[CONTRIBUTION FROM THE INSTITUTE OF PHYSICAL CHEMISTRY, UNIVERSITY OF PADUA, AND THE COATES CHEMICAL LABORATORY, LOUISIANA STATE UNIVERSITY]

Kinetic Currents in the Polarographic Reduction of Cadmium Nitrilotriacetic Acid Complexes

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Kinetic currents for the reduction of cadmium nitrilotriacetic acid in acetate buffer are studied (a) for correction for formation of cadmium acetate complexes, and (b) for determination of the instability constant from oscillographic current–potential curves at high rates of potential change. The corrected rate constant for the forward reaction $CdX^- + H^+ = Cd^{++} + HX^{--}$ is $(1.5 \pm 0.3) \times 10^6$ mole^{-1} l. sec.$^{-1}$ at the ionic strength 3, and $(1.3 \pm 0.3) \times 10^6$ for $\mu = 0.3$. The minor effect of ionic strength variation and of the resulting change in double layer structure is consistent with the electrode reaction mechanism.

The electrochemical reduction of cadmium nitrilotriacetic acid (represented as H_3X) complexes in buffered solution was investigated by Koryta and Kössler[1] with the streaming mercury electrode and by Morinaga and Nomura[2] by conventional polarography. Observations can be summarized as follows. (a) With proper concentrations of reactants, a double wave is observed which corresponds to the reduction of uncomplexed cadmium (or complexed with the components of the buffer mixture) and CdX^-. (b) The limiting current for the first wave (dropping mercury electrode) is controlled in part by the rate of $CdX^- + H^+ = Cd^{++} + HX^{--}$; and the kinetics of this reaction were studied by polarography.[2] (c) The total current for the dropping mercury electrode is diffusion controlled, *e.g.*, the current is proportional to the square root of the head of mercury. (d) The limiting current for the first wave at the streaming mercury electrode is determined by the equilibrium for the foregoing reaction,[1] and the resulting equilibrium constant K_{CdX^-} is in agreement with the values obtained by Schwarzenbach and co-workers by potentiometry.[3]

The author had almost completed an investigation of this system at the time the paper of ref. 2 appeared. Details will not be given therefore but two points not previously considered will be discussed: (a) correction for complexation with the buffer (acetate); (b) application of oscillographic polarography to the determination of the equilibrium constant K_{CdX^-} from current–potential curves at high rates of potential change (up to 100 volts. sec.$^{-1}$).

Correction for Formation of Cadmium Acetate Complexes.—The rate constant k for dissociation of CdX^- to Cd^{++} in well-buffered solution is obtained by application of Koutecky's method as described by Morinaga and Nomura.[2] However, in the acetate buffer used by these authors and in this work correction must be made for formation of cadmium acetate complexes (studied by Leden).[4] Thus

$$k = 1.31 K_{CdX} \cdot K_{HX} \cdot C_{HX^{--}} \cdot C^{-2}_{H^+} \cdot a \left(\frac{h_1}{h_2}\right)^2 t^{-1} \quad (1)$$

where the h's are the wave heights, t the drop time and a the correction factor for the formation of acetate complexes. Equation 1 is the same as in ref. 2 except for the factor a and the term h_2. The latter is now the difference between the diffusion current, as obtained in presence of HX, and the limiting current for the first wave. This factor will now be discussed.

Reduction of cadmium acetate complexes is diffusion controlled under polarographic conditions as shown by a normal dependence of limiting current on the head of mercury. This can be interpreted either by assuming that the complexes are reduced directly or that dissociation of complexes is so rapid that dissociation is not rate determining. One can then assume, in the absence of another complexing agent, that the total concentration of cadmium aquocomplex ion is $\sum_0^p C_0 K_i$ where the equilibrium constant K_i includes the complexant concentration and the subscript zero corresponds to the aquocomplex. The equilibrium constant K_{CdX} is now changed by the factor $a = C_0 / \sum_0^p C_i K_i$. The only modification in the kinetic treatment is thus the introduction of the factor a in eq. 1, it being assumed that equilibrium is achieved for the acetate complexes.

