14.- C.W. Kreuk, M. Sluyters and J.H. Sluyters. J. Electroanal Chem. 33(1971)267.

15.- K.J. Cantrell and R.H. Byrne. J. of Solution Chem. 16(1987)555.

16.- R.I. Machkhoshuili-Z.Neorg (Chem) 32(1987)1833.

17.- B.L. Bausal, S.K. Patil. J. Inorg. Chem. 26(1964)973.

18.- D.F. Peppard, G.W. Mason. J. Inorg. Chem. 24(1962)24.

19.- G. Chopin, W.F. Strazik, J. Inorg. Chem., 28(1967)32.

20.- Koryta-J.Electrochem. Acta. 6(1962)67.

21.- Matsuday Ayabe. Z. Electroche. 63(1959)1164.

22.- V. Almagro, V. López, J. Sancho, Anales de F.y Q. Real Sociedad. 73(1977)754.

23.- M.J. Peña and V. López. Anales de Química. 82(1986)28.

24.- Lingane J.J. Chem. Rev. 29(1941)1.

25.- M. Aihara, S. Misumi. Bull. Chem. Soc. Japan 481(1975)684

(Received 26 September 1988
Revised form 30 January 1989)

POLAROGRAPHIC BEHAVIOUR OF Cu(II)-LYSOZYME

J. Cornejo, C. Monleón and F. Vicente

Departamento de Química Física. Facultad de Química.

Universidad de Valencia. Burjassot-Valencia (Spain)

SUMMARY

Lysozyme acts as suppresor of polarographic minima of Cu(II) reduction dc wave. Lysozyme gives 1:1 and 1:2 complexes with the Cu(II) and catalyzes the reduction of copper ion. A structural change of the lysozyme with the pH is detected around pH = 3.5 which is related to carboxilic groups dissociation.

INTRODUCTION

The cation Cu(II) is reduced on the *dme* giving a polarographic wave whose reversibility depends on the nature of the media(1,2). In certain media, a polarographic minimum is detected in the diffusion zone of this reduction wave. Some authors consider is due to the reduction of the complex species negatively charged (3,4).

Polarographic methods can be used to elucidate the mechanism of the helix-coil transition of ionizable poliaminoacids. It

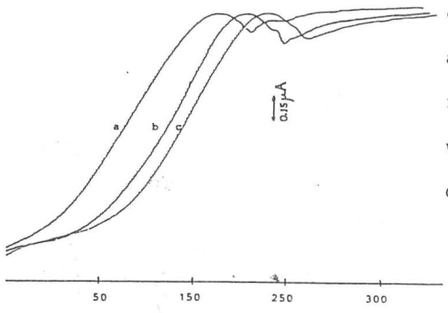

Fig.1.- dc reduction waves of $Cu(NO_3)_2(H_2O)_3$ in chloride media.
a) |KCl| =0.08M; b) |KCl| = =0.25M; c) |KCl| =0.40M.
T=298K; m=2.35mgxs^{-1}; t=0.6s; |Cu(II)| =4.0x10^{-4}M.

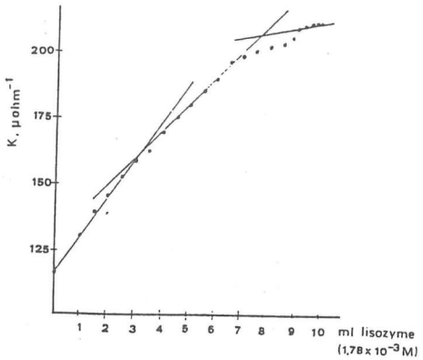

Fig.2.-Conductimetric titration of 10 ml $Cu(NO_3)_2(H_2O)_3$ 1.25x10^{-3} M.
T=298K; lysozyme 2.5% (1.78x10^{-3}M).

can be achieved by adding a small amount of any reducible metallic ion which can form stable complex with the polymer but does not disturb the polymer conformation (5,6).

