Note

Effect of Ascorbic Acid on the Chemiluminescence of Polyphenols

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The chemiluminescence of gallic acid by hydrogen peroxide had completely inhibited by the presence of ascorbate. After ascorbate had disappeared by oxidation, chemiluminescence returned. The concentration of gallic acid was virtually unchanged by the presence of ascorbate, but started to decrease after the disappearance of ascorbate. This might be attributable to the rapid reduction of quinone, which was the first product of the chemiluminescence reactions, to gallic acid by ascorbate or the donation of proton to the phenoxy radical from ascorbate to stop the chemiluminescence reaction at the first stage. The effects of ascorbate on the chemiluminescence of other polyphenols depended on their oxidation rate.

Key words: ascorbic acid; polyphenol; chemiluminescence; gallic acid

Polyphenols have recently been attracting strong attention as antioxidants,1) antiallergic agents,2) and anti-Alzheimer’s disease agents.3) Several methods have been reported to determine them such as HPLC-UV or FL,4) electrochemical detection,5) and evaporative light-scattering detection.6) The Trautz-Schorigin reaction is known as a chemiluminescence reaction of polyphenols, and this reaction has been applied to determine formaldehyde, proteins, tannins, pyrogallol,7,8) catechin and epigallocatechin gallate.9,10) The method was also been applied to estimate the antioxidation activity of foodstuffs by a very simple and rapid procedure.11) However, such compounds as ascorbic acid interfere with chemiluminescence.12) Since ascorbic acid is widely used for a food additive as a water-soluble antioxidant, it might cause errors in a chemiluminescence analysis using polyphenols. We describe here the effect of ascorbate on the chemiluminescence reaction of polyphenols by measuring the chemiluminescence development and a determination of the components of the reaction by capillary electrophoresis.

Gallic acid (1.25 mM) was used as a typical polyphenol component, and the chemiluminescence in the presence of hydrogen peroxide (25 mM) in a 250 mM KHCO$_3$–12.5% 2-propanol solution was observed by a CLS-LSI instrument (Tohoku Electronic Industrial Co., Sendai, Japan). The reaction was performed at 26 °C in a glass dish (16 mm i.d.) under magnetic stirring, and the total volume of the solution was 0.800 ml. The photons were counted with a 1-sec gate time. Acetaldehyde is usually used for the chemiluminescence reactions of polyphenols as a sensitizer. However, the chemiluminescence reaction was performed without acetaldehyde, because it was possible to observe the photons without it and it seemed too volatile for evaluating the change in concentration of components in the reaction solutions.

The chemiluminescence of gallic acid was apparent just after mixing the reagents, while it was delayed by the addition of ascorbate. This chemiluminescence lag time depended on the amount of ascorbate added (Fig. 1A). The relative total photon counts to the control (a, ascorbate = 0 mM) were 95% (b, 0.125 mM), 86% (c, 1.25 mM) and 48% (d, 12.5 mM). This means that the total photon count also decreased according to the amount of ascorbate added. It was noted that the total photon count was almost constant when the hydrogen peroxide concentration was between 20 and 25 mM, and the hydrogen peroxide concentration was maintained in this range for at least 3 h under the reaction conditions. Therefore, the decrease in total photon count by the addition of ascorbate was not attributable to a decrease in hydrogen peroxide.

Figure 1B shows the decrease in gallic acid and ascorbate in the reaction solutions with and without ascorbate. In the absence of ascorbate (solution a), gallic acid decreased just after mixing. On the other hand, the concentration of gallic acid remained constant in the presence of ascorbate and then started to decrease after 1000 sec when the ascorbate had completely disappeared from the solution and the chemiluminescent emission started. The determination of ascorbate and gallic acid was performed by a G-1600A-Z capillary electrophoresis instrument (Agilent, USA) under the following conditions: capillary, fused silica (50 μm i.d. × 64.5 cm, 56 cm effective length); background solution, 50 mM sodium dodecylsulfate–50 mM phosphate–120 mM borate buffer (pH 7.0); voltage, 17 kV; sample injection, 50 mbar for 5 sec; detection, UV at 256 nm.

