Nitroxy radical/Copper-Catalyzed Electrooxidation of Alcohols and Amines at Low Potentials

Kyoko Sugiyama, a, Yusuke Sasano, a,b Sachio Komatsu, a Kentaro Yoshida, c Tetsuya Ono, c Tsutomu Fujimura, a Yoshiharu Iwabuchi, b Yoshitomo Kashiwagi, c and Katsuhiko Sato* a,d

a Faculty of Pharmaceutical Science, Tohoku University; 4–4–1 Komatsushima, Aoba, Sendai 981–858, Japan; b Graduate School of Pharmaceutical Sciences, Tohoku University; 6–3 Aoba, Aramaki, Aoba-ku, Sendai 980–8578, Japan; c School of Pharmaceutical Sciences, Ohu University; 31–1 Misumido, Tomita-machi, Koriyama, Fukushima 963–8611, Japan; and d Department of Creative Engineering, National Institute of Technology, Tsuruoka College; 104 Sawada, Inooka, Tsuruoka, Yamagata 997–8511, Japan.

Received May 11, 2021; accepted July 15, 2021

Nitroxy radicals, such as 2,2,6,6-tetramethylpiperidine N-oxyl (TEMPO), can catalyze the electrochemical oxidation of alcohols and amines. Because the oxidation current obtained in this process depends on the concentration of alcohols and amines, this process can be applied to their sensing. However, the relatively high oxidation potentials required by nitroxy radicals can induce interfering oxidation currents from various reductive substances in biological samples, which affects the accuracy of analyte measurements. In this study, we examined the electrooxidation of alcohols and amines at a low potential by applying cooperative oxidation catalysis using a nitroxy radical and a copper salt. Nortropine N-oxyl (NNO), which showed higher catalytic activity than TEMPO was used as the nitroxy radical. An increase in the oxidation current was observed at the low potential, and this increase depended on the alcohol concentration. In the case of the electrooxidation of amines, a positive correlation between oxidation current and amine concentration was observed at low amine concentrations. Therefore, low-potential cooperative catalysis can be applied to alcohol and amine electrooxidation for the development of accurate sensors suitable for clinical settings.

Key words electrooxidation; nitroxy radical; copper; electrochemical sensor; nortropine N-oxyl

Introduction

Various analytical methods, including immunological assays (e.g., enzyme-linked immunosorbent assay (ELISA)), fluorescence polarization immunoassay (FPIA)), HPLC, and LC-MS, 1–5 are used to monitor therapeutic drugs in clinical practice. However, these methods have some drawbacks; for example, immunological assays are often affected by the cross-reactivity of antibodies and LC-based methods require complicated pretreatment operations, such as extraction for noise reduction, as well as skilled professionals to operate complex equipment. 6,7 Therefore, an analytical detection method that provides rapid results and is simple to operate is in demand.

Analytical methods based on electrochemical measurements are simple to execute and suitable for use in clinical settings. For instance, enzyme-based glucose sensors are used to measure blood glucose levels in patients with diabetes. These sensors quantify glucose by electrolyzing the hydrogen peroxide produced during the oxidation of glucose in a blood sample by glucose oxidase. Electrochemical sensors (biosensors) based on enzymatic reactions have high substrate specificity and temporal resolution derived from the enzymatic reactions, which enables highly reliable and rapid measurements without pretreatments. 8,9 However, these sensors are less versatile because of the poor long-term stability and high cost of enzymes. Moreover, enzymatic reactions are applicable to only a limited number of substances. Therefore, enzyme-free electrochemical sensors, such as metal oxide-coated electrodes, 10,11 supramolecular host/guest compound-modified electrodes, 12 and phenylboronic acid-modified electrodes, 13,14 have also been developed.

