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Peroxyoxalate Chemiluminescent Assay of Ascorbic Acid Based on Autoxidation with Oxygen in Reversed Micelle

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A highly sensitive chemiluminescence (CL) method was developed for the determination of ascorbic acid (AA) based on the conversion of AA to hydrogen peroxide (H_2O_2) in a cationic reverse micellar hexadecyltrimethylammonium chloride : chloroform medium. AA was indirectly determined by measuring the H_2O_2 generated by the peroxyoxalate CL method. The CL intensity was linearly correlated with AA concentration over the range from 5.0×10^{-8} to 1.0×10^{-6} M.

The oxidation of ascorbic acid (AA) with oxygen has been studied in order to obtain information on the role of AA in biological systems. AA is rapidly oxidized by oxygen in the presence of metal ion catalysts such as copper(II), resulting in the formation of H_2O_2 and dehydroascorbic acid (DHAA).[1] In the presence of reverse micellar media, we have found that AA in aqueous solution can be partially converted into H_2O_2 in spite of the absence of metal ion catalyst. Further, light emission was observed from the subsequent CL reaction of the H_2O_2 formed with bis(2,4,6-trichlorophenyl)oxalate (TCPO) and perylene dissolved in the cationic reverse micellar system, i.e. hexadecyltrimethylammonium chloride (CTAC) in chloroform. The CL intensities were dependent on the AA concentration. A highly sensitive CL method for the determination of AA was thus developed.

The general CL experimental procedure consisted of pipetting 2.5 cm^3 of an aqueous AA solution into a glass cuvette in a luminometer. The AA solution was prepared with 5.0×10^{-2} M(1 M = 1 mol dm^{-3}) imidazole buffer (pH 7.5). A CL reagent was prepared by dissolving 1.0×10^{-2} M CTAC, 7.0×10^{-3} M TCPO and 1.5×10^{-2} M perylene in chloroform. CTAC can associate in chloroform to form a reversed micelle.[2] Next, a 0.3 cm^3 portion of the CL reagent was injected into the cuvette. This injection of the CL reagent initiated to the CL reaction. The light emission was detected using a photomultiplier tube. Vigorous agitation by a magnetic stirrer was continued during the reaction. The resultant photocurrent was converted to

a voltage and displayed on a chart recorder. The maximum light emission was corrected for the light emission of a background blank without AA. These background-corrected CL values was referred to as the CL intensity. All measurments were made at 25 °C.

Typical CL response curves are shown in Fig.1 for the cases when the CL reagent was injected into a 1.0×10^{-6} M H_2O_2 solution or 5.0×10^{-6} M AA solution. The light emission reached a maximum within 10 s and then began to rapidly decay. In the case of AA, the CL reaction proceeded regardless of addition of or not H_2O_2. It is known that H_2O_2 is required to produce dioxetanediones which are a key intermediates required for the peroxyoxalate CL. The intermediates are capable of transferring their energy to a suitable fluorescer such as perylene, thus resulting in the light emission from the exited fluorescer.[3] Therefore, the result shown in Fig.1 (curve 2) suggests that AA is partially oxidized by oxygen to form DHAA and H_2O_2 in order for CL to be observed.

Next, the oxidation of AA with oxygen using various reaction media was examined. The experiments were carried out using the following conditions. A 10 cm^3 portion of a 5.0×10^{-5} M AA solution was mixed with a 1.2 cm^3 portion of an organic solvent alone, an organic solvent containing 2.0×10^{-2} M CTAC or an aqueous solution containing 2.0×10^{-2} M CTAC. Chloroform, hexane and tetrachloromethane were selected as the organic solvent. The determination of H_2O_2 generated was carried out by a spectrophotometric method[4] after 5 min from the initiation of the reaction. The oxidation of AA with oxygen only occurred in the presence of organic solvents containing CTAC. The concentrations of H_2O_2, thus formed, were 2.6×10^{-6} M for chloroform, 1.6×10^{-5} M for hexane and 2.7×10^{-6} M for tetrachloromethane, respectively. However, no H_2O_2 was produced in the pure organic solvents alone nor in the normal micellar assemblies of CTAC in water alone.

The effect of charge-type of surfactant micelles on the oxidation of AA with oxygen was next examined. The AA solution was mixed with chloroform containing 2.0×10^{-2} M

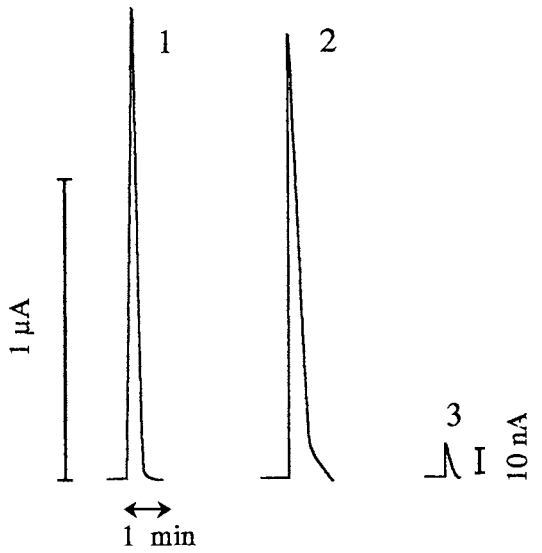

Fig.1. Typical CL response curves.
[CTAC]=5.0×10^{-3} M, [TCPO]=7.0×10^{-3} M,
[perylene]=1.5×10^{-2} M,
1: [H_2O_2]=1.0×10^{-6} M,
2: [AA]=5.0×10^{-6} M, 3: blank.

