Polarographic Evidence for the Reduction of Nitrite to Nitrogen Monoxide by Iron(II) Ions in Neutral Buffer Solutions and Characteristics of Cathodic Waves of Nitrogen Monoxide in the Presence of Iron Ions and Citrate

By

Yasuhiko Komatsu, Masuro Yamaguchi, and Masanosuke Takagi*

(Department of Agricultural Chemistry, College of Agriculture,
University of Osaka Prefecture, Sakai, Osaka 591)

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Synopsis

The reduction of NO_2^- to NO by Fe^{2+} in neutral buffer solutions containing excess citrate has been substantiated polarographically. Nitrogen monoxide exhibited three cathodic waves having a characteristic maximum in the presence of iron ions and citrate. The two waves ($E_{1/2} = -0.56$ and $-0.75V$ vs SCE at pH 7) were of kinetic current, whereas the third wave ($E_{1/2} = -1.10V$) having a maximum was a diffusion-controlled one. The NO concentration dependence of the limiting currents of the three waves showed a relation with the form of Langmuir's adsorption isotherm.

In the course of our experiments to study the biological reduction of inorganic substances by soil micro-organisms in a closed system of a soil suspension buffered to pH 7, the chemical reduction of NO_2^- to NO by Fe^{2+} has been suggested to play an important role in over-all process (1). Although the standard potential for $\text{NO}_2^- \rightleftharpoons \text{NO}$ has long since been studied by several investigators and been cited in textbooks (2, 3), the reported equilibrium potentials are little helpful for estimating the feasibility of the reaction between these nitrogen compounds with metal ions under biological conditions.

We attempted direct polarographic detection to demonstrate the formation of NO from NO_2^- through reduction by Fe^{2+} in the presence of excess citrate in neutral buffer solutions.

Experimental

Apparatus A Yanagimoto polarograph, type PA-102, was used. Characteristics of the capillary used were $m = 1.63$, $t = 4.36$ at open circuit in the buffer solution pH 7 at 25°C , with mercury head 60 cm. The electro-

* To whom all the communications should be addressed.

lytic cell was a modified Thunberg tube with a KCl saturated agar bridge, convenient for mixing reactants, isolating from atmospheric oxygen. All the experiments were carried out at $25 \pm 0.1^\circ\text{C}$.

Results and Discussion

Reduction of NO_2^- to NO by Fe^{2+} The polarogram of a mixture of Fe^{2+} and Fe^{3+} in the presence of excess citrate is of reversible character involving one-electron transfer (4, 5). To follow the reduction of NO_2^- to NO by Fe^{2+} in buffer solutions containing sufficient citrate, the increase of Fe^{3+} reduction wave with time may be utilized, though NO_2^- shows no reduction wave at DME in neutral solutions (Fig. 1 a). When a small amount of FeCl_2 crystals was added to the deoxygenated citrate-phosphate buffer containing excess KNO_2 , the oxidation of Fe^{2+} to Fe^{3+} was observed and at the same time relatively complicated cathodic waves appeared (Fig. 1 b). The newly appearing reduction waves were found to be due to the NO formed from NO_2^- , since same waves were obtained when NO gas of 95% purity (purchasable for GC standard) was bubbled in the same buffer solution containing ferric ions (Fig. 2). As the solubility of NO in aqueous solution is small, the reduction waves completely disappeared, when N_2 gas was bubbled through the solution for

Fig. 1 Polarograms (a) before and (b) after the addition of Fe^{2+} to excess NO_2^- in McIlvaine buffer pH 7 at 25° KNO_2 ; 0.1M, FeCl_2 ; $1.7 \times 10^{-4}\text{M}$.

Fig. 2 Polarograms (a) before and (b) after the addition of Fe^{3+} to buffer pH 7 containing 0.1M KNO_2 and dissolved NO. FeCl_3 added; ca. $1 \times 10^{-3}\text{M}$.

a few minutes. In contradistinction, when N_2O gas was also bubbled in the same buffer solution, no reduction waves were observed, irrespective of the presence of iron ions. The half-wave potentials of the NO waves were -0.56 , -0.75 and possibly -1.10V , respectively, but the last wave was not clearly observed because of a large maximum as seen in Figs. 1 and 2. When gelatin was added, the first and the second waves disappeared but the maximum remained almost unchanged.

The increase of the reduction waves due to NO and that of Fe^{3+} wave with time were followed as presented in Fig. 3. It was revealed that the NO reduction waves cannot be ascribed to direct discharge of the NO formed stoichiometrically at the expense of Fe^{2+} , since the limiting current of each wave is very large compared with that of the Fe^{3+} wave at any reaction time; if we assume diffusion current for each limiting current, ignoring the difference of diffusion coefficient between Fe^{3+} and NO, the number of transferred electrons for the first wave should be more than 4 at the early reaction time. Also the time course of NO wave formation was different in shape from that for Fe^{3+} formation.