The oxidation of gallic acid to quinone is the first step in a series of chemiluminescence reactions for gallic...
acid by hydrogen peroxide in an alkaline solution, with subsequent oxidative polymerization and degradation to give chemiluminescence.\(^7\) The results of this present study strongly suggest that ascorbate quickly reduced the quinone produced from gallic acid to gallic acid or donated a proton to the phenoxy radical to stop the chemiluminescence reaction at the first stage. The addition of 0.100 ml of a 10 m M ascorbate solution to the photon-emitting solution 600 sec after mixing the reagents soon quenched the chemiluminescence, and the solution emitted chemiluminescence again (second emission) 1600 sec after the addition (Fig. 2A). The ascorbate added had completely disappeared at this time (Fig. 2B).

The photon count is effected by the total volume of the solution, so it was necessary to correct the photon count for the second emission by comparing the total photon counts with and without ascorbate. Since the total photon count for the second emission in Fig. 2A agrees with that for the same concentration of a gallic acid solution (0.22 m M) with the same total volume (0.900 ml), the corrected total photon count for the second emission should be that of the solution containing 0.25 m M gallic acid in a 0.800-ml solution. After correcting for the dilution effect, the total photon count was about 17% lower than that of the corresponding solution without adding ascorbate. These results might suggest that ascorbate also directly affected photon emission by quenching such active species as singlet oxygen. Even if the chemiluminescence reaction proceeded partly in the presence of ascorbate, the photon emission was irreversibly inhibited by ascorbate. This might be partly attributable to the total photon count decreasing according to the amount of ascorbate added.

Figure 3 shows the chemiluminescence development of some polyphenols with and without 1.25 m M ascorbate. As with gallic acid, most cases of polyphenol chemiluminescence development were delayed by the addition of ascorbate. The lag times (\(y\)) for gallic acid, caffeic acid, quercetin, pyrogallol and 2,3-hydroxybenzoic acid were inversely correlated with the polyphenol oxidation rate (\(x\)), this being evaluated by the decrease in polyphenol during 5 min after mixing according to the equation of 
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\sqrt{y} = -173.43x + 44.54 \text{ and } r^2 = 0.9975.
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Since a quinone of polyphenol was quickly reduced to the initial polyphenol by ascorbate, the decrease in the rate of ascorbate was accelerated by polyphenol oxidation. If polyphenol had a higher oxidation rate, the ascorbate in the solution would also have been oxidized faster to reduce the lag time for chemiluminescence development by ascorbate.

On the other hand, catechin and epicatechin showed different chemiluminescence patterns. Their chemiluminescence was slightly accelerated by the addition of
ascorbate (Figs. 3D and G). Without ascorbate, their chemiluminescence emission started about 2000 sec after mixing, which was much later than with the other polyphenols, while the decrease in catechin started just after mixing. This result suggests that the non-emission reaction process of the catechin and epicatechin chemiluminescence reactions was much slower than that of the other polyphenols. On the other hand, the decrease of catechin in the reaction solution wasn’t completely stopped by ascorbate, which had disappeared completely within 1700 sec, but was slowed down only for initial 600 sec. Therefore, the non-emission reaction process would proceed in spite of the presence of ascorbate, and the ascorbate-oxidized products might accelerate the chemiluminescence reaction by the oxidation of polyphenols. Moreover, the added ascorbate had disappeared completely before the light emission process started. These events might be the reason why the chemiluminescence was accelerated by ascorbate.

Therefore, with some analytical methods such as a flow injection analysis, it would be necessary to take into account the effect of ascorbate. Since the effect depended on the polyphenol oxidation rate, it might be possible to reduce or eliminate the effect of ascorbate on polyphenol chemiluminescence by accelerating the polyphenol oxidation reaction rate by the addition of such metal ions as Co$^{2+}$, Cr$^{3+}$, Fe$^{3+}$ and Mn$^{2+}$ which accelerated both polyphenol oxidation and the chemiluminescence reaction (Naoko INOUÉ, Kazuaki AKASAKA, Hirokazu ARIMOTO, and Hiroshi OHRIU, unpublished results).

In conclusion, ascorbate stopped the chemiluminescence reaction of polyphenols by a synergistic effect and by quenching such active species as singlet oxygen to delay the start of the chemiluminescence emission of most polyphenols.

References

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