The Studies on Brdicka's Catalytic Hydrogen-Wave

IV. The Role of Amino and Carboxyl Groups on the Catalytic Hydrogen-Waves.

by

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Introduction

It must be naturally considered that the nature of the catalytic hydrogen wave may depend on the structure of complexant forming with cobalt and some active groups in catalyst. It is probable that the sulfhydryl or disulfide group has a stronger tendency to react with cobalt than the amino group or the carboxyl group. The important role of sulfhydryl group was elucidated skilfully by Hata¹⁾. It is a well known fact that most compounds containing sulfhydryl or disulfide group and amino group and/or carboxyl group give the catalytic effect in cobaltous or cobaltic solution²⁾.

Shinagawa et al.³⁾ studied on the possibility of the Bis-cysteinate diaquacobaltate(III)⁴⁾ as a catalytically active complex. The complex compound was considered to form by coordination of nitrogen in amino group and sulfur atom in sulfhydryl group to trivalent cobalt.

Although several amino acids without sulfhydryl group have also been found to give catalytic effect in cobaltous or nickelous solution, these waves have quite different patterns from those of cysteine or cystine.

In this study, in order to learn more of the relation between the role of active group in the compound and the catalytic effect, L-cystine ($-\text{NH}_2$, $-\text{S}-\text{S}-$, and $-\text{COOH}$, i.e., three active groups), N,N'-diacetyl-L-cystine ($-\text{NHCOCH}_3$, $-\text{S}-\text{S}-$ and $-\text{COOH}$, i.e., defect of $-\text{NH}_2$) and L-cystine-diethylester ($-\text{NH}_2$, $-\text{S}-\text{S}-$ and $-\text{COOC}_2\text{H}_5$, i.e., defect of $-\text{COOH}$) were selected and studied in both divalent and trivalent cobalt solution. Cystine-diethylester containing two amino and a disulfide group gave a catalytic wave in both divalent and trivalent cobalt solution, but no catalytic wave was seen with diacetyl-L-cystine containing two carboxyl and a disulfide group. As for the catalytic hydrogen wave of cystine, it was found that amino group played an important role as well as sulfhydryl or disulfide group.

Experimental

A Yanagimoto D.C. and A.C. polarograph Model PA-102 was used for this study. Characteristics of the capillary used; $m=1.83 \text{ mg. sec}^{-1}$, $\tau=3.02 \text{ sec.}$ at -1.0 V. , $h=57 \text{ cm.}$ in the solution containing $0.1M \text{ NH}_3$ and $0.1M \text{ NH}_4\text{Cl}$. Pure nitrogen gas, which had been bubbled through a solution of $0.1M \text{ NH}_3$ and $0.1M \text{ NH}_4\text{Cl}$, was used to remove dissolved

oxygen from the electrolytic solution before running polarograms. Potentials were measured against a saturated calomel electrode by connecting with salt bridge having a low resistance (230 ohm). All polarograms were recorded at temperature of $25 \pm 0.2^\circ\text{C}$.

L-Cystine, $(-\text{SCH}_2\text{CH}\cdot\text{NH}_2\text{COOH})_2$, purified by Merck and Co., Inc., Rahway, N.J. was used to prepare the stock solution in 0.1M HCl. L-Cystine diethylester, $(-\text{S}\cdot\text{CH}_2\text{CH}\cdot\text{NH}_2\cdot\text{COOC}_2\text{H}_5)_2$, was synthesized from L-cystine described above and N,N'-diacetyl L-cystine, $(-\text{S}\cdot\text{CH}_2\text{CH}\cdot\text{NHCOCH}_3\cdot\text{COOH})_2$, was presented by Dr. T. Ikenaka. These synthesized cystine derivatives were identified as L-cystine diethylester and N,N'-diacetyl-L-cystine by the method of Infrad Red Spectrometry. All chemicals used were of reagent grade.

