ELECTROANALYTICAL STUDIES OF COMPOUNDS OF BIOCHEMICAL IMPORTANCE

by

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LIST OF ABBREVIATIONS

ATP.....Adenosine Triphosphate

CoA.....Coenzyme A

$(CoAS)_2$.....Coenzyme A, oxidized

CoASH.....Coenzyme A, reduced

CSH.....Cysteine

CSSC.....Cystine

E.....Potential vs. saturated calomel electrode

$E_{1/4}$.....Quarter-wave potential

$E_{0.22}$.....Quarter-wave potential after current reversal

$E_{1/2}$.....Half-wave potential

E_p.....Peak potential

$Hg(CS)_2$.....Mercuric cysteinate

HgCS, $Hg_2(CS)_2$.....Mercurous cysteinate

i.....Current in μamps

i_d.....Diffusion current in μamps

i_o.....Current density

SCE.....Saturated calomel electrode

t.....Time in seconds

τ.....Transition time

GENERAL INTRODUCTION

The sulfur containing amino acids L-cysteine and L-cystine are of great importance in biological chemistry. These amino acids are important constituents of protein and function in some important oxidation/reduction reactions. Cysteine serves as a catalytic residue in the mechanism of action of certain enzymes. Cysteine and cystine can be interconverted both chemically and biologically by the oxidation/reduction reaction given below:

$$2CSH \rightarrow CSSC + 2\,H^{+} + 2\,e^{-} \qquad (1)$$

This reaction serves as the basis for explaining the function of these amino acids and compounds containing these amino acids in a number of important biological phenomena. Thus the formation of disulfide bonds from cysteine residues in a protein results in a covalent cross-linking between specific points in peptide chains. These disulfide bonds make a significant contribution to the three-dimensional structure of proteins. A number of enzymes such as papain are inactive if the cysteine residue at the active site is oxidized to a disulfide structure, but can be reversibly activated by reduction to the sulfhydryl form. Because of the importance of this reaction in these and other phenomena, it is important that the properties of this reaction be thoroughly understood. Consequently, in this laboratory it was felt that an investigation of the electrochemical properties of this reaction be undertaken.

In 1930 Heyrovsky and Babicka (13) reported on polarographic studies involving an albumin wave. Albumin is a protein containing

1.4% cystine and 1.0% cysteine (8). They observed a cathodic wave appearing at -1.6 V. (0.4 V. before the wave due to the deposition of sodium) and found the limiting current of the wave to be dependent upon the concentration of albumin in a solution which was 0.146 M in ammonium chloride and 0.02 M in lithium chloride. Albumin from the following substances gave positive results: serum, egg, and urine; flour of corn, barley, wheat, peas, beans, Brazil nuts, rice, and other unspecified grains; and liquids like milk and beer. None of the following substances produced the albumin wave: glycine, asparagine, cellulose, starches, lecithin, gelatin, sugars, fats, oils, high alcohols, and fatty acids.

These authors concluded that the phenomenon observed was caused by the binding of the ammonium ion to the albumin in an unspecified way, weakening the proton-ammonium bond which is illustrated by the following reaction:

$$NH_4^+ \rightarrow H^+ + NH_3 \qquad (2)$$

The wave was thought to be due to the albumin catalyzing the reduction of protons from the ammonium ion which the authors considered to be simply a complex of protons about the nitrogen atom.

While investigating the polarographic properties of cobalt salts in ammoniacal solutions, Brdicka (3) in an attempt to suppress a polarographic maximum added serum which contained albumin to cobalt-containing solutions, and observed an unusual phenomenon. The polarographic maximum of the cobalt deposition wave ($Co(II) \rightarrow Co^0$) entirely vanished,

and there appeared two waves (which he called protein waves) which are not a characteristic of serum solutions. From the data he obtained from a solution of 2.4 g./l. "blood albumin", 0.002 M $Co(NH_3)_6Cl_3$, 0.1 M ammonium chloride, and 0.1 M ammonia, an estimated value for the half-wave potential of the first wave is -1.35 V. vs. SCE, while that of the second wave is -1.6 V. vs. SCE. The slope of the first wave was almost perpendicular, while that of the second wave was quite drawn-out. The sum of the limiting currents of the two waves increased nonlinearly with increasing protein concentration. The current of the first wave approached a limiting value before the second wave was fully developed. At the highest concentration of "blood albumin" tested (2.4 g./l.), the ratio of the limiting currents of the second/first waves was 2/1. The addition of the serum displaced the deposition potential of the cobalt anodically by about 0.2 V. The use of Co(II) instead of the cobalt hexammine complex did not significantly alter the results. A linear dependency between the limiting current of the protein waves and both the cobalt ion concentration and the ammonium concentration was observed.