Fig. 3 Time course of formation of NO waves and Fe^{3+} wave. Reaction conditions were the same as for Fig. 1. Scale on the right ordinate is only for peak current.

Characteristics of NO waves in the presence of iron ions and citrate.

To obtain the NO concentration dependence of the NO reduction waves, we utilized the limiting current of Fe^{3+} wave as the value proportional to the concentration of NO formed by the reaction of NO_2^- with Fe^{2+} . Each limiting current of the first and the second waves and the total current at the

peak of maximum were plotted against the limiting current of Fe^{3+} wave during the reaction time. Figure 4 a reminds us of Langmuir's adsorption isotherm, if we may describe the relation as

Fig. 4 Relations between NO wave heights and diffusion current of Fe^{3+} formed in NO_2^- reduction by Fe^{2+} . In Fig. (a) scale on the right ordinate is only for peak current.

$$I_l = \frac{ab I_{d, \text{Fe}}}{1 + b I_{d, \text{Fe}}} \quad (1)$$

where I_l and $I_{d, \text{Fe}}$ are limiting current of NO wave and the diffusion current of Fe^{3+} wave, respectively, and a and b are constants.

The relation (1) can be written as

$$\frac{I_{d, \text{Fe}}}{I_l} = \frac{1}{ab} + \frac{I_{d, \text{Fe}}}{a} \quad (2)$$

This relation was verified by obtaining the linear relations presented in Fig. 4 b.

The dependence of each limiting current of NO waves on the mercury head was as seen in Fig. 5. The first and the second waves seems to be mainly kinetically controlled, whereas the peak current of the maximum suggests to involve the character of diffusion controlled wave. Also the shape of the waves changed with pH. In citrate buffer pH 9, the first and the second waves disappeared and the maximum sharpened (Fig. 6).

Consideration on the electrode reaction of the NO waves

In comparison of the limiting current of each NO wave with that of Fe^{3+} wave, the total wave should involve more than 10 transferred electrons, if simple diffusion current were assumed for each of the waves. This is, however, difficult to consider, since the reduction of NO even to H_2NOH requires only 3 electrons. Further, the first and the second waves were found to be mainly kinetically controlled. The NO concentration dependence of each wave took a form of adsorption isotherm. From these results, the NO reduction waves may be most probably ascribed to catalytic current similar to Brdicka's protein waves, though the possibility of direct discharge of NO at DME would not be completely excluded for the third wave.

Fig. 5 The dependence of each limiting current of NO waves on mercury level.

Mercury levels: (a) 75 cm, (b) 60 cm, (c) 45 cm, (d) 30 cm.

Fig. 6 Polarogram of NO wave taken in citrate buffer pH 9.

Reaction conditions other than pH were the same as for Fig. 1.

For the interpretation of the catalytic current due to NO in the presence of iron ions and citrate in neutral buffer solutions, it may be worthwhile to consider its polarographic characteristics in comparison with those of other related compounds. Kolthoff and Toren (6) and Masěk (7) reported three reduction waves of nitroprusside in buffer solutions which involved some anomalies and they interpreted each wave as kinetic or diffusion current. Also Silvestroni and Ceciarelli described similar polarographic behavior of NO in the presence of excess cobaltbisthistidine and ascribed the phenomenon to the reduction of NO as a complex with cobaltbisthistidine (8). The present NO waves resemble the above nitroprusside waves and cobaltbisthistidine complex waves in many points except the large maximum, though none of the above authors described the catalytic waves of the compounds. In the present case, therefore, we may consider some ternary complex of NO, iron ions, and citrate, which may catalyze the hydrogen discharge at DME, though

no definite numbers of co-ordination of the ligands are available.

As for the iron ions, either ferric or ferrous ions may afford to exhibit the catalytic waves under the present experimental conditions, but no experiments strictly without Fe^{3+} could be carried out.

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中性緩衝液中で二価鉄による亜硝酸塩の一酸化窒素への還元のパールログラフ的証拠および、鉄イオンとクエン酸塩の存在下での一酸化窒素の陰極波の特徴

小松泰彦，山口益郎，高木正之助

大阪府立大学農学部農芸化学科（堺市百舌鳥梅町 〒591）

過剰のクエン酸を含む中性緩衝液中で Fe^{2+} によって NO_2^- が NO へ還元されることをパールログラフ的に証明した。一酸化窒素は鉄イオンとクエン酸の存在下で 3 陰極波を示し、その第1波は特徴のある極大波を示した。第2波 ($E_{1/2} = -0.56$ および -0.75 V vs SCE , pH 7) は反応電流であるが、第3波 ($E_{1/2} = -1.10 \text{ V}$) は極大を持ち拡散波である。第3波の限界電流の NO 濃度依存性は Langmuir の吸着等温式の型の関係を示した。