Results and Discussion

Polarography of the Cystine Derivatives in the Solution Containing 0.1M Ammonia and 0.1M Ammonium Chloride. For the study on the mechanism of Brdicka's catalytic hydrogen wave, most papers have treated the catalytic effect which is observed in the range of -0.8V . to

Fig. 1. D.C. and A.C. polarographic waves of cystine, diacetyl-cystine and cystine diethylester in the solution of 0.1M NH_3 and 0.1M NH_4Cl . Polarograms A : 0.2 mM cystine diethylester by D.C. polarograph, and A' by A.C. polarograph. B : 0.2 mM diacetyl cystine by D.C. polarograph and B' by A.C. polarograph. C : 0.2 mM cystine by D.C. polarograph and C' by A.C. polarograph.

—2.0V. However it has been already known that the reaction of sulfhydryl or disulfide group with mercury electrode shows a polarographic wave at a more positive potential than that for the catalytic wave.

D.C. and A.C. polarographic waves of cystine and its derivatives in the solution of 0.1 M NH_3 and 0.1M NH_4Cl are illustrated in Fig. 1. The half-wave potentials of the first waves of cystine, acetyl cystine and cystine ester were -0.57 V. , -0.59 V. , and -0.65 V. , respectively. The wave-heights of these compounds at the concentration of 0.2 mM were $0.16\text{ }\mu\text{A}$ for cystine, $0.27\text{ }\mu\text{A}$ for acetyl cystine, and $0.97\text{ }\mu\text{A}$ for cystine ester, respectively. The second reduction waves of cystine and the two derivatives had drawn out forms. The relation between the first wave-heights and the concentrations were shown in Fig. 2. The results obtained with diacetyl cystine and cystine ester did not show a linear relation as well as those obtained with cystine. It may be considered that cystine derivatives also

Fig. 2. The relation between the height of the prewave and the concentration of cystine, diacetyl cystine and cystine diethylester in the solution of 0.1M NH_3 and 0.1M NH_4Cl .

—●— : Cystine
 —◐— : Diacetyl cystine
 —○— : Cystine diethylester

Fig. 3. The D.C. and A.C. polarographic waves of cystine-diethylester in $0.1M$ NH_3 and $0.1M$ NH_4Cl .
Cystine diethylester : $0.4 mM$ in $0.1M$ NH_3 and $0.1M$ NH_4Cl .

Fig. 4. The influence of the concentration of ammonia on the half-wave potential and the reduction current of prewave. The electrolytic solution, $0.1M$ NH_3 and $0.1M$ NH_4Cl ; the concentration of cystine, acetyl cystine and cystine-ethylester, $0.2 mM$.
(A) —●— : Cystine, (B) —○— : Diacetyl cystine, (C) —○— : Cystine diethylester. Solid line ; the reduction current of prewave and dotted line ; half-wave potential of prewave.

react with mercury electrode and their waves correspond to the prewave of cystine.

The half-wave potentials of cystine and its derivatives shifted to more negative value with increasing concentrations. The values were $-0.55V$. to $-0.58V$. for cystine, $-0.59V$. to $-0.61V$. for acetyl cystine, and $-0.65V$. to $-0.66V$. for cystine ester. These values of the half-wave potentials were closed to one another and indicate the similarity due to the groups containing sulfur and at the same time the slight differences due to different groups involved. The A.C. polarographic waves of cystine and the derivatives showed different potentials, such as $-0.59V$., $-0.61V$. and $-0.69V$. for cystine, diacetyl cystine and cystine ester, respectively.