We have studied enzyme-free electrochemical sensing of alcohols and amines based on nitroxy radical catalysts acting as organocatalysts. 15–18 In this sensing mode, the nitroxy radical is oxidized by an electrode into oxoammonium ion 1 (Fig. 1a), which oxidizes a substrate (an alcohol is shown as an example) to afford its corresponding oxidation product (a carbonyl compound is shown as an example) and hydroxylamine 2. Hydroxylamine 2 is re-oxidized into oxoammonium ion 1 to form a catalytic cycle, which increases the oxidation current. This increase in the oxidation current is proportional to the alcohol/amine concentration in the sample solution; therefore, an electrochemical measurement system (e.g., cyclic voltammetry, CV) can be used for the quantification of alcohols and amines. Among the nitroxy radicals we examined, nortropine N-oxyl (NNO) showed much higher catalytic activity than 2,2,6,6-tetramethylpiperidine N-oxyl (TEMPO), a conventional nitroxy radical, for alcohol oxidation (Fig. 2). TEMPO is less reactive because of the four methyl groups adjacent to its nitroxy radical moiety, which is necessary to stabilize TEMPO. In contrast, NNO lacks two alkyl groups, and its bicyclic skeleton contributes to its stabilization. This less-hindered bicyclic nitroxy radical exhibits higher catalytic activity than TEMPO. 19,20 Moreover, NNO has a hydroxy group, and the electron-withdrawing nature of this group increases the catalytic activity of NNO. Although the oxidation current arising from the electrochemical oxidation of alcohols in the presence of the NNO catalyst is large enough for analytical sensor applications, the relatively high oxidation potentials of nitroxy radicals (around +0.6 V vs. Ag/AgCl) in aqueous so-
Solutions can induce interfering oxidation currents from various reductive substances in biological samples, such as vitamin C, acetaminophen, and hydroquinone analogs, which affects the accuracy of sensor measurements.

Electroanalytical systems that provide electrical signals at low potentials have been reported. In a recent study using CV by Badalyan and Stahl, the increase in oxidation current due to alcohol oxidation catalyzed by a nitroxyl radical/copper salt co-catalyst was observed at a lower potential than that in the absence of the copper salt. The oxidation current at the lower potential is assigned to the oxidation of copper(I) to copper(II), and copper(II) alkoxide complex $\text{Cu}^{II}$ (Fig. 1b) is proposed as a key reaction intermediate. In the presence of nitroxyl radical $\text{N}^\cdot$, copper complex $\text{Cu}^{II}$ collapses into copper(I) and a carbonyl compound with hydroxylamine, thus completing a catalytic cycle. Although the relationship between the increase in oxidation current and the alcohol concentration was evaluated by Stahl et al. for their mechanistic study, a wide range of alcohol concentrations for analytical applications has not been investigated. Moreover, the catalytic activity of NNO for cooperative catalysis with a copper salt and electrochemical oxidation of amines with nitroxyl radical/copper catalysis are underdeveloped. The NNO/copper salt co-catalyst is expected to exhibit higher catalytic activity for the electrooxodization of alcohols and amines than the TEMPO/copper catalyst. Therefore, cooperative catalysis with NNO/copper salt is applicable to a wider range of substances. In the present study, the electrochemical detection of alcohols and amines at low potentials has been examined with the cooperative catalysis of NNO and a copper salt.

Experimental

Materials NNO and 9-azabicyclo[3.3.1]nonane $N^\cdot$-oxyl (ABNO) were synthesized as reported previously. Copper(I) trifluoromethanesulfonate toluene complex (CuOTf) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.), TEMPO, tetrabutylammonium perchlorate (TBAP), 2,2′-bipyridyl (bpy), and 2,6-lutidine were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The reagents were used without purification for the measurements.

Electrochemical Measurement CV was conducted on an electrochemical analyzer (ALS model 660B, BAS, Tokyo, Japan) in a three-electrode cell comprising a GC electrode (diameter: 3 mm) as the working electrode, a platinum wire as the counter electrode, and an Ag/Ag$^{+}$ reference electrode. The obtained cyclic voltammograms were plotted with reference to the redox potential of ferrocene (Fc/Fc$^{+}$). For the electrochemical measurements, tetrabutylammonium perchlorate (TBAP, 100 mM in acetonitrile) was used as the supporting electrolyte. The CV cycle was recorded three times, and the third CV cycle was plotted. For the calibration curve, measurements were performed several times, and the average values were plotted. All the experiments were performed at room temperature (approx. 20 °C).

Results and Discussion

Cyclic Voltammograms of Alcohol Oxidation with Nitroxyl Radical/Copper Cooperative Catalysis Electrochemical alcohol oxidation catalyzed by nitroxyl radicals with a copper salt was evaluated using CV. Initially, cyclic voltammograms of 1 mM solutions of TEMPO, CuOTf, and bpy in acetonitrile (containing 100 mM TBAP) were measured in the presence and absence of 10 mM benzyl alcohol (Fig. 3a), according to the conditions of Badalyan and Stahl. Unlike Stahl’s conditions where they used triethylamine as a base, we used 2,6-lutidine (20 mM) in place of triethylamine, which could potentially be oxidized in the electrochemical conditions. To confirm the effect of the copper salt, cyclic voltammograms of the solutions without CuOTf were also measured (Fig. 4a). As a result, oxidation currents were observed at approximately +0.3 V and −0.2 V vs. Fe/Fe$^{+}$. The oxidation current at +0.3 V was assigned to the oxidation of the nitroxyl radical into its corresponding oxoammonium ion because this oxidation current was observed even in the absence of the copper salt. The oxidation current at the lower potential was observed only in the presence of the copper salt, which indicated that this oxidation current corresponded to the oxidation of the copper ion. Both of the observed oxidation currents increased with the addition of 10 mM benzyl alcohol. The increase in the oxidation current at a higher potential indicates
the oxidation of the alcohol with the TEMPO catalyst alone, and that at a lower potential indicates alcohol oxidation with TEMPO/copper cooperative catalysis.