Protein waves were observed in solutions prepared from albumin, globulin, whey, egg white, and some varieties of wine-vinegar. The waves were absent in solutions prepared from gelatin, fermented vinegar, and aqueous extracts of silk. In an effort to find out what constituent of protein gave rise to these waves, glycine, asparagine, creatine, creatinine, arginine, leucine, and tyrosine were tested and found to give negative results. Cystine gave results identical to the waves which had been called "protein waves". The effect on the polaro-

graphic behavior by protein in the presence of Co(II) ions was concluded to be due to the sulfhydryl groups contained in the protein molecule (4). Both the sulfhydryl and the disulfide groups will produce protein waves since the disulfide will be reduced to the sulfhydryl form at this potential at the dropping mercury electrode.

The authors concluded that the protein wave effect on the polarographic behavior of Co(II) in aqueous ammonia could be explained as a catalysis of hydrogen evolution. In modern terminology the phenomenon described earlier as protein waves is called either "catalytic hydrogen waves" or "Brdicka waves". From a historical point of view, it has been of interest to include the discovery of catalytic hydrogen waves in this introduction, because their beginning was also the first polarographic study of cysteine/cystine. However, since catalytic hydrogen waves require a complex mixture of cysteine/cystine, ammonia, and Co(II), and are not a property of cystine or cysteine alone, they remain outside of the scope of the research here reported and will not be discussed further.

One of the difficulties in interpreting the polarographic behavior of sulfhydryl compounds is the complexity of the interaction of these compounds with the mercury surface of the dropping mercury electrode. In 1929, Michaelis, et al., (1) studied the reaction of cysteine with mercury. These investigators showed that when solutions of cysteine buffered at various pH values between pH 2 and pH 12 were shaken in the presence of air and metallic mercury, a white substance precipitated. Treatment of a solution containing the white precipitate with hydrogen sulfide gave the expected black precipitate of mercuric sulfide. The

white precipitate which was obtained by the interaction of mercury and cysteine was concluded to be mercuric cysteinate because of its similarity to the solid obtained by the addition of mercuric ion to cysteine.

These authors considered cysteine to be a mono-substituted derivative of hydrogen sulfide. By analogy, mercurous cysteinate was considered to be labile, transient product disproportionating according to:

$$Hg_2S \rightarrow Hg + HgS \quad (3)$$

$$Hg_2(CS)_2 \rightarrow Hg + Hg(CS)_2 \quad (4)$$

Michaelis observed that when a dilute solution of mercurous nitrate was added to a cysteine solution, a brown, cloudy precipitate formed which gradually faded away. The addition of mercurous nitrate to the above solution resulted in the slow appearance of white needles which they thought were probably mercuric cysteinate. The graying of the solution was attributed to the accumulation of colloidal metallic mercury. The authors interpreted the transient brown substance as being mercurous cysteinate, which decomposed yielding mercuric cysteinate and mercury.

The first polarographic study of the amino acid cysteine in the absence of catalytic wave producing conditions was done by Kolthoff and Barnum in 1940 (15). They found that the oxidation of cysteine by air at a mercury surface produced mercuric cysteinate. In 0.1 M perchloric acid, cysteine gave a broadened polarographic peak, rather than a

reversibly shaped wave. The plateau of the peak was interpreted as being due to diffusion since the limiting current was proportional to concentration over the range of 2.5×10^{-4} M to 2×10^{-3} M. The half-wave potential of this peak at pH = 1 was -0.05 V. vs. SCE. From the Ilkovic equation, the authors calculated that cysteine oxidation requires one electron per molecule at the dropping mercury electrode. Assuming one electron per molecule in the oxidation mechanism at the dropping mercury electrode, the diffusion coefficient for cysteine was calculated from the Ilkovic equation (3.1×10^{-5} cm.^{2}/sec.).

Another phenomenon which Kolthoff observed is what he termed the "false diffusion" plateau. The "false diffusion" plateau is a prewave which has also been attributed to adsorption (12) and therefore also called an "adsorption prewave". The current at the "false diffusion" plateau at pH values greater than pH 2 ($E_{\frac{1}{2}}$ = -0.2 V. vs. SCE at pH 6.0) was attributed to the formation of a film of mercurous cysteinate around the drop. This film interferes with further interaction between the cysteine and the mercury. The limiting current on the "false diffusion" plateau was independent of concentration above 0.00025 M in cysteine. At this concentration and below, the current is proportional to concentration and was called "true diffusion" current. The film of mercurous cysteinate was concluded to be soluble below pH 1 and above pH 9 because in these two pH ranges a diffusion wave was observed. Further evidence for mercurous cysteinate formation was obtained when a plot of log i_d-i/i vs. E for one polarogram at pH 1 yielded a slope of 56 mV. The slope obtained from a similar plot for a polarogram made from a similar solution (pH 13) is 49 mV. Apparently the latter case was ignored since

the value of 56 mV. was closer to the expected theoretical value of 59 mV. From this agreement and a constancy of half-wave potential, it was concluded that either ($RSH \rightleftarrows RS^{\bullet} + H^{+} + e^{-}$; $2\ RS^{\bullet} \rightarrow RSSR$) or ($2\ RSH + 2\ Hg \rightarrow (RSHg)_2 + 2\ H^{+} + 2\ e^{-}$) is the mechanism of cysteine oxidation on mercury. The solution after oxidation above a quiet mercury pool in the absence of oxygen at +0.1 V. vs. SCE at a pH which was not specified gave a positive test for mercury.

