Biosci. Biotechnol. Biochem., **63** (2), 421–423, 1999

Note

Antioxidative Activity of a Cathodic Solution Produced by the Electrolysis of a Dilute NaCl Solution

Kazuo MIYASHITA,* Manami YASUDA, Toru OTA, and Tetsuya SUZUKI

Faculty of Fisheries, Hokkaido University, 3-1-1 Minatocho, Hakodate 041-8611, Japan

Received July 30, 1998; Accepted October 26, 1998

The effectiveness was evaluated of a cathodic solution prepared by the electrolysis of an NaCl solution in inhibiting the aqueous oxidation of ethyl linoleate and ethyl docosahexaenoate. The decrease in unoxidized substrate and the formation of total peroxides during oxidation indicate that the cathodic solution completely inhibited the oxidation of both ethyl esters, while these lipids were easily oxidized in an NaCl solution and in distilled water. The antioxidative activity of the cathodic solution was confirmed after open incubation for 3 days and 7 days at 37°C, although the scavenging ability of the cathodic solution toward DPPH radicals disappeared during this incubation.

Key words: electrolyzed NaCl solution; cathodic solution; antioxidative activity; DHA; linoleate

The solution produced in the cathodic compartment by electrolyzing a dilute NaCl solution exhibits low dissolved oxygen and high dissolved hydrogen.[1] The properties of this cathodic solution suggest its antioxidative activity toward lipid oxidation. Shirahata *et al.*[1] have demonstrated that the cathodic solution had superoxide dismutase-like activity and catalase-like activity; however, the antioxidative effect of this cathodic solution on polyunsaturated lipids had not been examined.

In the present study, we oxidized ethyl linoleate (ethyl LA) and ethyl docosahexaenoate (ethyl DHA) in aqueous micelles, which had been prepared by dispersing them with Ttriton X-100 in a cathodic solution, NaCl solution, and distilled water, to evaluate the antioxidative activity of the cathodic solution toward lipid oxidation. We also determined the relationship between the antioxidative activity of the cathodic solution and its low dissolved oxygen content and scavenging effect on the DPPH radical.

Ethyl LA (18:2n-6; 99+% purity) was purchased from Nu-Chek Prep (Elysian, MN, U.S.A.). Ethyl DHA (22:6n-3; 99+% purity) was kindly donated by Harima Chemical Co. (Osaka, Japan). Each ethyl ester was refined by silicic acid column chromatography just before its use. Each refined ethyl ester was found to be free of tocopherols as determined by HPLC,[2] and its peroxide value was less than 1.0 as determined by the colorimetric iodine method.[3]

Ethyl stearate (99% purity), Triton X-100 and $FeSO_4$ were obtained from Nacalai Tesque (Kyoto, Japan), and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), L(+)-ascorbic acid (AsA), NaCl and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Each ethyl ester was mixed homogeneously with Triton X-100 in chloroform. After removing the chloroform by gently sweeping with nitrogen, distilled water, a 0.1% (w/v) NaCl solution, or a cathodic solution was added to the mixture. The cathodic solution was prepared by electrolyzing an NaCl solution with a Super Water Mini device (Altech, Kanagawa, Japan). This device was divided into two compartments (cathode and anode) by an ion-exchange diaphragm, and the electrolyzed NaCl solution produced in the cathode was used as the cathodic solution.

Since ethyl DHA is oxidatively more stable than ethyl LA in an aqueous system,[4] the oxidation of ethyl DHA was induced by the strong radical initiator, AAPH, while Fe(II)–AsA was used as a catalyst for ethyl LA. For the oxidation with Fe(II)–AsA, a substrate solution (8 ml) was pipetted into a flat-bottomed glass tube (30 ml, 2.6 cm i.d.), and oxidation was then initiated by adding 40 μl of an $FeSO_4$–AsA aqueous solution. When oxidation was induced by AAPH, 1 ml of an AAPH solution was added to 9 ml of an ethyl DHA solution. The final concentrations of the substrate, Triton X-100, $FeSO_4$, AsA, and AAPH were 1.0 mM, 0.1% (w/v), 1.0 μM, 20.0 μM, and 0.25 mM, respectively. Oxidation was done in the dark at 37°C, and the oxidative stability of each ethyl ester was followed by the thiocyanate method and GC analysis, the thiocyanate method[5] being used for monitoring peroxide formation and the decrease of substrate during oxidation being evaluated by GC.[4]