In this work, the behaviour of lysozyme and its interaction with Cu(II) by dc and dp polarographic methods, in aqueous media, is studied.

EXPERIMENTAL

A Metrohm Polarecord E-506 was employed. Reference and auxiliary silver/silver chloride electrodes, together with a KCl saturated bridge were used. A Phillips Conductimeter 522 was used. The conductimeter cell had a geometrical constant of 0.64 cm^{-1}. The pH was measured with a PHM62 Radiometer pH meter.

Water and mercury were double distilled. Tricristallyzed, dialyzed and liophyllized lysozyme

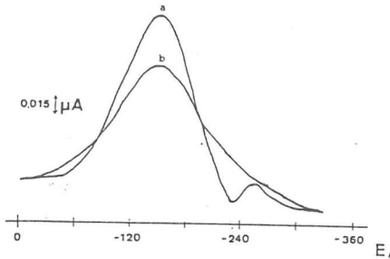

Fig.3.- dp reduction waves of Cu(II).
a) |KCl| 0.8M; |Cu(II)|= =8x10^{-5}M. b) |KCl| 0.8M; |lysozyme|=5x10^{-6}M.
T=291K;m=2,35mgxs^{-1};t=0.6s; ΔE=22mV.

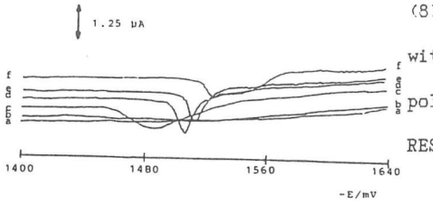

Fig.4.- Apparent minima (dp anodic waves) of Cu(II)--lysozyme-KCl system.
|KCl|=0.2M; |lysozyme| = =1.4x10^{-5}M.
a) |Cu(II)| = 0M
b) |Cu(II)| =5x10^{-3}M
c) |Cu(II)| =1x10^{-2}M
d) |Cu(II)| =1.5x10^{-2}M
e) |Cu(II)| =2x10^{-2}M
f) |Cu(II)| =2.5x10^{-2}M
T=291K; m=0.88mgxs^{-1}; t=0.6s;ΔE=22mV.

(Muramidase, Micoreptide N-acetylmuramoyl hydrolase), from egg-white (98%) was supplied by Sigma S.A.. Cu $(NO_3)_2$ $(H_2O)_3$, KCl, as well as phosphoric, acetic and boric acids, of an analytical grade were supplied by Merck S.A..

Double layer capacity(7), C_{dl}, values were obtained adapting the zero charging potential at -650 mV. In general the values of the half-width parameter, $w_{1/2}$ (mV), in dp polarography were obtained from direct measurements in polarograms (8), and they are in accordance with the αn obtained from dc polarograms.

RESULTS AND DISCUSSION

Reduction of Cu(II)

The polarographic reduction wave of $Cu(NO_3)_2(H_2O)_3$ in KCl solution presents a polarographic minimum at the diffusion zone of the wave. Its depth depends on theconcentration of chloride ions (Fig.1). The height of the wave

Table 1.- pH effect on the dp and dc reduction waves of Cu(II) in presence of lysozyme.

I=0.3M; T=291K; m=2.35mg. 5^{-1}, t=0.65, ΔE_{dp}=-22mV

pH	2.7	3.2	4.2	5.8	7.6
i_l(μA)	2.0	0.20	0.20	0.86	2.50
$E_{1/2}$(mV)	150	210	168	152	165
i_p(μA)	0.42	0.02	0.05	0.16	0.02
E_p(mV)	142	170	151	146	149

remains practically constant, which indicates that only one species is reduced. However, the $E_{1/2}$ potentials are shifted to more negative values when the concentration of KCl increases.

The slope $\Delta E_{1/2}$ / Δln(KCl) is 37 ± 3mV.