Next, the activities of the less-hindered bicyclic nitroxyl radicals, namely, ABNO and NNO, were evaluated. As reported by Badalyan and Stahl, ABNO increased the oxidation current in the presence of benzyl alcohol (Figs. 3b, 4b), and the increase in the oxidation current was greater than that induced by TEMPO.23) These observations indicated that ABNO promoted a higher reaction rate for alcohol oxidation, most likely because the steric hindrance around the nitroxyl radical moiety was reduced compared with that in TEMPO.19,24) The same conditions were carried out with NNO (Figs. 3c, 4c), and its catalytic activity was compared with that of TEMPO and ABNO. The increase in oxidation current ($\Delta I_p$) upon the addition of 10 mM benzyl alcohol in the absence of the copper salt for TEMPO, ABNO, and NNO were 1.9, 3.6, and 175 $\mu A$, respectively (Fig. 4). In comparison, the $\Delta I_p$ values in the presence of the copper salt at the lower potential for TEMPO, ABNO, and NNO were 2.2, 4.6 and 4.1 $\mu A$, respectively (Fig. 3).

Interestingly, the relative catalytic activity of NNO itself compared with TEMPO and ABNO was significantly different from that of NNO/copper catalysis compared with TEMPO/copper and ABNO/copper catalysis. In the absence of a copper salt, NNO produced a much larger $\Delta I_p$ value than TEMPO and ABNO, which indicated that the oxidation of benzyl alcohol was more efficient with NNO than with the other catalysts. However, in the presence of the copper salt, NNO produced a $\Delta I_p$ value that was only approximately twice that of TEMPO and almost the same as that of ABNO, which indicated that the reaction rate of alcohol oxidation with NNO/copper catalysis was almost the same as that with ABNO/copper catalysis. This difference can be attributed to the difference between the reaction mechanisms of the two distinct catalytic systems. When the nitroxyl radical acts as an independent catalyst, an oxoammonium ion is generated as the active species (Fig. 1a). An electron-withdrawing group, such as a hydroxy group, introduced on the catalyst structure increases the electrophilicity of the oxoammonium ion, thus increasing the reaction rate of the oxoammonium-promoted oxidation25) (Fig. 5). In contrast, when a nitroxyl radical and a copper salt act as a cooperative catalyst, such electrophilic active species are not generated (Fig. 1b), and thus the positive effect of an electron-withdrawing group is absent.26) Although a significant advantage was not gained with NNO/copper catalysis compared with ABNO/copper catalysis, the facile synthesis of NNO is a clear advantage over the relatively complex synthesis of ABNO. NNO is synthesized in only one step from commercially available nortropine, whereas ABNO is synthesized in three steps from acetonedicarboxylic acid.15,24)

Relationship between Oxidation Current and Alcohol Concentration for Nitroxyl Radical/Copper-Catalyzed Electrooxidation of Primary and Secondary Alcohols

To obtain more detailed data on the activity of NNO/copper catalysis, the relationship between oxidation current at the lower potential and alcohol concentration was evaluated. CV was conducted in acetonitrile solutions containing 100 mM TBAP and 1 mM of nitroxyl radicals (TEMPO or NNO), CuOTf, and bpy. Benzyl alcohol and 2,6-lutidine (2 equivalents (equiv) to the alcohol) were added successively to each of the solutions to final concentrations ranging from 0 to 100 mM (Figs. 6a, b). Both TEMPO and NNO showed increases in $\Delta I_p$ depend-

![Fig. 3. Cyclic Voltammograms of (a) TEMPO, (b) ABNO, and (c) NNO and a Copper Salt in the Absence (Dotted Line) and Presence (Solid Line) of 10mM Benzyl Alcohol (Containing 20mM 2,6-Lutidine); Concentrations: 1 mM Nitroxyl Radical, 1 mM Bpy, and 1 mM CuOTf in Acetonitrile (Containing 100mM TBAP); Sweep Rate: 50mV/s](image)

