*On the Model Form of the Catalytic Wave of Proteins**

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In order to extend the knowledge about the nature of the catalytic wave of protein[1,2] a model experiment was undertaken, and the following three points were emphasized in this experiment: 1) hydrogen sulfide and cystine were used as typical sulfhydryl compounds; 2) the influence of methyl cellulose and gelatin on the catalytic wave of cystine was examined polarographically; and 3) the influence of glucose, sucrose, glucosamine and glycogen on the cystine wave was similarly examined. In the latter case, these sugars were used as model compounds for the carbohydrates contained in the mucoprotein molecule, which is said to be an effective substance for the demonstration of the serum filtrate.

From such experiments, the various wave patterns analogous to those of the protein wave were obtained by regulating the amounts of each of the constituents in the test solutions (Table I).

Single Rounded Cystine-like Wave.—Hydrogen sulfide, which may be considered as one of the simplest of the sulfhydryl compounds, was examined polarographically over a large range of concentrations in the buffered cobaltous solutions, i.e. 0.1 M NH_4OH, 0.1 M NH_4Cl and 10^{-3} M $CoCl_2$. When hydrogen sulfide was added to the solution containing gelatin, the single rounded wave analogous to the cystine wave appeared and the diffusion current of cobalt disappeared. When hydrogen sulfide was added to the solution, and then gelatin was added, the single rounded wave did not appear. If the solution contains large amounts of gelatin, the wave analogous to that of cystine did not appear and the diffusion current of cobalt disappeared (Fig. 1A). This cystine-like wave was observed even at the limiting concentrations of hydrogen sulfide as low as 10^{-7} M.

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1) R. Brdicka, *Research* (*London*), 1, 25—35 (1948).
2) M. Brezina and P. Zuman, "Die Polarographie in der Medizin, Biochemie und Pharmazie", Akademische Verlagsgesellschaft, Leipzig (1956), pp. 532—618.

TABLE I. POLAROGRAPHIC EFFECTS OF GELATIN AND CARBOHYDRATES ON THE CATALYTIC WAVE OF HYDROGEN SULFIDE AND CYSTINE IN THE AMMONIACAL BUFFERED COBALTOUS SOLUTION

Case	Geratin	Carbo-hydrate	Sulfhydryl compound	Single wave (or 2nd wave), V.	Double wave	Unknown wave, V.
1	—	—	H	—	—	—
2	G	—	H	−1.6	—	−0.95
3	G	—	H	—	—	—
4	—	M	H	−1.6	—	−0.7
5	—	Gl	H	−1.6	—	−0.7
6	—	—	C	poorly defined	—	—
7	G	—	C	−1.75	—	−1.0
8	—	M	C	−1.75	well defined	−1.0
9	G	M	C	−1.75	well defined	−1.0
10	—	Gl	C	−1.75	poorly defined	−1.0
11	—	S	C	−1.75	poorly defined	−1.0
12	—	Gs	C	−1.75	poorly defined	−1.0
13	—	Gg	C	−1.75	poorly defined	−1.0

C: 10^{-3} M Cystine Gl: 0.1% Glucose M: 0.1% Methyl cellulose
G: 0.1% Gelatin Gs: 0.1% Glucosamine S: 0.1% Sucrose
Gg: 0.1% Glycogen H: Hydrogen sulfide

Protein-like Wave.—When methyl cellulose was added to the buffered cobaltous solution, the polarographic double wave similar to the protein double wave appeared as shown Fig. 1B. Other carbohydrates such as glucose, sucrose, glucosamine and glycogen were examined in order to determine whether their activities were similar to that of methyl cellulose on the formation of the wave analogous to the protein wave (Table I). Further quantitative experiments were initiated in order to examine the influence of methyl cellulose and gelatin on the cystine wave (Fig. 2).

It seems that Brdicka's mechanism[3], generally accepted since 1933, is unsatisfactory to explain these phenomena for the following reasons: 1) It might be difficult to supply hydrogen ions to the electrode surface from the bulk of an ammoniacal buffered solution at about pH 9. 2) At a potential of about −1.6 V. where the two peaks of the double wave appear, the cobalt-ammine ion is rapidly reduced to metallic cobalt at the electrode surface but the cobaltocysteinate ion may not be reduced to metallic cobalt. So the most delicate point in the interpretation of the whole catalytic electrode process involved in the polarographic "protein wave" consists in the elucidation of the role of cobalt. 3) Even if these waves depend on the catalytic reduction of hydrogen it is difficult to explain how, in the case of protein, two maxima are observed.