In 1955, Kolthoff, Stricks and Tanaka (18) continued their polarographic study of cysteine and obtained a better value (8.2×10^{-6} cm.^{2}/sec.) for the diffusion coefficient of cysteine. Dithiodiglycolic acid changed the apparent shape of the polarographic waves of cysteine at pH 4.9 from one of two ill-defined waves and an adsorption prewave to one well-defined oxidation wave. Dithiodiglycolic acid was thought to eliminate the mercurous cysteinate film which had been postulated to form on the surface of the mercury drop. From their observations, they calculated that the film at the mercury electrode was composed of a monomolecular layer of mercurous cysteinate.

In 1953 Grubner reported on the polarography of cysteine (12). The limiting current of the two waves of cysteine (pH 3.15) was found to increase with an increase in concentration, with that of the more anodic wave nonlinearly increasing faster. At pH 5.2, the half-wave potential of the more cathodic wave (the first wave) was -0.50 V. vs. SCE, while that of the more anodic wave (the second wave) was -0.41 V. vs. SCE. The composition of the supporting electrolyte was not specified; it was described only as being a buffer. The two anodic waves merged in the interval between pH 2 and pH 3, giving rise to a single anodic wave

below pH 2. The half-wave potential of the anodic wave below pH 2 was independent of concentration and changed "only a little" with pH. The half-wave potentials of both anodic waves moved cathodically with increasing pH in the amount of 60 mV./pH unit. The oxidation of cysteine at the dropping mercury electrode was concluded to be a one electron process because the limiting current of the anodic wave in alkaline medium (pH not stated) was about half the limiting current for the reduction of an equimolar concentration of cystine.

The behavior of the polarographic waves of cysteine upon the addition of metallic cations like Hg(II), Ag(I), Cu(I), and Cu(II), and similar cations was studied. These cations remove cysteine from the solution by forming slightly soluble compounds. The anodic wave decreased with the addition of these heavy metal ions. After oxidation of cysteine at a quiet mercury pool at an unspecified potential, no cystine was detected in the anode compartment.

In 1966 cysteine was studied chronopotentiometrically and polarographically in 1 M sulphuric acid at platinum electrodes by Davis and Bianco (6) producing a single drawn-out chronopotentiometric oxidation wave and one polarographic oxidation peak. In several different supporting electrolytes at various pH values, $i_o\tau^{1/2}/C$, decreased with increasing cysteine concentration. At "moderate current densities", $i_o\tau^{1/2}/C$ did not change with changing current densities at a given concentration of cysteine. However, at high current densities, the value of $i_o\tau^{1/2}/C$ was observed to decrease with increasing i_o values.

Controlled potential electrolysis of cysteine at +1.0 V. vs. SCE at a platinum electrode in 1 M sulphuric acid resulted in values for the

number of electrons, n, which varied between 0.8 and 1.6. This variability in n was thought to be due to the electrode reaction being suppressed by an oxide film which forms on the platinum surface. Cystine was identified by infrared analysis as the major oxidation product of cysteine on platinum. The chronopotentiogram of cysteine possessed a drawn-out slope which was ascribed to a kinetic reaction. This kinetic effect was thought to result from the deprotonation reaction prior to oxidation. At pH 5, the quarter-wave potential is +0.68 V. vs. SCE for the anodic wave. The slope of the straight line plot of log $(\tau^{\frac{1}{2}}-t^{\frac{1}{2}})$ vs. E gave an αn of 0.19. The authors assumed that the primary oxidation reaction which takes place is represented by the following equation:

$$CSH \rightarrow CS^{\bullet} + H^{+} + e^{-} \qquad (5)$$

As the result of these experiments they concluded that the oxidation of cysteine on platinum is electrochemically irreversible.

Sister et al., (29) studied the oxidation of thiophenol at the dropping mercury electrode. The polarogram of thiophenol below a concentration of 2×10^{-4} M, with 1 M sodium hydroxide as the supporting electrolyte, showed two waves, the first with a half-wave potential of -0.63 V. vs. SCE and the second wave with a half-wave potential of -0.45 V. vs. SCE. With increasing concentration, the limiting current of both waves increased, that of the second wave increased more rapidly than that of the first wave. In 5×10^{-4} M thiophenol, the value of $i/h^{\frac{1}{2}}$ was not constant, indicating the kinetic character of adsorption.