Chronopotentiometric and Polarographic Behavior of Complex of Ferric Ion with Flavinmononucleotide

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It has been reported in many publications that some metal ions form complexes with riboflavin and its related compounds.¹⁻⁶⁾ Chemical analysis, titration, spectrophotometry, fluorometry, ion-exchange technique and other methods were used in those studies. Recent polarographic studies have shown the formation of complex of mercury ion with riboflavin and flavinmononucleotide (FMN)⁷⁾.

In the present study, the author found that ferric ion forms a stable complex with FMN in a neutral medium containing oxalate and that two ferric ions combine with one FMN molecule. There appeared in polarogram a cathodic wave of kinetic nature due to the reduction of Fe^{3+} ion liberated from Fe^{3+} -FMN complex at the electrode surface. Chronopotentiometric and polarographic behavior of the complex of ferric ion with FMN will be described and discussed.

Experimental

The apparatus used and experimental conditions for chronopotentiometry were the same as reported previously⁸⁾. Polarographic measurements were carried out by use of Polarograph PA102 (YANAGIMOTO MFG. Co., Ltd.) with a DME, the characteristic of which was: $m=0.96$ mg/sec., $t=10.1$ sec. in distilled water at open circuit. Basal solution (pH=6.9) was 0.1M sodium oxalate + 0.05M alkali biphosphate buffer solution ($\text{H}_2\text{KPO}_4 + \text{HNa}_2\text{PO}_4$). All measurements were carried out at room temperature (26°C). Reference electrode used was Ag-AgCl in 0.1M KCl for chronopotentiometry and SCE for polarography.

The reason why oxalate medium was used as basal solution in the present study is that Fe^{3+} is reduced at more positive potential than FMN in neutral oxalate medium and the wave of Fe^{3+} is isolated from that of FMN with a potential difference enough to analyze the voltammetric curves.

Experimental Results and Discussion

As shown in Fig. 1, the system in which 10^{-3} M Fe^{3+} and 10^{-3} M FMN are present in the oxalate medium gives two waves, *i.e.* (b) and (c), the total height of which is equal to the sum of height of (a) and (d). The wave (c), $E_{1/2}$ of which is close to that of (a), shows the character of kinetic current as described below. The wave height of (c) was independent of mercury reservoir height between 80 and 40 cm, and temperature coefficient of the wave height was about 10%/deg., while that of (b)+(c) was about 1.6 to 2%/deg., and $(\bar{i}_{b+c} - \bar{i}_d) \times t^{1/2}$ was constant, *i.e.* $(\bar{i}_{b+c} - \bar{i}_d)$ is diffusion-controlled (t , drop time of DME) (refer to Table I). Thus it is considered that Fe^{3+} forms a stable complex with FMN, the reduc-

Fig. 1. Polarograms of Fe^{3+} and FMN, and of the mixture of these two.

Table 1. The relationship between drop-time of DME and limiting current of (b)+(c), (d) and (b)+(c)-(d).

t sec $\frac{1}{2}$	$i_{b+c} \times t^{\frac{1}{2}}$ $\mu\text{A} \cdot \text{sec}^{\frac{1}{2}}$	$i_d \times t^{\frac{1}{2}}$ $\mu\text{A} \cdot \text{sec}^{\frac{1}{2}}$	$(i_{b+c} - i_d) \times t^{\frac{1}{2}}$ $\mu\text{A} \cdot \text{sec}^{\frac{1}{2}}$
1.79	9.45	5.75	3.71
1.90	9.46	5.76	3.71
2.07	9.56	5.84	3.73
2.28	9.60	5.88	3.72
2.53	9.49	5.78	3.72

tion potential of which is very close to that of FMN, and (c) is the reduction wave of Fe^{3+} liberated from the complex in chemical reaction: $\text{Fe}^{3+} - \text{FMN} \rightarrow \text{Fe}^{3+} + \text{FMN}$. This is more clearly verified by the fact that the value of $i_c/(i_{b+c} - i_d)$ is directly proportional to square root of t as shown in Fig. 2⁹⁾.