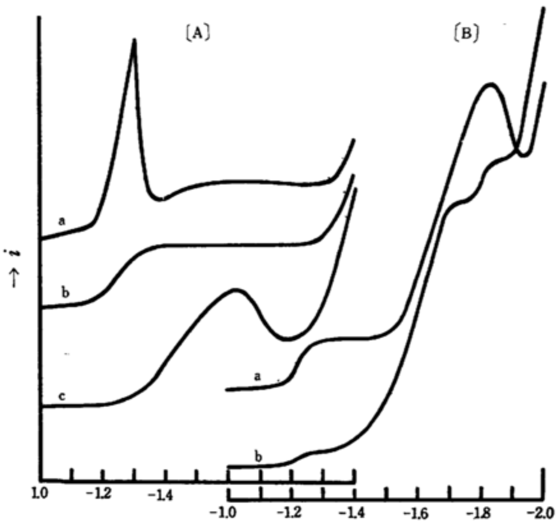

Fig. 1. Polarograms demonstrating the catalytic wave.
(A) a) $1\cdot10^{-2}$ M $CoCl_2$+1 M NH_4OH 1 ml. +1 M NH_4Cl 1 ml.+H_2O 7 ml.
b) (a)+1% gelatin 5 drops
c) (b)+H_2S
(B) a) (A, b)+$1\cdot10^{-2}$ M cystine 1 ml.
b) (a)+1% methyl cellulose 10 drops

However, the coexistence of constituents (cobalt ion, cystine, carbohydrate, gelatin and ammonia) in the ammoniacal buffered solution is necessary to obtain the wave analogous to the protein wave. In view of the above experimental facts, it seems reasonable to assume that these five essential constituents in the ammoniacal buffered

3) R. Brdicka, *Collection Czechoslov. Chem. Communs.*, 5, 148—164 (1933).

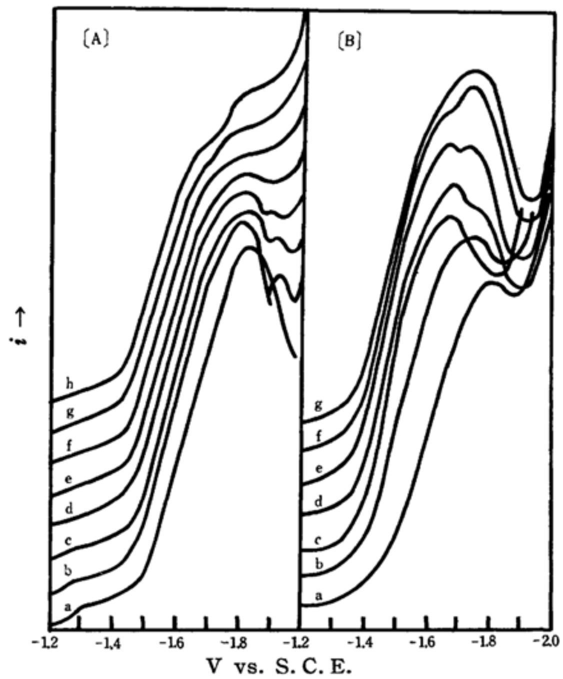

Fig. 2. Polarograms demonstrating the influence of methyl cellulose and gelatin on the cystine wave.

(A) 1% methyl cellulose 30 drops+10^{-2} M $CoCl_2$ 0.5 ml.+1 M NH_4OH 1 ml.+1 M NH_4Cl 1 ml.+H_2O: total volume 10 ml. $2\cdot10^{-2}$ M cystine:
a) 0.03 ml., b) 0.06 ml., c) 0.12 ml., d) 0.18 ml., e) 0.24 ml., f) 0.30 ml., g) 0.36 ml., h) 0.42 ml.

(B) a) $2\cdot10^{-2}$ M cystine 0.1 ml.+10^{-2} M $CoCl_2$ 0.5 ml.+1 M NH_4OH 1 ml.+ 1 M NH_4Cl 1 ml. + H_2O: total volume 10 ml.
b) (a)+1% methyl cellulose 1 drop
c) (a)+1% methyl cellulose 2 drops
d) (c)+1% gelatin 2 drops
e) (d)+1% gelatin 2 drops
f) (e)+1% gelatin 2 drops
g) (e)+1% gelatin 2 drops

system make a certain contribution to the electrode reaction either through their chemical interactions in the vicinity of the electrode surface and their adsorptive activities at the surface.

In conclusion, this mechanism may be considered in terms of the two following ideals: a) There may be some competition among the constituents which react with the cobaltous ions to yield complex ions or salts of extremely low solubility. Such competition in the vicinity of the electrode surface has a great effect on the currents of electroreduction and reoxidation of cobalt. b) The surface activities of these competing constituents have characteristic effects on the currents.

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