CHEMISTRY LETTERS, pp. 1719-1722, 1991.

Enhancement of Lucigenin Chemiluminescence by Periodate Oxidation of Adrenaline in Cationic Surfactant Micelles

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Aqueous solutions of cationic micellar hexadecyltrimethylammonium hydroxide (CTAOH) containing periodate enhanced markedly the intensity of chemiluminescence (CL) emitted from the reaction of lucigenin with adrenaline (AD). Detection limit for AD was improved by factors of 20 compared to that in micellar CTAOH alone.

The determination of such catecholamines (CAs) as dopamine, noradrenaline and adrenaline (AD) in biological fluids is primarily useful for the diagnosis for various diseases and evaluation of their medical treatments. We have previously proposed the lucigenin (10,10'-dimethyl-9,9'-biacridinium dinitrate : Luc) chemiluminescence (CL) method for the determination of CAs.[1)] In addition, the sensitivity of the Luc CL methods for CAs was increased by the use of hexadecyltrimethylammonium hydroxide (CTAOH).[2)] Detection limits for CAs in cationic micellar CTAOH were improved by factors of 1.6 to 10 compared to those in water.[2)]

In the course of our studies on the improvement of the sensitivity of the Luc CL methods for CAs, we have found that periodate gave the CL enhancement effect, the extent of which was comparable to that in micellar CTAOH. Further, the CL intensity is markedly enhanced by the combined use of periodate with micellar CTAOH. Based on this finding, a highly sensitive Luc CL method for AD has been developed.

The general CL experimental procedure consisted in pipetting 250 μl of the solution containing NaOH and CTAOH micelles, and 250 μl of $NaIO_4$ solution into a 1-cm glass cell in the fluorometer. Next, 250 μl of Luc solution and 250 μl of AD solution were injected simultaneously through Teflon tubing into the cell by using an automatic injector. Thus the CL reaction was initiated and the light emission was detected by a photomultiplier tube without wavelength discrimination. 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 blank containing no AD. These background-corrected CL values were referred to as the CL intensities. The detection limit for AD was defined as the concentration yielding an analytical signal equal to two times the standard deviation of the blank.

The effect of oxidizing agents concentration on the CL response curve was investigated. Measurements were carried out under following conditions: a 6.0×10^{-5} M (= mol dm^{-3}) Luc solution and a 1.0×10^{-4} M AD solution were injected into the solution containing 0.20 M NaOH in water alone or the solution containing 0.20 M NaOH and an apporopriate oxidizing agents between 1.0×10^{-6} M and 1.0×10^{-2} M. Some oxdizing agents were tested: $NaIO_4$, KIO_4, NaClO, $KMnO_4$, and $K_2S_2O_8$. The CL enhancement was only observed for periodate. Typical CL response curves for AD in $NaIO_4$ are shown in Fig.1. The CL intensities of the first peak increased markedly with an increase in the $NaIO_4$ concentration in the range of 1.0×10^{-6} M to 5.0×10^{-4} M of $NaIO_4$. The CL intensity increases to a maximum value, after which it decreases rapidly above 5.0×10^{-4} M $NaIO_4$. On the other hand, the CL intensity of the second peak decreases gradually with an increase in $NaIO_4$ concentration and could not be detected at concentrations below 5.0×10^{-4} M of $NaIO_4$. Thus, the intensity of the maximum light emission at the first peak was measured for the determination of AD. The CL response curves for AD in KIO_4 are

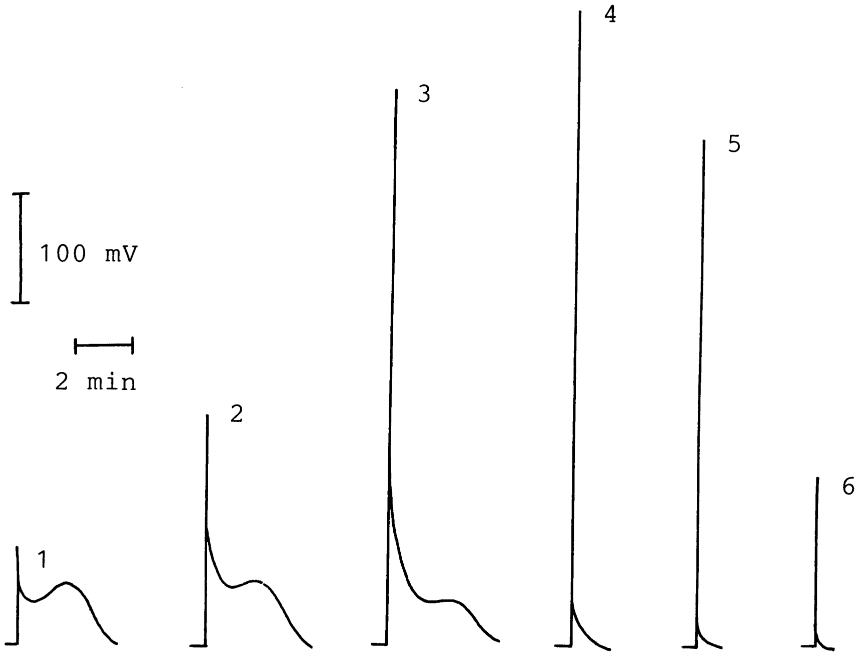

Fig.1. Typical response curve for AD.
[AD]=1.0×10^{-4} M, [Luc]=6.0×10^{-5} M, [NaOH]=0.2 M,
$NaIO_4$/M : 1=0, 2=1.0×10^{-6}, 3=1.0×10^{-4}, 4=5.0×10^{-4}, 5=1.0×10^{-3}, 6=1.0×10^{-2}

the same as that shown in Fig.1. Sodium periodate was thus chosen as an oxdizing agent.