The polarographic patterns of cystine and acetyl cystine with increasing concentration showed a behavior similar to each other, but cystine ester gave a rounded maximum wave which

followed by the first wave. The D.C. and A.C. polarographic waves are illustrated in Fig. 3. The wave-height increased with increasing concentration of cystine ester, but began to decrease at the higher concentration. The height of A.C. polarographic wave of cystine, acetyl cystine and cystine ester at the concentration of 0.2 mM showed 53 μ mho (0.79 μ A), 52 μ ohm (0.78 μ A) and 284 μ ohm (4.26 μ A), respectively. It is considered that the first

Fig. 5. The D.C. and A.C. polarographic waves of cystine, diacetyl cystine and cystine diethylester in ammoniacal divalent cobalt solution.
The electrolytic solution: 1 mM CoCl_2 in 0.1 M NH_3 and 0.1 M NH_4Cl .
I. D.C. polarograms: (A) 6×10^{-6} M cystine, (B) 6×10^{-6} M diacetyl cystine and (C) 6×10^{-6} M cystine diethylester.
II. A.C. polarograms: (A) 1×10^{-5} M cystine, (B) 1×10^{-5} M diacetyl cystine and (C) 1×10^{-5} M cystine diethylester.

Fig. 6. The D.C. and A.C. polarographic waves of diacetyl cystine in ammoniacal divalent cobalt solution.

The electrolytic solution : $1mM$ $CoCl_2$ in $0.1M$ NH_3 and $0.1M$ NH_4Cl .
D.C. polarograms : (A) and (A') $8 \times 10^{-5} M$ diacetyl cystine (C) and (C') $2 \times 10^{-4} M$ diacetyl cystine.
A.C. polarograms : (B) and (B') $8 \times 10^{-5} M$ diacetyl cystine, (D) and (D') $2 \times 10^{-4} M$ diacetyl cystine.

Fig. 7. The relation between the wave heights and the concentrations of cystine, diacetyl cystine and cystine diethylester.

The electrolytic solution : $1mM$ $CoCl_2$ in $0.1M$ NH_4Cl
—●— : Cystine, —○— : Diacetyl cystine,
—○— : Cystine diethylester.

wave is due to the adsorption of cystine ester at the mercury electrode and a rapid reduction to cystine ester.

In order to know the influence of variation of ammonia concentration, it was changed from $0.01M$ to $0.1M$. The behavior of the first wave observed is illustrated in Fig. 4. The half-wave potentials of the first wave shifted to more negative value with increasing concentration of ammonia and the wave height of cystine decreased with increasing concentration of ammonia, but the heights of the derivatives decreased at about $0.1M$ of ammonia.

Polarography of Diacetyl cystine and Cystine Diethylester in the Solution of Ammoniacal Ammonium Chloride Containing Divalent Cobalt or Trivalent Cobalt. Although the catalytic wave of cystine in ammoniacal divalent cobalt solution has been satisfactorily studied by several authors, the comparison of cystine with its derivatives, such as acetyl cystine and cystine ester, has not been known.

The cystine ester in the ammoniacal divalent cobalt solution gave a wave similar to catalytic wave shown by cystine, but acetyl cystine showed no catalytic effect. These D.C.

and A.C. polarographic waves are illustrated in Fig. 5-I and II.

The A.C. polarographic waves revealed that a characteristic maximum wave of cobalt in the presence of cystine, cystine ester and acetyl cystine consisted of two reduction steps. The A.C. polarograms at the concentration of $1 \times 10^{-5}M$ are illustrated in Fig. 5-II. Since acetyl cystine did not show any catalytic wave at the same concentration as cystine or cystine ester did, higher concentrations were examined. The D.C. and A.C. polarograms are illustrated in Fig. 6. The polarograms of acetyl cystine with increasing concentration showed decrease of the maximum wave-height of cobalt (wave III). On the other hand, an ill defined wave (wave II, $E_{1/2} = -1.05V.$) and another reduction wave at more negative potential (-1.6 to $-1.8V.$) appeared. The A.C. polarographic wave appeared as peak waves corresponding to the D.C. polarographic waves, and the nature of wave I was considered as adsorption effect of acetyl cystine at the mercury electrode.