Varing the drop time, the diffusion current, i_d, is proportional to $t^{0.5}$, as it corresponds to processes controlled by diffusion. The reduction process is irreversible ($n\alpha$ =0.8±0.1 was calculated from semilogarithmic treatament of the sigmoidal waves). Thus, from the dc waves it is found that varing the drop time(9) from t=0.4 s to t=3.0 s the rate constant of the electrode transfer, k = $5x10^{-7}$ cm s^{-1} is calculated.

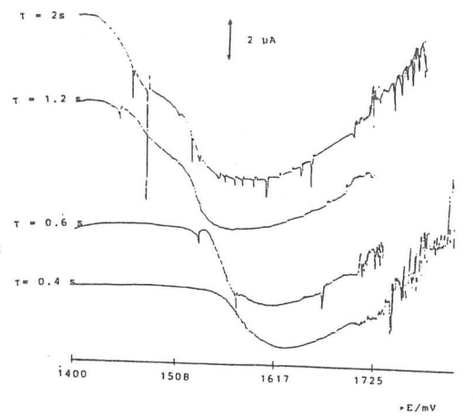

Fig.5.- Drop time effect on the dc anodic waves of Cu(II)-lysozime.
I=0.6M; T=291K; m=0.88 $mgxs^{-1}$; t=0,6s; |Cu(II)|=$2,5x10^{-2}$M; |lysozyme| =$1.4x10^{-5}$M

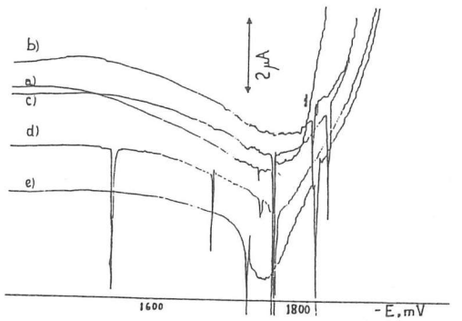

Fig.6.- lysozyme concentration effect on the anodic waves of Cu(II)-lysozyme.
I=0.8M; T=291K; m=0.88 $mgxs^{-1}$; t=0.6s; |Cu(II)|= $1,10^{-2}$M.
a) |lysozyme| =$7.1x10^{-5}$M; b) $1.4x10^{-4}$M; c) $2.8x10^{-4}$M; d) $4.3x10^{-4}$M; e) 7.1x10-4M.

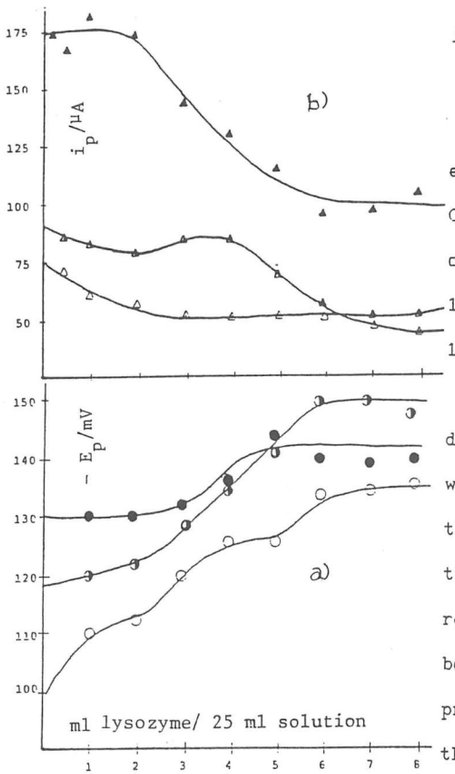

Fig.7a.- Effect of the lysozyme concentration on the peak-potentials of the dp reduction wave of $Cu(NO_3)_2(H_2O)_3$. I=0.4M; T=291K; m=2.35mgxs^{-1}; t=0.6s; ΔE=22mV.
●)lysozyme 2.5%; |Cu(II)| = =2.5x10^{-4}M. ◑)lysozyme 1.25%; |Cu(II)| =1.25x10^{-4}M. ○)lysozyme 0.82%; |Cu(II)| =8.2x10^{-5}M.