Dissolved oxygen and the pH value of the cathodic solution were measured with a dissolved oxygen meter (TOX-90i; Toco Chemical Laboratories Co., Tokyo, Japan) and pH meter (model PH82; Yokogawa Electric Co., Tokyo, Japan), respectively. The scavenging effect of the cathodic solution on DPPH radicals was evaluated according to the method of Tanaka and Nakagawa.[6]

When the oxidative stability of ethyl linoleate in the three kinds of aqueous solution was compared by determing the decrease in unoxidized substrate during oxidation (Fig. 1), ethyl linoleate was easily oxidized in distilled water and in the NaCl solution with about a 60% loss of the substrate after only 24 h of oxidation. On the

* To whom correspondence should be addressed. Tel: +81-138-40-8803; Fax: +81-138-40-8802; E-mail: kmiya@pop.fish.hokudai.ac.jp

Abbreviations: ethyl LA, ethyl linoleate; ethyl DHA, ethyl docosahexaenoate; AsA, ascorbic acid; AAPH, 2,2′-azobis(2-amidinopropane)dihydrochloride

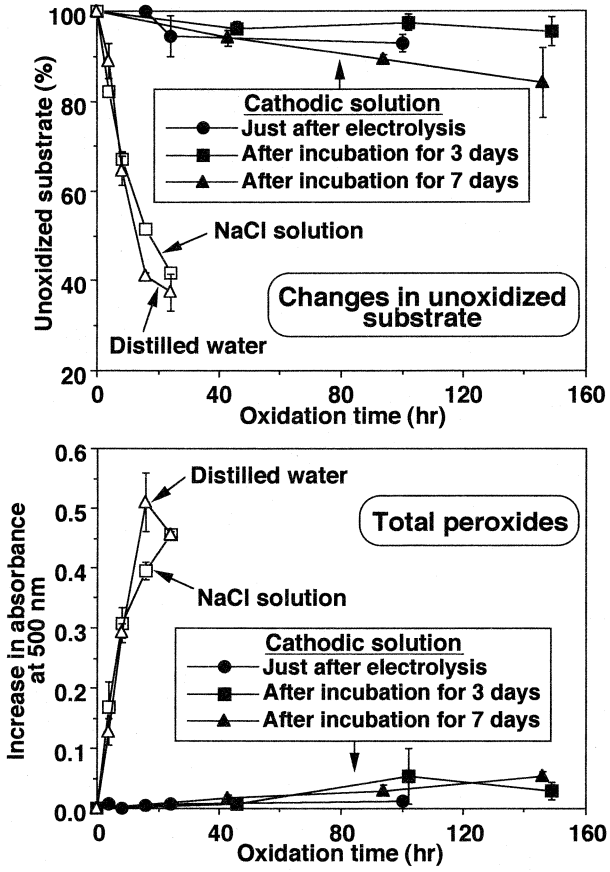

Fig. 1. Antioxidative Activity of a Cathodic Solution toward the Oxidation of Ethyl LA in Aqueous Micelles.

Ethyl LA (1.0 mM) was incubated in the dark at 37°C with Fe(II) (1.0 μM) and AsA (20.0 μM) in three kinds of aqueous solution containing 0.1% (w/v) of Triton X-100. The decrease in unoxidized substrate content and the formation of total peroxides were determined by GC and the thiocyanate method, respectively. Each data value is expressed as the mean±S.D. derived from the results of three independent experiments.