![Fig. 4. Cyclic Voltammograms of (a) TEMPO, (b) ABNO, and (c) NNO in the Absence (Dotted Line) and Presence (Solid Line) of 10mM Benzyl Alcohol (Containing 20mM 2,6-Lutidine); Concentration: 1 mM Nitroxyl Radical in Acetonitrile (Containing 100mM TBAP); Sweep Rate: 50mV/s](image)

![Fig. 5. Plausible Effect of an Electron-Withdrawing Group on an Oxoammonium Ion](image)
ing on the concentration of benzyl alcohol. The increase in the oxidation current was more pronounced for NNO than for TEMPO. These observations suggest that NNO is more suitable for electrochemical sensors than TEMPO. CV was then conducted under the same conditions using 1-phenylethanol, a secondary alcohol, instead of benzyl alcohol (Figs. 6c, d). A concentration-dependent increase in the oxidation current was observed for NNO, but no change was observed for TEMPO. Notably, this difference was also observed in nitroxyl radical/copper-catalyzed alcohol oxidation with molecular oxygen as a terminal oxidant. 27–30) Therefore, the less-hindered nitroxyl radicals oxidized secondary alcohols more efficiently than TEMPO.

Construction of Calibration Curves toward Alcohol Sensor Application

To facilitate the application of NNO/copper-catalyzed electrochemical alcohol oxidation to alcohol sensing, we constructed calibration curves for several alcohols. The relationship between $\Delta I_p$ and alcohol concentration was plotted for the NNO/copper-catalyzed electrochemical oxidation of benzyl alcohol, 1-phenylethanol (a secondary benzylic alcohol), 1-butanol (a primary aliphatic alcohol), and 2-propanol (a secondary aliphatic alcohol) (Fig. 7). In all cases, $\Delta I_p$ increased with increasing alcohol concentration, and linear increases were observed at concentrations above 1 mM.

Electrochemical Oxidation of Amines Using NNO/Copper Catalysis

To expand the applicability of electrocatalytic oxidation with NNO/copper catalysis, we investigated the NNO/copper-catalyzed electrochemical oxidation of amines using CV and prepared calibration curves for amines based on oxidation. CV was performed under the same conditions as for alcohol oxidation (Fig. 6) using various amines as substrates, and calibration curves were prepared based on the $\Delta I_p$ values at the lower potential. In the NNO/copper-catalyzed electrooxidation of primary, secondary, and tertiary aliphatic amines, namely, propylamine, diethylamine, and triethylamine, a linear increase in $\Delta I_p$ with increasing amine concentration was observed at concentrations below 1 mM. However, in contrast to the results of alcohol oxidation, the $\Delta I_p$ values decreased sharply when the amine concentration increased to more than 10 mM. Similar results were obtained for the electrooxidation of benzylic amines, namely, benzylamine, $N$-methylbenzylamine, and $N,N$-dimethylbenzylamine (Fig. 8b). The decrease in the $\Delta I_p$ values at higher amine concentrations is likely attributed to the amine molecules inhibiting the coordination of nitroxyl radicals to copper ions.

Conclusion

We have demonstrated, using CV, that NNO/copper cooperative catalysis induced an increase in oxidation currents at lower potentials, which was dependent on the concentration
of various types of alcohols and amines. A linear correlation between oxidation current and alcohol concentration was observed over a wide concentration range. The catalytic activity of the NNO/copper co-catalyst for the electrooxidation of alcohols was higher than that of the TEMPO/copper co-catalyst and almost the same as that of the ABNO/copper co-catalyst. We have also demonstrated, using CV, that NNO/copper catalysis was suitable for the oxidative detection of amines, and a positive linear correlation was observed between oxidation current and amine concentration at low amine concentrations. In general, electrochemical oxidation at a lower potential is less susceptible to interference from substances in biological and clinical samples.\(^{31}\) We believe that the electrooxidation system developed herein provides useful insights for the development of simple electrochemical sensors for therapeutic drugs bearing alcohols and/or amines. Investigations into the electrooxidation of alcohols and amines with NNO/copper catalysis in aqueous solutions are underway.

Acknowledgments This work was partially supported by JSPS KAKENHI Grant Nos. 20K06984 and 20K06961.

Conflict of Interest The authors declare no conflict of interest.