Fig.7b.- Effect of the lysozyme concentration on the peak intensities of the dp reduction wave of $Cu(NO_3)_2(H_2O)_3$. I=0.4M; T=291K; m=2.35 mgxs^{-1}; t= 0 .6s; ΔE=22mV.
▲)lysozyme 2.5%; |Cu(II)| =2.5x x10^{-4}M; ▲)lysozyme 1.25%; |Cu(II)|= 1.25x10^{-4}M; △)lysozyme 0.82%; |Cu(II)| =8.2x10^{-5}M.

Effect of lysozyme

Conductimetrically, the existence of two types of lysozyme-Cu complexes is detected (Fig.2): one corresponds to stoichiometry 1:1 and the other to stoichiometry 1:2.

Since the reduction of disulphide bonds yields kinetic waves which are very dependent on the drop time, pH, concentration of the depolarizer (10-13), etc., the reduction waves of the disulphide bonds, -S-S, of the lysozyme protein are not well detected at this level of concentrations (14-15) and, therefore, they practically do no interfere in the morphology of the polarographic wave of Cu(II). A change in the heigts of the waves is observed as a consequence of the formation of the lysozyme-copper complexes. The lysozyme is a suppresor of the polarographic minima (Fig.3).

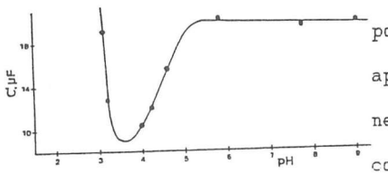

Fig.8.- pH effect on the capacity of the double layer(Calculated from the charging current of $Cu(NO_3)_2(H_2O)_3$ reduction wave). I=0.3M; T=291K; m=2.35mgxs^{-1}; t=0.6s.

By maintaining constant the concentration of lysozyme as well as the other polarographic magnitudes, and adding excesses of Cu(II), it is proved, that, at certain concentration of Cu(II), a polarographic apparent minimum appears which is shiffted to negative potentials when the concentration of Cu(II) increases (fig.4). Initially, the depth of the minimum increases, but at higher concentrations of Cu(II) the depth decreases and enlarges as if they would correspond to two apparent minima. In dc polarography, at drop times longer than 1 s, two well defined anodic waves are obtained (Fig.5). It seems that in fact this apparent minimum is a process of oxidation. At drop time of 3 s, from E vs $\ln(i/i_1-i)$ of the two anodic waves, $(n\alpha_m)_1$ = 1.9 and $(n\alpha_m)_2$ = 2.0 values are obtained, respectively. The intervention of Cu(II) in the global process is confirmed experimentally. The $Cu(0)_{amalg}$ formed in the previous reduction may be reoxidized favoured by the formation of the corresponding complex

$$Cu(0)_{amalg} + 2\ e^- \leftrightarrows Cu(II)_{complex}$$

The Cu(II)$_{complex}$ formed is quickly reduced at these negative potentials. The residual current and shape of these anodic dc waves are affected by the lysozyme concentration (Fig.6). Possibly the nonreducted disulphide bridges are reduced by the Cu(O) amalgamated on the dme and form complex with the denaturalized lysozyme.

Conformational study of lysozyme

S. Inoue and coworkers (5) use dc polarography to detect a conformational transition of poli(α,L-glutamic)acid. The results obtained are comparable to those obtaidedby other techniques (16). This polarographic method consists of measuring the variation of i_l with the pH, in the polarographic reduction of copper-polymer complex. The authors detect a lowering of i_l which starts at pH=3.5 and reaches stability at pH=5. That is attributed to a decrease in the polarographic diffusion coefficient of Cu(II). In lysozyme this fenommenon is situated at slightly more acid pH than that S. Inoue and coworkers attribute to a conformational change of the poly(α,L-glutamic) acid (Table 1), precisely in the zone of pH where a structural change of lysozyme has been detected by other techniques. The lowering of limiting current at pH=4 is accompanied by an increase at a more acidic pH, with maximum around pH=7.