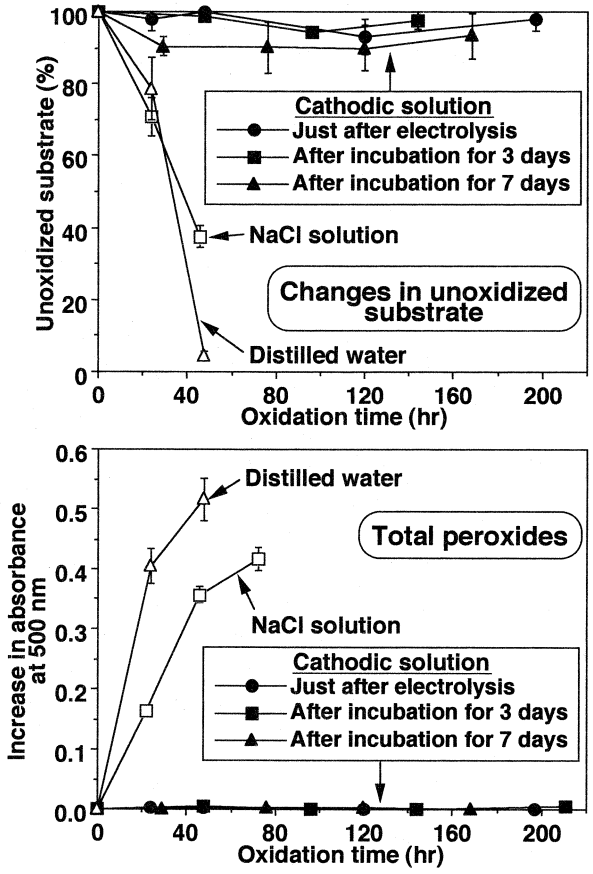

Fig. 2. Antioxidative Activity of a Cathodic Solution toward the Oxidation of Ethyl DHA in Aqueous Micelles.

The reaction conditions were similar to those described for Fig. 1, except that AAPH (0.25 mM) was used as a radical initiator. Each data value is expressed as the mean±S.D. from three separate experiments.

other hand, in the cathodic solution, it was very stable to oxidation, more than 90% of the substrate remaining unchanged after more than 100 h of oxidation. The antioxidative activity of the cathodic solution toward ethyl linoleate oxidation was also confirmed by measuring the increase in total peroxides.

The strong inhibiting effect of the cathodic solution on lipid oxidation was also apparent with the aqueous oxidation of ethyl DHA (Fig. 2). Dispersing ethyl DHA in a cathodic solution with an emulsifier resulted in the decrease in unoxidized substrate and the formation of peroxides being mostly inhibited, while it was oxidized rapidly in distilled water and in the NaCl solution with high rates of substrate disappearance and peroxide accumulation.

The antioxidative activity of the cathodic solution may have depended on its low dissolved oxygen level. The cathodic solution just after electrolysis showed 3.95 mg/l of dissolved oxygen, but this value increased to almost the same level as that found in distilled water (7.71 mg/l) after dispersing each ester with Triton X-100 in that solution. Therefore, it is apparent that the low dissolved oxygen level in the cathodic solution just after electrolysis did not contribute to the antioxidative activity that was found.

Lipid peroxidation proceeds via a free radical chain reaction consisting of chain initiation, propagation and termination processes. The key event in initiation is the formation of a lipid radical (L·). This can occur by abstraction of the hydrogen radical (H·) from the substrate lipid (LH). Under the present experimental conditions, hydrogen abstraction was induced by the O_2 species activated by the AsA-Fe(II) or organic peroxide radical generated from AAPH. The resulting lipid free radicals (L·) reacted with oxygen to form peroxy radicals (LOO·). In this propagation process, LOO· reacted with more LH to form lipid hydroperoxides (LOOH). The most important mechanism for antioxidation is to break this chain reaction by reacting with free radicals to form nonradical products. Therefore, another factor responsible for the antioxidative activity of the cathodic solution may have been its scavenging ability toward free radicals, which could be expected from the high dissolved hydrogen level with reducing potential in the cathodic solution.

Table 1. Change in the Scavenging Effect of a Cathodic Solution on the DPPH Radical during Incubation

Incubation period (day)	Amount (ml) of the cathodic solution in the reaction mixture (5 ml)	Scavenging effect (%)
0	0.0	0.0
	0.1	1.4
	0.5	25.4
	1.0	37.2
3	0.0	0.0
	0.1	0.0
	0.5	5.9
	1.0	10.9
7	0.0	0.0
	0.1	0.0
	0.5	0.0
	1.0	0.0

The experimental procedure is described in the Materials and Methods section. Scavenging effect (%) was calculated from the absorbance ratio at 517 nm of each sample solution to a blank solution containing no cathodic solution.

Table 1 shows the scavenging effect of the cathodic solution on DPPH radicals which are often used as the free radical to evaluate the antioxidative activity of some natural materials. The cathodic solution just after electrolysis showed a high scavenging effect, but this effect was decreased by incubation at 37°C when exposed to air, and finally disappeared after incubation for 7 days. This decrease in the scavenging effect of the cathodic solution during incubation may have been due to the instability of hydrogen in an aqueous solution.