Values of a at ionic strength of 3 were calculated from Leden's e.m.f. data.[4] Thus, $a = 2.0 \times 10^{-1}$, 3.3×10^{-2}, 1.6×10^{-3} for acetate concentrations of 0.1, 0.3 and 1 M, respectively. The factor a for 3 M acetate solution was determined from the shift of half-potential as the acetate concentration is changed from 1 to 3 M. Thus[5] at 25°

$$\Delta E_{1/2} = 0.0295 \log \frac{(C_{Cd^{++}})_1 \times D_3^{1/2}}{(C_{Cd^{++}})_3 \times D_1^{1/2}} \quad (2)$$

where 1 and 3 refer to the data for 1 and 3 M acetate. One has $\Delta E_{1/2} = 9.5$ millivolts and $(D_3/D_1)^{1/2} = 0.76$, *e.g.*, $a = 6.6 \times 10^{-4}$ for 3 M acetate.

Results are summarized in Table I for 3 different pH's. The average value $k = (1.5 \pm 0.3) \times 10^6$ mole^{-1} l. sec.$^{-1}$ is quoted. Similar determinations at the ionic strength of 0.3 yielded $k = (1.3 \pm 0.3) \times 10^6$ mole^{-1} l. sec.$^{-1}$. The influence of ionic strength is quite small and cannot be attributed to the effect of the change in the double layer structure in the sense discussed by Delahay and co-workers.[6] This conclusion supports the interpretation based on the reaction $CdX^- + H^+ = Cd^{++} + HX^{--}$. There should indeed be no effect of the double layer structure for this reaction in the polarographic determination of k (see ref. 6).

Finally, it is noted that equation 1 is quite general and holds when several complexing agents are in solution provided that equilibrium is achieved

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[1] J. Koryta and I. Kössler, *Collection Czechoslov. Chem. Communs.*, **15**, 241 (1950).

[2] K. Morinaga and T. Nomura, *J. Chem. Soc. Japan*, **79**, 200 (1958).

[3] (a) G. Schwarzenbach and E. Freitag, *Helv. Chim. Acta*, **34**, 1492 (1951); (b) G. Schwarzenbach, G. Anderegg, W. Schneider and H. Senn, *ibid.*, **38**, 1147 (1955).

[4] I. Leden, *Svensk. Kem. Tid.*, **58**, 129 (1946).

[5] P. Papoff and M. Caliumi, *Gazz. chim. ital.*, **84**, 1006 (1954).

[6] M. Breiter, M. Kleinerman and P. Delahay, THIS JOURNAL, **80**, 5111 (1958).

TABLE I

RATE CONSTANT FOR DISSOCIATION OF CdX^- AND RELATED DATA AT IONIC STRENGTH OF 3^a AT 25°

pH	4.62	5.10	5.35	5.67
$C_{Cd^{++}}$, mmole l.$^{-1}$	1	1	1	0.5
C_{HAc}, mole l.$^{-1}$	0.1	1.0	0.01	0.1
C_{NaAc}, mole l.$^{-1}$	0.1	0.3	0.1	1
$C_{HX^{--}}$, mmole l.$^{-1}$	1.44^b to 15.7	0.6^b to 12.0	0.5^c to 12.4	7
k, 10^6 mole^{-1} l. sec.$^{-1}$ (uncor.)	7.2 ± 0.6	4.1 ± 0.3	8.0 ± 0.5	..
k, 10^6 mole^{-1} l. sec.$^{-1}$	1.4 ± 0.1	1.3 ± 0.2	1.6 ± 0.1	1.7 ± 0.1

[a] Ionic strength adjusted with sodium perchlorate. [b] 5 concentrations. [c] 6 concentrations.

except for only one complex. When one cannot assume that $C_{Cd^{++}} << C_{CdX^-}$ one has

$$k = 1.31 K_{CdX} \cdot K_{HX} C_{HX^{--}} a\, t^{-1} \times \frac{h_1 - \dfrac{(h_1 + h_2)}{K_{CdX^-} K_{HX^{--}} \cdot C_{HX^{--}} C_{H^+}{}^{-1} + 1}}{h_2} \quad (3)$$