Fig. 8. The A.C. polarograms of cystine, diacetyl cystine, and cystine diethylester in ammoniacal divalent cobalt solution. The electrolytic solution: $1 mM$ $CoCl_2$ in $0.1M$ NH_3 and $0.1M$ NH_4Cl (A) $5 \times 10^{-5}M$ cystine, (B) $5 \times 10^{-5}M$ diacetyl cystine, (C) $5 \times 10^{-5}M$ cystine diethylester and (D) $1 mM$ $CoCl_2$.

The relations between the wave-heights and the concentration of cystine and the derivatives are illustrated in Fig. 7. The heights of catalytic waves of cystine and cystine ester showed adsorption curves. The heights of maximum waves of cobalt decreased, and wave IV of acetyl cystine

began linearly to increase from $4 \times 10^{-5} M$, showing the simple process of the reduction of this compound.

In order to learn more of the behavior of cobalt-maximum wave in D.C. polarographic waves, the A.C. polarographic waves at the concentration of $5 \times 10^{-5} M$ were taken and compared with those of cystine and its derivatives. As illustrated in Fig. 8, it has been found in A.C. polarograms that the reduction wave of cobalt in the D.C. polarograms which accompanied a rounded maximum wave, consisted of two reduction steps; the peak potentials were $-1.1V$. with cystine, $-1.02V$. with acetyl cystine and $-1.08V$. with cystine ester, respectively. These reduction waves were considered as the reduction of complex of cobalt with cystine or its derivative.

In spite of the fact the reduction waves of the complexes of cobalt with cystine or its derivatives appeared similarly, it is notable that only diacetyl cystine does not show any catalytic effect. A species of complexant forming with cobalt and some active groups in cystine or the derivatives seems to be considered as a important factor for the origin of the catalytic hydrogen wave. Therefore, the origin of the catalytic wave shown by cystine in divalent cobalt solution may be understood as a cooperative activity of amino and disulfide group, and this suggests the possibility of the existence of Bis-cysteinate diaquocobaltate (III) as the catalytically active complex described by Shinagawa et al.³⁾

On the A.C. polarographic waves illustrated in Fig. 8 the nature of a sharp minimum designated as wave III in cystine or cystine ester was not explained. The maximum peak (Wave IV) and the minimum peak (Wave V) corresponding to the ascending and descending parts in catalytic wave were shown, i.e., it seemed to be a differential patterns of the catalytic wave.

In the solution of ammoniacal ammonium chloride containing hexammine cobaltic

Fig. 9. The polarographic waves of cystine, diacetyl cystine, and cystine diethylester in ammoniacal trivalent cobaltic solution. The electrolytic solution : $1 mM Co(NH_3)_6Cl_3$ in $0.1 mM NH_3$ and $0.1 M NH_4Cl$. (A) $0.6 mM$ cystine, (B) $0.6 mM$ diacetyl cystine and (C) $0.6 mM$ cystine diethylester.

Fig. 10. The relation between the height of the catalytic waves of cystine diethylester and the concentration of ammoniacal trivalent cobalt solution.

The electrolytic solution : 1 mM $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ in 0.1M NH_3 and 0.1M NH_4Cl .

chloride, the behavior of cystine was described in previous paper³⁾. The cystine ester gave a wave similar to that of cystine, but acetyl cystine did not give catalytic effect. These polarographic waves were illustrated in Fig. 9. Both cystine and cystine ester showed two rounded maximum waves in the range of -1.2V. to -1.8V. , and cystine ester showed a different pattern from that of cystine before -1.2V. The relation between the height of two maximum waves and the concentration of cystine ester were illustrated in Fig. 10. The heights of the two maximum waves showed adsorption curve with increasing concentration of cystine ester. The relation between the height of the first maximum wave (i_{p1}) and the second maximum wave (i_{p2}) showed a result similar to "crossing effect of protein" described by Tropp⁶⁾, but the phenomenon was not given by cystine.

From the results described above, it was found that the origin of catalytic wave of cystine in trivalent cobalt solution

depended on the cooperative role of disulfide and amino groups contained in the catalyst.