It has been shown in chronopotentiometry that the value of $i_0\tau/nF$ corresponds to the density of adsorbed FMN molecules on the surface of mercury electrode when the concentration of FMN in the bulk is small (i_0 , current density; τ , transition time).⁸⁾ It may be predicted from the polarographic behavior of the system that the concentration of free FMN is negligibly small compared with that of FMN combined with Fe^{3+} when the concentration of Fe^{3+} is appreciably higher than FMN in the bulk. Consequently, the value of $i_0\tau/nF$ for the system should correspond to the density of the adsorbed FMN plus the density of Fe^{3+} combined with the adsorbed FMN. Let's have $\tau_{\text{FMN}+\text{Fe}^{3+}}$ for the system of $\text{FMN}+\text{Fe}^{3+}$ and τ_{FMN} for the system of FMN alone. Then, $i_0(\tau_{\text{FMN}+\text{Fe}^{3+}} - \tau_{\text{FMN}})/1 \times F$

Fig. 2. Variations of the ratio of the height of i_c and that of $i_{b+c}-i_d$ with square root of drop-time of DME. (Extrapolated line along the plot cuts the origin.

Fig. 3. Chronopotentiograms of FMN+ Fe^{3+} (a) and FMN (b). FMN, $1 \times 10^{-5} M$; Fe^{3+} , $1 \times 10^{-4} M$. Starting potential, $-0.1 V$ vs. Ag-AgCl in $0.1 M$ KCl; Current density, $6.69 \times 10^{-4} A/cm^2$.

Table 2. Comparison of the transition time for (a) and (b) in Fig. 2 at different current density.

Current density (A/cm^2)	Transition time (sec)	
	$1 \times 10^{-4} M$ $\text{Fe}^{3+} + 1 \times 10^{-5} M$ FMN	$1 \times 10^{-5} M$ FMN
1.44×10^{-3}	6.0×10^{-3}	3.0×10^{-3}
6.69×10^{-4}	1.4×10^{-2}	7.5×10^{-3}
4.49×10^{-4}	2.0×10^{-2}	1.1×10^{-2}
3.47×10^{-4}	2.7×10^{-2}	$1.3_5 \times 10^{-2}$
2.80×10^{-4}	$3.8_5 \times 10^{-2}$	1.9×10^{-2}

should correspond to the density of Fe^{3+} combined with FMN at the electrode surface, while $i_0 \tau_{\text{FMN}} / 2F$ corresponds to the density of FMN at the electrode surface as mentioned above. Therefore one has the following equation;

$$2 \times (\tau_{\text{FMN}+\text{Fe}^{3+}} - \tau_{\text{FMN}}) / \tau_{\text{FMN}} = \text{Number of mole of } \text{Fe}^{3+} \text{ combined with a mole of FMN} \quad (1)$$

As shown in Fig. 3 and Table 2, the values of transition time of (a) (Fe^{3+} —FMN) are twice

Fig. 4. Chronopotentiograms of anodic process.
(a) $1 \times 10^{-5} M$ FMN + $1 \times 10^{-4} M$ Fe^{3+} , (b) $1 \times 10^{-5} M$ FMN. Starting potential, $-0.6V$ vs. Ag-CgCl in $0.1 M$ KCl, Current density, $6.69 \times 10^{-4} A/cm^2$.

Fig. 5. Comparison of polarograms for the system of $Fe^{2+} + FMN$ and of $Fe^{3+} + FMN$.
(1) $1 \times 10^{-3} M$ Fe^{2+} ; (2) FMN added to (1); (3) After air-oxidation of (2). Concentration of FMN in (2) and (3) is approximately $2 \times 10^{-4} M$. Dotted line on each polarogram is galvazero line.

as large as those of (b) (free FMN), which means that two ferric ions combine with one FMN molecule.