In Fig.1, the appearance of the first peak is probably ascribable to the reaction of Luc with the oxidation product formed during the early stage of the oxidation of AD with periodate. Semiquinone radicals, quinone and adrenochrome are accepted to be main oxidation products by sequential one-electron oxidations of AD during the early stage of oxidation.3) However, the chemical species concerned to the CL are still not clear. The concentration of the oxidation products increases in any event with an increase in periodate concentration, resulting in an effective enhancement of the CL intensity of the first peak. On the other hand, the decrease of the CL intensity above 5.0×10^{-4} M $NaIO_4$ is probably attributable to the decrease of the concentration of the chemical species concerned to the CL reaction, because the oxidation of AD will be accelerated with an increase in $NaIO_4$ concentration.

Next, we examined the effect of periodate on the micelle-enhanced CL. A 1.0×10^{-4} M Luc solution and a 1.0×10^{-4} M AD solution were injected into the solution containing CTAOH alone or the solution containing CTAOH and 5.0×10^{-4} M $NaIO_4$. Figure 2 shows the CL intensity-CTAOH concentration profiles with or without periodate. The CL intensity in the absence of periodate was about the same as that in water alone (results not shown), when CTAOH concentrations were well below the critical micelle concentration (cmc), which was 9×10^{-4} M for CTAOH.4) On the other hand, the CL intensity in the presence of periodate was greater than that in water alone, even if the concentration of CTAOH was below cmc. The increase of the CL intensity below cmc is attributable to the CL enhancement by periodate. The increase in the CL intensity commenced at a CTAOH concentration close to cmc. The CL intensity increases to a maximum value, after which it either levels off in CTAOH alone or decreases rapidly in the mixture of CTAOH and periodate with increasing CTAOH concentration.

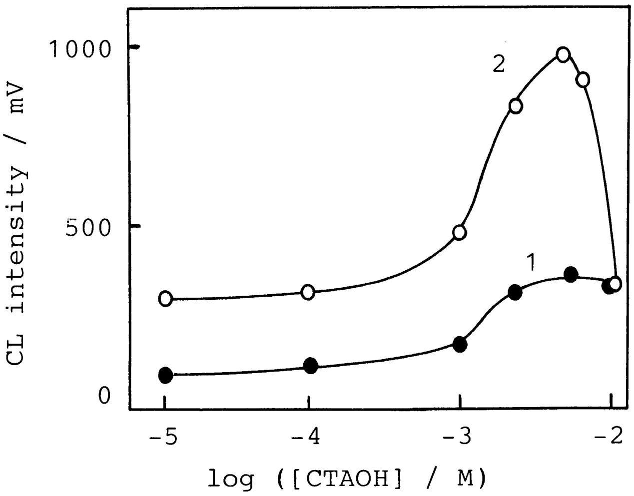

Fig.2. Effect of CTAOH concentration on CL intensity.
[AD]=1.0×10^{-4} M, [Luc]=1.0×10^{-4} M
[NaOH]=0.1 M,
$NaIO_4$/M : 1=0, 2=5.0×10^{-4} M

The remarkable CL enhancement above cmc may be interpreted in terms of the electrostatic interation of the deprotonated forms of AD and periodate with a cationic micelle pseudophase in which Luc is solubilized. Consequently, the formation of oxidation product concerned to the appearance of the first peak proceeds more effectively in micellar assemblies than in water alone. Thus, the CL intensity is increased in the combined use of CTAOH and periodate.

Analytical calibration curves were prepared under the optimized experimental conditions. Logarithmic calibration curves of AD in periodate and in the combined use of CTAOH and periodate were linear over the range form the detection limit of $1\text{x}10^{-8}$ M and $5\text{x}10^{-10}$ M up to $1\text{x}10^{-4}$ M with slopes of 0.92 and 0.80, respectively. The use of periodate improves the sensitivity of the Luc CL assays for AD by factors of 10 compared with that in water alone. The sensitivity with periodate was comparable to that with micellar CTAOH. Further, detection limit for AD in micellar CTAOH containing periodate is improved by factors of 20 compared to that in micellar CTAOH or periodate alone. The relative standard deviation of five successive experiments in periodate and in the combined use of CTAOH and periodate was 3% at $1.0\text{x}10^{-6}$ M of AD.

In conclusion, the best sensitivity of the CL reaction of Luc with AD is achieved by the use of an aqueous CTAOH micellar solution containing periodate. The sensitivity of the proposed method for AD is comparable to that of the electrochemical detection method which is being accepted to be a highly sensitive method. Further studies on the mechanism of the CL enhancement and extension to other CAs are under way.

This work was partly supported by a Grant-in-Aid from the Ministry of Education, Science and Culture (No.03650596).

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(Received July 4, 1991)