sodium dodecylsulfate (SDS) or 2.0×10^{-2} M polyoxyethylene(23)dodecanol (Brij-35). The oxidation of AA also proceeded in those surfactant micelles, and the concentrations of H_2O_2 generated were 1.3×10^{-6} M for nonionic Brij-35 and 2.4×10^{-6} M for anionic SDS, respectively.

The results obtained in various reaction media suggest that the fromation of the reversed micelle assemblies in the mixture of the aqueous AA solution and the CL reagent solution play an important role in the formation of H_2O_2 because no H_2O_2 was fromed in the pure organic solvents nor in the normal micellar assemblies. It is known that superoxide anion and DHAA radical are produced as intermediates in the catalytic oxidation of AA with oxygen.[5] These active species could be more stable in the presence of both of these micellar surfactants and organic solvents compared to in the other reaction media, resulting in the formation of H_2O_2. However, the exact function of surfactant reverse micelles upon this reaction are still not clear.

Next, CL measurements were carried out by adding 7.0×10^{-3} M TCPO and 1.5×10^{-2} M perylene to different charge-type surfactant reversed systems. CL was only observed in the micellar reversed CTAC/chloroform system. These results are probably due to the instability of TCPO in tetrachloromethane and hexane.[6] As a result, it was found that the most promising surfactant and organic solvent for the reversed micellar mediated determination of AA were CTAC and chloroform.

In subsequent studies, the optimum conditions for the quantification of AA were determined by measuring the CL intensities, so as to be maximal under optimum conditions. Figure 2 shows the dependence of CL intensity upon the CTAC concentration. The increase of the CL intensity commenced at surfactant concentrations above the critical micelle concentration, which was 3×10^{-3} M for CTAC in chloroform.[2] The CL intensity increased to a maximum value, after which it decreased gradually with increasing CTAC concentration. Thus, the optimum CTAC concentration was chosen to be 1.0×10^{-2} M.

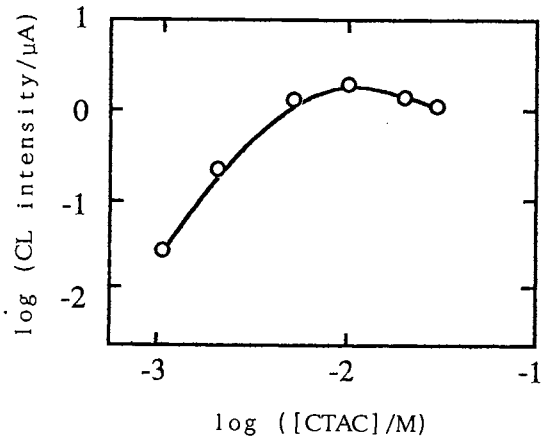

Fig.2. Effect of CTAC concentrations on CL intensity.
[AA]=5.0×10^{-6} M, [TCPO]=7.0×10^{-3} M, [perylene]=1.5×10^{-2} M.

The effect of pH in the AA solution was examined in the 6.2 - 8.0 pH range, in which ascorbate anion is the predominant species, since the pKa values of AA are 4.2 and 11.8.[5] The CL intensity exhibited a broad maximum at pH 7.5. Thus, a pH of 7.5 was chosen for the recommended procedure. The influence of TCPO concentration was tested in the range 2.0×10^{-3} - 1.4×10^{-2} M. The CL intensity increased with increasing TCPO concentration up to 7.0×10^{-3} M and then decreased gradually with increasing TCPO concentration. The optimum TCPO concentration was determined to be 7.0×10^{-3} M. The dependence on the perylene concentration was also investigated in the range 3.0×10^{-3} - 1.5×10^{-2} M. The CL intensity increased with increasing perylene concentration. The optimum perylene concentration was chosen as 1.5×10^{-2} M, because of the limited solubility of perylene in chloroform.

Analytical calibration curve was prepared under the optimized experimental conditions. Logarithmic calibration curve of AA was linear over ranging in initial concentration from the detection limit of 5.0×10^{-8} to 1.0×10^{-6} M with slope of 0.85. The relative standard deviation of five successive experiments was 2.5% at 5.0×10^{-7} M of AA.

In conclusion, the best sensitivity of AA is achieved by the use of reverse micellar CTAC containing TCPO and perylene dissolved in chloroform. The sensitivity of the proposed method for AA is comparable to that of a reported electrochemical detection method which has gained wide acceptance as high sensitive method.[7] Further studies concerning the oxidation mechanism of AA in reverse micellar CTAC are underway.

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