Determination of K_{CdX^-} from Oscillographic Current-Potential Curves at High Rates of Potential Change.—In view of the work of Koryta and Kössler[1] discussed in the Introduction, it was thought that the equilibrium constant K_{CdX^-} could be obtained from the ratio of the peak heights h_2/h_1 for current-potential curves obtained at high rates of potential change (oscillographic polarography). This was indeed the case. At sufficiently high rates of potential change the kinetic contribution becomes quite negligible, and the ratio h_2/h_1 is determined by K_{CdX^-}. Results are summarized in Table II. Values of pK_{CdX} of 9.8 (μ = 0.1), 9.9 (μ = 0.3) and 10.3 (μ = 3) which were deduced are in good agreement with the data of Schwarzenbach, *et al.*[3]

TABLE II

DETERMINATION OF K_{CdX^-} FROM OSCILLOGRAPHIC DATA AT 25°

V, v. sec.$^{-1}$	h_2/h_1 (μ = 0.1)	h_2/h_1 (μ = 0.3)	h_2/h_1 (μ = 3)
0.26	1.4	1.4	0.9
1.0	2.6	2.6	1.7
2.5	5.5	..	..
6.1	9.1	2.5	3.9
10.0	9.6	3.2	4.2
25.0	15.2	5.0	4.9
40.0	17.6	6.3	5.1
57.0	19.0	7.6	5.1
100.0	19.7	10.0	5.1

Conditions for 3 μ's: pH 4.13, 4.62 and 5.62, respectively; $C_{Cd^{++}}$ = 1 mmole l.$^{-1}$; C_{HAc} = 0.3 mole l.$^{-1}$; C_{NaAC} = 0.1, 0.3, 3 mole l.$^{-1}$; C_{H_3X} = 5 mmole l.$^{-1}$.

Experimental

Conventional polarographic techniques were applied. No maximum suppressor was needed. Drop times were measured in the plateau range of the second wave. Several capillaries were utilized with drop times varying between 2.9 and 5 sec. Oscillographic recordings were made with an improved version of the instrument described by Favero and Vianello.[7]

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(7) P. Favero and E. Vianello, *Ricerca sci.*, **25**, 1415 (1955); see also paper in course of publication.

ADDED IN PROOF.—Reference is also made to the very recent paper by J. Koryta, *Z. physik. Chem.* (*Leipzig*), Special issue, 157 (1958).

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The Configuration of Ribonuclease at Low pH in 2-Chloroethanol and in 2-Chloroethanol-Water Mixtures[1]

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Imahori, Klemperer and Doty have shown that ribonuclease dissolved in 2-chloroethanol acquires a configuration characterized by a high content of helical polypeptide structure. This paper shows that the transition from native aqueous ribonuclease to the highly helical structure occurs in two stages, through an intermediate with about the same helix content as aqueous ribonuclease but with a high intrinsic viscosity and flexibility, which suggests that its non-helical portions are more or less randomly coiled. The highly helical structure in chloroethanol is presumably the result of intramolecular hydrogen bonding; the compact configuration in aqueous solution is ascribed primarily to hydrophobic forces.

It has been shown recently by Doty, Imahori and Klemperer[3] that 2-chloroethanol is a good solvent for many globular proteins. Both optical rotation and infrared absorption have indicated that protein molecules in this solvent have a strong tendency, much stronger than in water solution, to form intramolecular hydrogen bonds so that the major portion of their polypeptide chains may possess a helical configuration. The purpose of the present paper is to measure the intrinsic viscosity of one globular protein, ribonuclease, in the solvent 2-chloroethanol, and to investigate the transition

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(1) Abstracted from the Ph.D. Thesis of Robert E. Weber, State University of Iowa, 1958. Presented at the 134th meeting of the American Chemical Society, Chicago, Ill., September 1958.

(2) Union Carbide (Bakelite Division) Fellow, 1957-1958.

(3) P. Doty, K. Imahori and E. Klemperer, THIS JOURNAL, **81**, in press (1959). We are grateful to these authors for making some of their data available to us in advance of publication.