Thus, for the system of $Fe^{3+} + FMN$ in neutral oxalate medium, the electrode process may be postulated as follows:

In Fig. 1, it is observed that the reduction wave of Fe^{3+} combined with FMN overlapped closely with that of FMN. This phenomenon may be naturally expected, since when FMN is reduced at the electrode, the resulting leuco-FMN functions as electron donor to Fe^{3+} combined with FMN, and consequently the reduction of FMN and the combined Fe^{3+} occur simultaneously. When the starting potential is set at the potential in the region of diffusion current of FMN, the adsorbed species (both FMN and Fe) are to be in the reduced form and the anodic process may be observed by applying anodic current. Chronopotentiometric curves of the anodic process show only a slight difference of τ between the system of $Fe^{3+} + FMN$ and of FMN alone as shown in Fig. 4, which means that Fe^{2+} -leuco-FMN complex is not so

stable as Fe^{3+} -FMN. It was also found in polarography that Fe^{2+} does not combine with FMN so strongly as Fe^{3+} as shown in Fig. 5. Namely, after air oxidation of Fe^{2+} to

Fe^{3+} , the wave height of (a) decreases, while that of (b) increases.

In oxalate medium Fe^{3+} is apparently present as oxalate complex but it is not known whether Fe^{3+} combines with oxalate when Fe^{3+} -FMN is formed. But oxalate interacts with FMN and catalyzes photochemical reduction of FMN, and therefore Fe^{3+} -FMN feasibly carries oxalate with Fe^{3+} and/or FMN. The structure of Fe^{3+} -FMN complex can not be stated at present.

Summary

The system of Fe^{3+} +FMN in neutral oxalate medium was studied. It was found that two ferric ions combine with one FMN molecule, and that the system produces a kinetic current wave of Fe^{3+} liberated from Fe^{3+} -FMN complex.

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Literature

- 1) A. Albert, *Biochem. J.*, **54**, 646 (1953).
- 2) W.O. Foye and W.E. Lange, *J. Am. Chem. Soc.*, **76**, 2199 (1954).
- 3) W.J. Rutter, *Acta chem. scandinavica*, **12**, 438 (1958).
- 4) E. Walaas, *ibid.*, **12**, 528 (1958).
- 5) I.F. Baarda and DE.. Metzler, *Biochem. Biophys. Acta*, **50**, 463 (1961).
- 6)
- 7) B. Breyer and T. Biegler, *Collection Czech.Chem. Commun.*, **25**, 3348 (1960); M. Senda, M. Senda and I. Tachi, *This Review*, **10**, 149 (1962); M. Maruyama, T. Eguchi and M. Nishiyama, paper read at the 9th annual meeting of the Society of Analytical Chemistry of Japan (1960).
- 8) Y. Takemori, *This Review*, **12**, 63 (1964).
- 9) M. Senda, *This Review*, **6**, 95 (1958).

第二鉄イオン—フラビンモノニュクレチド錯塩のクロノポテンシオ メトリーおよびポーラログラフ的挙動

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シュウ酸塩を含む中性媒質 ($\text{pH}=6.9$) 中で等濃度の FMN および Fe^{3+} が共存する系のポーラログラムを記録すると, Fe^{3+} -FMN 錯塩 $\rightarrow \text{Fe}^{3+}$ +FMN なる解離反応により生じる遊離の Fe^{3+} の還元による kinetic current が現われる, ポーラログラフ的挙動から, 上記反応系は平衡状態では左にかたよっていると考えられ, 従って Fe^{3+} の濃度を FMN に比して大過剰に共

存させれば, 上記反応系において FMN は事実上すべて Fe^{3+} -FMN 錯塩として存在するであろう. 著者は前報⁹⁾の結果を応用し, クロノポテンシオメトリーにより Fe^{3+} イオン 2 個が FMN 分子 1 個と結合することおよび Fe^{2+} と Leuco-FMN の結合は上記の酸化系程安定ではないと考えられる結果を得た.