To elucidate the relationship between the scavenging effect of the cathodic solution and its strong antioxidative property, the oxidative stability of ethyl LA and ethyl DHA in the cathodic solution just after electrolysis was compared with that in a cathodic solution that had been incubated for 3 days or 7 days (Figs. 1 and 2). Although the scavenging effect of the cathodic solution on the DPPH radicals disappeared after incubation (Table 1), no significant difference in the antioxidative activity was apparent between the cathodic solution just after electrolysis and that after incubation for 3 days or 7 days, suggesting that the scavenging effect on free radicals found in the cathodic solution just after electrolysis could not be the main reason for its strong antioxidative activity. The maintenance of antioxidative activity of the cathodic solution during incubation also shows its usefulness for a variety of applications.

The cathodic solution just after electrolysis had a pH value of 11.9, but the pH values of cathodic solutions incubated for 3 days and 7 days fell to 10.2 and 8.4, respectively. Despite a such change in pH during incubation, the strong antioxidative activity of the cathodic solution was maintained after incubation for 3 days and 7 days (Figs. 1 and 2); therefore, the high pH of the cathodic solution would have been independent of its antioxidative activity. Several reports have been published on the effect of pH on lipid oxidation.[7-9] The results in these reports indicate that the oxidative stability of a lipid in an aqueous solution tended to decrease with increasing pH value, although it depended on the buffer system. The effect of pH on the aqueous lipid oxidation found in these studies also suggest that the high pH value of the cathodic solution was not responsible for its antioxidative activity, but was rather a prooxidative factor.

This study has shown that a cathodic solution prepared by electrolyzing an NaCl solution had strong antioxidative activity. The scavenging effect of a cathodic solution on free radicals derived from its high dissolved hydrogen concentration has been thought to be the most likely factor responsible for its antioxidative activity; however, another reason could also be expected from the fact that the antioxidative activity of the cathodic solution was maintained after incubation for 3 days and 7 days, while its scavenging effect disappeared after incubation.

Shirahata *et al.*[1] have reported that a cathodic solution had the ability to scavenge active oxygen species such as the superoxide anion radical and hydrogen peroxide. They attributed this scavenging effect to the amount of dissolved hydrogen in the cathodic solution, although the mechanism was not clear. However, such active oxygen species would not play an important role under the present experimental conditions. In future studies on the antioxidative activities of cathodic solutions, it is, therefore, imperative that a comparison of these activities be made under various oxidation conditions to elucidate the mechanism for the effect of the cathodic solution on lipid peroxidation.

References

1) Shirahata, S., Kabayama, S., Nakano, M., Miura, T., Kusumoto, K., Gotoh, M., Hayashi, H., Otsubo, K., Morisawa, S., and Katakura, Y., Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage. *Biochem. Biophys. Res. Commu.*, **234**, 269–274 (1997).
2) Miyashita, K. and Takagi, T., Tocopherol content of Japanese algae and its seasonal variation. *Agric. Biol. Chem.*, **51**, 3115–3118 (1987).
3) Takagi, T., Mitsuno, Y., and Masumura, M., Determination of peroxide value by the colorimetric iodine method with protection of iodine as cadmium complex. *Lipids*, **13**, 147–151 (1978).
4) Hirano, S., Miyashita, K., Ota, T., Nishikawa, M., Maruyama, K., and Nakayama, S., Aqueous oxidation of ethyl linoleate, ethyl linolenate, and ethyl docosahexaenoate. *Biosci. Biotechnol. Biochem.*, **61**, 281–285 (1997).
5) Mitsuda, H., Yasumoto, K., and Iwami, K., Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Shokuryo* (in Japanese), **19**, 210–214 (1966).
6) Tanaka, M. and Nakagawa, M., Antioxidant activity of thiocholesterol on copper-induced oxidation of low-density lipoprotein. *Lipids*, **30**, 321–325 (1995).
7) Marbrouk, A. F. and Dugan, L. R., A kinetic study of the autoxidation of methyl linoleate and linoleic acid emulsions in the presence of sodium chloride. *J. Am. Oil Chem. Soc.*, **37**, 486–490 (1960).
8) Huang, S.-W., Frankel, E. N., Schwarz, K., and German, J. B., Effect of pH on antioxidant activity of α-tocopherol and trolox in oil-in-water emulsions. *J. Agric. Food Chem.*, **44**, 2496–2502 (1996).
9) Saunders, D. H., Coleman, J. E., Hampson, J. W., Wells, P. A., and Riemenschneider, R. W., Autoxidation of fatty materials in emulsions. I. Pro-oxidant effect of histidine and trace metals on the oxidation of linoleate esters. *J. Am. Oil Chem. Soc.*, **39**, 434–439 (1962).