTA (200 ppm) was added. The optical densities at 233 nm and 265 nm were used for the measurement of the LA hydroperoxide formed and the AsA remaining, respectively.

RESULTS AND DISCUSSION

Effects of AP and DHAP on the LA peroxidation

Figure 1 shows the results of LA peroxidation catalyzed by the addition of AP or DHAP. AP suppressed the LA peroxidation in both 10% and 20% EtOH. When the A_{265} due to AsA disappeared after several hours of incubation, the amounts of LA hydroperoxide started to increase gradually. Notable suppression of LA peroxidation catalyzed by AP was observed in both 10% and 20% EtOH solutions, as compared with control values without catalysts shown in column C in Fig. 1.

Fig. 1. Peroxidation of linoleate catalyzed by AP (A) or DHAP (B). The reaction mixture contained 10 mM LA, 180 μ M of catalyst and 10% EtOH (●) or 20% EtOH (○) in phosphate buffer. A sample (0.3 ml) of the reaction mixture was withdrawn and added to 3 ml of 60% EtOH solution containing EDTA (200 ppm), and the UV absorption spectra of the solution was measured. C shows the control values without AP or DHAP.

However, the duration of the suppression was longer in 20% EtOH than in 10% EtOH. After the apparently perfect suppression, peroxidation began to proceed more quickly with both AP and DHAP.

The rates of LA peroxidation catalyzed by DHAP in 10% and 20% EtOH solutions were similar to those catalyzed by AP, but the duration of the suppression of LA peroxidation by DHAP was somewhat shorter than that by AP. Furthermore, in the system with 10% EtOH a rather strong suppression of LA peroxidation was observed even in the case of DHAP; this is quite a different result from the LA peroxidation catalyzed by AsA in 10% EtOH.

Effects of Ac-AsA and Ac-DHA on the LA peroxidation

The effects of Ac-AsA and Ac-DHA on the peroxidation of LA were examined as shown in Fig. 2. Addition of Ac-AsA caused only an acceleration of LA peroxidation in 10% EtOH. With 20% EtOH solution, the amounts of LA hydroperoxide increased gradually after the complete disappearance of the absorption peak due to AsA (data not shown). That is, Ac-AsA suppresses LA peroxidation in 20% EtOH solution for several hours, then accelerates it after the consumption of the Ac-AsA in the reaction mixture.

Addition of Ac-DHA to the reaction mixture showed only an acceleration in 10% EtOH solution, but both suppression and acceleration of LA peroxidation were seen in 20% EtOH solution, similar to AP. The pattern of acceleration of LA

Fig. 2. Peroxidation of linoleate catalyzed by Ac-AsA (A) or Ac-DHA (B). Experimental conditions were the same as for Fig. 1. ●, 10% EtOH; ○, 20% EtOH.

Fig. 3. Peroxidation of linoleate catalyzed by PDHB (A) or PDOB (B). Experimental details were the same as for Fig. 1. ●, 10% EtOH; ○, 20% EtOH. The broken line shows the control values in 20% EtOH solution.

peroxidation catalyzed by Ac-AsA in 10% EtOH was similar to that catalyzed by Ac-DHA. Likewise, Ac-AsA and Ac-DHA in 20% EtOH showed almost the same pattern of suppression, followed by acceleration of LA peroxidation.

In the 20% EtOH, Ac-DHA had almost the same duration of suppression of LA peroxidation as Ac-AsA, then acceleration of LA peroxidation was seen.

Furthermore, the degree and the rate of acceleration catalyzed by Ac-AsA were almost the same as those catalyzed by Ac-DHA.

Effects of PDHB and PDOB on LA peroxidation

Figure 3 shows the results of LA peroxidation catalyzed by PDHB in the systems with 10% and 20% EtOH. Addition of PDOB to the reaction mixture containing 10% EtOH solution only accelerated LA peroxidation from the beginning of the reaction, but in the system with 20% EtOH solution, a prominent suppression of the LA peroxidation which lasted more than 5 h was observed, which then changed to an acceleration like AP.

Addition of PDOB accelerated the LA peroxidation in the system with 10% EtOH solution from the beginning of the reaction, but in the system with 20% EtOH solution, a suppression of LA peroxidation for 1 h, followed by a gradual acceleration of LA peroxidation, was observed.

Based on these results, we propose that 6-*O*-acetyl- and phenyl-derivatives of AsA or DHA have almost the same effects on the peroxidation of LA. Furthermore, these two derivatives of AsA or DHA accelerate LA peroxidation in the 10% EtOH solution, just like that induced by AsA or DHA alone. On the other hand, in the system with 20% EtOH solution, the type of LA peroxidation catalyzed by 6-*O*-acetyl- and phenyl-derivatives of AsA or DHA is similar to those catalyzed by AP or DHAP, which suppress the LA peroxidation notably for several hours, followed by a gradual acceleration.

These results suggest that the solubilities of the catalysts in the reaction mixture are very important for the peroxidation of LA. Considering the results of the two opposite effects on LA peroxidation, the acceleration and suppression are probably dependent on the dispersion of LA (12). Therefore, the effects of AsA on LA peroxidation are greatly influenced by the partition of AsA or DHA into the water layer or oil layer in the oil-water dispersion system of the reaction mixture (2).

Finally, some comments on the appearance of the reaction mixtures are necessary. Colloidal mixtures of LA and additives in the buffer solutions had considerably different appearances, suggesting the different miscibility of each component in the mixture. The factors of partition of each component in the oil and water phases must be estimated, if we are to discuss this phenomenon more quantitatively. To eliminate this factor, we used lipophilic derivatives instead of pure AsA and DHA. However, although some new information on AP and DHAP was obtained as described above, little additional understanding of lipophilic derivatives of AsA and DHA was gained, as compared with reactions catalyzed by AsA or DHA without a lipophilic moiety. This suggests the complexity of the acceleration and suppression of LA peroxidation catalyzed by AsA or DHA or its lipophilic derivatives in an aqueous solution containing other solvents.

We express our sincere thanks to Dr. Y. Tsujimoto, Osaka City Institute of Public Health and Environmental Sciences, for the kind gift of 4-phenyl-2,3-dihydroxy-2-buten-4-

olide and its derivative.

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