Proteins are very complex and unstable systems and, therefore, the application of Ilkovics's expression must be verified carefully. From a comparative point of view, this expression can be very useful in order to stablish the difference between ionic sizes of proteins. At pH=3.5 the calculated diffusion coefficient of lysozyme-Cu(II) complex is $D = 1\times10^{-5} cm^2.s^{-1}$. At pH=5.8. $D = 3.4\times10^{-4} cm^2.s^{-1}$ is

sigmoidal curves corresponding to each stoichiometries. At higher concentrations the results are more difficult to explain since the lysozyme-lysozyme interaction and lysozyme adsorption fenomena occur simutaneously at the Cu(II) reduction.

Theichberg and coworkers determine a association constant of 1:1 complex, $K = 1.8 \times 10^{-2}\ M^{-1}$, in buffered solutions of lysozyme 0.1M and pH = 5. In their oppinion the Cu(II) is not united simustaneously to the catalytic centers Asp-52 and Glu-35 as other investigators had previously suggested (19). From polarographic studies we can say that is possible the existence of other complexes, because the behaviour of lysozyme depends on the temperature, medium and dilution grade of the lysozyme.

REFERENCES

1) L. Meites, J. Am.Chem. Soc., 72(1950)180.

2) I.M. Koltoff and J.J. Lingane: Polarography Vol I Chap.12(1952) Interscience New York.

3) S. González, S.V. alvarez, and A. Arevalo, Electrochim. Acta, 28 (1983)535.

4) S. González,S.V. Alvarez, E. Fatas and A. Arevalo, Anales de Química (R.S.E.Q.), 78(1982)344.

5) S. Inoue, K. Yamaoka and M. Miura, Bull, Chem. Soc. Japan, 44(1971) 1443.

6) H. Takesada and H. Yamazaki, Biopolymers, 4(1966),713.

7) A.J. Bard and L.R. Faulkner: "Electrochemical methods", p.156(1980) Ed. Jhon Wiley. New York-Toronto.

8) F. Vicente and C. Sanz, Electrochim. Acta, 29(1984)1659.

9) J. Koryta, Chem. Zvesti, 8(1954)64.

10) G.J. Patriarche, J.C. Voie, C. Mairene-Ducharmois, J.L. Vanderbalk and G.D. Christian, Biochem. and Bioenergetics, 6(1979)147.

11) P. Mader, Coll, Czech. Chem. Commun.,36(1971)1035.

12) J.M. Issa, A.A. Samahy, R.M. Issa y J.M. Temerik, Electrochim. Acta, 17((1972)1615.

13) C. Sanz, C. Monleón, J. Trijueque and F. Vicente, J. Electroanal. Chem.251(1988)173.

14) J. Cornejo, C. Sanz and F. Vicente: Primer Congreso de la Sociedad Española de Biofísica(1987). Valladolid.

15) C. Sanz, J. Trijueque and F. Vicente. Química e Industria,34(1988) 21.

16) P. Doly, A. Wada, T.T. Yang and E.R. Blont,J. Polym. Sci.,23(1957) 851.

17) K. Kedda and K. Hamaguchi, J. Biochem., 71(1972)256.

18) S. Kuramitsu and K. Hamaguchi, J. Biochem.,87(1980)1215.

19) V.I. Teichberg, N. Sharon, J. Moult, A. Smilansky and A. Yonath,J. Mol. Biol., 87(1974)357.

(Received 6 September 1988
Revised form 16 February 1989)

METHIONINE - GLYCINE - COPPER (II) MIXED COMPLEXES. A CYCLIC VOLTAMMETRIC STUDY

V. López Fernández
Departamento de Química
Universidad Autónoma de Madrid
Canto Blanco, 28049-MADRID-SPAIN

SUMMARY

Cyclic voltammetric studies on the reduction of the title complexes were carried out in acetic acid-sodium acetate buffer at the HMDE. The mechanism of these processes is discused. The complex species in solution of this system have been determined, depending on methionine and glycine concentrations, pH values and potential scan rate.