References

1) Truffot A., Jourd J. E., Seitz-Polski B., Malvezzi P., Brglez V., Stanke-Labesque F., Gautier-Veyret E., Clin. Biochem., 87, 60–66 (2021).
2) Farin D., Piva G. A., Gozl I., Kitzes-Cohen R., J. Pharm. Biomed. Anal., 18, 367–372 (1998).
3) Oyaert M., Peersman N., Kieffer D., Deiteren K., Smits A., Allegaert K., Spriet I., Van Eldere I., Verhaegen J., Vermeersch P., Clin. Chim. Acta, 441, 63–70 (2015).
4) Bileveld Y., de Haan T., Toersche J., Groenendaal F., Dijk P., van Heijst A., Gavilanes A. W. D., de Jonge P., van Straaten H., Rijken M., Zonnenberg I., Cools, Badalyan A., Stahl S. S., J. Org. Chem., 80, 78–82 (2003).
5) Truffot A., Jourd J. E., Seitz-Polski B., Malvezzi P., Brglez V., Stanke-Labesque F., Gautier-Veyret E., Clin. Biochem., 87, 60–66 (2021).
6) Ciobanu M., Taylor D. E. Jr., Wilburn J. P., Clift D. E., Anal. Chem., 80, 2717–2727 (2008).
7) Wilson J. F., Davis A. C., Tobin C. M., J. Antimicrob. Chemother., 52, 78–82 (2003).
8) Ciobanu M., Taylor D. E. Jr., Wilburn J. P., Clift D. E., Anal. Chem., 80, 2717–2727 (2008).
9) Otani Y., Yasukawa T., Mizutani F., Bunseti Kagaku, 59, 721–725 (2010).
10) Wei M., Qiao Y., Zhao H., Li J., Li T., Luo Y., Lu S., Shi X., Lu W., Sun X., Chem. Commun., 56, 14553–14569 (2020).
11) Dhara K., Mahapatra D. R., Mikrochim. Acta, 185, 49 (2018).
12) Egawa Y., Ishida Y., Yamauchi A., Anzai J., Suzuki I., Anal. Sci., 21, 361–366 (2005).
13) Kikuchi A., Suzuki K., Okabayashi O., Hoshino H., Kataoka K., Sakurai Y., Okano T., Anal. Chem., 68, 823–828 (1996).
14) Takahashi S., Aok N., Haraguchi N., Fujita H., Seki E., Ono T., Yoshida K., Anzai J., J. Environ. Sci., 23, 1027–1032 (2011).
15) Sato K., Ono T., Yoshida K., Ito T., Kashiwagi Y., Electroanalysis, 27, 2272–2274 (2015).
16) Sato K., Ono T., Sasano Y., Sato F., Kumano M., Yoshida K., Daiaka T., Iwabuchi Y., Kashiwagi Y., Catalysis, 8, 649 (2018).
17) Kashiwagi Y., Ono T., Sato F., Kumano M., Yoshida K., Daiaka T., Sasano Y., Iwabuchi Y., Sens. Biosens. Res., 27, 100302 (2020).
18) Komats S., Sasano Y., Sugiyama K., Watanabe K., Kumano M., Yoshida K., Ono T., Iwabuchi Y., Fujimura T., Sato K., Kashiwagi Y., Int. J. Electrochem. Sci., 16, 21027 (2021).
19) Shibuya M., Tomizawa M., Suzuki I., Iwabuchi Y., J. Am. Chem. Soc., 128, 8412–8413 (2006).
20) Iwabuchi Y., Chem. Pharm. Bull., 61, 1197–1213 (2013).
21) Bayat S., Kat S., Willner I., J. Am. Chem. Soc., 124, 14724–14735 (2002).
22) Bollella P., Gorton L., Antiochia R., Sensors, 18, 1319 (2018).
23) Badalyan A., Stahl S. S., Nature (London), 535, 406–410 (2016).
24) Shibuya M., Tomizawa M., Sasano Y., Iwabuchi Y., J. Org. Chem., 74, 4619–4622 (2009).
25) Rafee M., Miles K. C., Stahl S. S., J. Am. Chem. Soc., 137, 14751–14757 (2015).
26) Sasano Y., Yamauchi A., Sasaki R., Nagasawa S., Iwabuchi Y., Chem. Pharm. Bull., 60, 88–97 (2011).
27) Steves J. E., Stahl S. S., J. Am. Chem. Soc., 135, 15742–15745 (2013).
28) Sasano Y., Nagasawa S., Yamauchi A., Shibuya M., Park J., Iwabuchi Y., Angew. Chem. Int. Ed., 53, 3236–3240 (2014).
29) Sasano Y., Kogure N., Nishiyama T., Sasano Y., Iwabuchi Y., Chem. Asian J., 138, 1276–1277 (2016).
30) Sasano Y., Kogure N., Sasano Y., Kasabata K., Iwabuchi Y., Org. Lett., 20, 6104–6107 (2018).
31) Wang J., Chem. Rev., 108, 814–825 (2008).