The relation between the structure of complex of cobalt with cystine or the derivatives and the catalytic action, the difference in adsorption at mercury electrode between cystine and the derivatives, which is considered from the influence of amino, acetyl, carboxyl and ethylester groups on a disulfide group, must be considered as the leading factors of the problem in future.

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Brdicka 接触水素波に関する研究〔IV〕

接触水素波における $-\text{NH}_2$, $-\text{COOH}$ 基の役割

砂 原 広 志

接触水素波を生じさせる化合物はその殆んどが $\text{SH}-$, $-\text{S}-\text{S}-$ 基をもち、その重要な役割は既知の如くである。しかしそれらの化合物とコバルトにより錯化合物が形成される場合の中心的役割を果す他の基については接触波出現との関係において詳しく論じられていない。

著者は $-\text{S}-\text{S}-$ 基以外の $-\text{NH}_2$, $-\text{COOH}$ 基の役割を明らかにするためにシスチンをえらび、 $-\text{S}-\text{S}-$ 基はそのままにして $-\text{NH}_2$ を $-\text{NHCOCH}_3$ にしたアセチル・シスチン、 $-\text{COOH}$ を $-\text{COOC}_2\text{H}_5$ にしたシスチンエステルを合成し、 Co(II) 又は Co(III) の塩化アンモニウム-アンモニア性溶液中でシスチン及びその誘導体の直流及び交流ポーラログラフ波を検討した結果を報告する。

上記三種の化合物は $-\text{S}-\text{S}-$ 基をもつ点は共通しており、 $0.1M \text{NH}_3 + 0.1M \text{NH}_4\text{Cl}$ 溶液中での水銀電極面への反応を考慮して実験をすすめた。直流及び交流ポーラログラフ波が第1図に示してある。シスチン及びその誘導体の 0.2 mM の同濃度を用いた所、特に交流ポーラログラフ波では水銀面への吸着による特異性がみられる。之れは $-\text{S}-\text{S}-$ 基のみならず $-\text{NH}_2$, $-\text{COOH}$ 基の影響も考慮される。第2図は上記三種の化合物の濃度増加と波高の関係で吸着的性格を示している。特にシスチンエステルは濃度増加により還元波も変化し第3図の如く極大波が生じる。上記三種化合物の還元波に対してアンモニアの濃度を変化させた場合、第4図にその結果が示してある。シスチン及び

その誘導体の第1波の $E_{1/2}$ はいずれもアンモニア濃度増加により負電位側に移行し、その波高は減じている。

Co(II) の塩化アンモニウム-アンモニア性溶液中ではシスチン及びその誘導体の直流及び交流ポーラログラフ波が第5図 I, II に示してある。シスチン及びシスチンエチルエステルは接触波を示すがアセチルシスチンは示さない。又交流ポーラログラムにおいてもアセチルシスチンは $-1.0V. \sim -1.2V.$ あたりに1つのピーク波を示すのみであり、 $-0.4V. \sim -0.5V.$ あたりの水銀面への吸着がみられる。更にアセチルシスチン高濃度の場合が第6図に示してある。コバルトの極大波高が減少し図中D'にみられる様に一段波から二段波となつてくることが交流ポーラログラムよりわかる。以上シスチン及びその誘導体の濃度変化による各波高の変化がまとめて第7図に示してある。

Co(III) の塩化アンモニウム-アンモニア性溶液中でのシスチン及びその誘導体の直流ポーラログラフ波が第9図に示してある。アセチルシスチンのみはやはり接触波を示さない。シスチンエチルエステルは、シスチンの場合よりも更に複雑な挙動を示している。又それら化合物の濃度変化による接触波波高の変化が第10図に示してあるごとく、シスチンエチルエステルは Tropp の報告したごとく蛋白波にみられた "Crossing Effect" を示した。以上の結果によりシスチンの場合の接触水素波の起因の1つとして $-\text{S}-\text{S}-$ 基と $-\text{NH}_2$ 基のコバルトに対する錯化学的役割が注目された。