International Journal of Applied Research 2021; 7(8): 464-465

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2021; 7(8): 464-465
www.allresearchjournal.com
Received: 05-07-2021
Accepted: 06-08-2021

Dr. Lalan Kumar Jha
Guest Assistant Professor,
B.M. College, Rahika,
Madhubani, Bihar, India

Corresponding Author:
Dr. Lalan Kumar Jha
Guest Assistant Professor,
B.M. College, Rahika,
Madhubani, Bihar, India

Study of Eu (III)-lysozyme (HEW) in presence and absence of Zn (II)

Dr. Lalan Kumar Jha

Abstract
In the present study we have taken-op the hetero bimetallic complexation of Eu (III) with lysozyme in presence and absence of Zn (II) has been explored using cyclic-voltammetry, to reveal the intricacies of electron transfer mechanism of biochemistry and coordination chemistry of lysozyme.

Keywords: Eu (III), Zn (II), lysozyme

Introduction
Lysozyme (HEW) is a polypetide having enzymatic and is structurally related to Glutathione molecule. The Molecule is very closely related to human lysozyme. It plays significant role in inherent antibacterial activity is cow and human milk.
It is observed that denaturation of an enzyme proceed via two stage mechanisms
1. A reversible denaturation (unfolding of the molecule) and
2. An irreversible denaturation.

The change in pH of medium, nature of solvents or solvent compositions, temperature and presence of metal ions do induce 1. Changes between the allowed sequence of core residue which plays significant role in the denaturation process of lysozyme (Seleim *et al*., 1981). The three dimensional structure of Gd (IUII)-lysozyme is well known (Dick, 1982). It has two Gd (III) ion binding sites. At higher coordinating sites, Gd (III) coordinates with carboxylic group of Asp-52 and Glu-35. These two groups are too far apart bind simultaneously with same mental ion, except through intermediate water molecules and thus proposed non-rigid site model for lysozyme (Bolenand and Santoro, 1988). In the present study we have chosen Eu (III), the hard metal ion and Zn (II), the soft metal ion. Both the metal ions have different tendency to interact with Lysozyme (HEW). Hence hetero bimetallic complexation of Lysozyme (HEW) with hard metal use. Eu (III) and soft metal ion Zn (II) has been explored using cyclic-voltammetric technique. The electrode kinetic parameters were calculated and proposed mechanism is discussed.

Material and Methods
The solvents and chemicals used were of A. R/ G. R grade from E. Merck. Lysozyme (HEW) is procured from Aldrich Chemical Co. U.S.A and Europium Chloride from Indian Rare Earths Ltd., India. The stock solution of (0.1 M) Europium and Zinc were prepared in double distilled water and standardized by EDTA volumetric method. Both the solutions were kept at 5^0C in the inert chamber. A freshly prepared solution of lysozyme was used for each experiment.
All the voltammograms were recorded on the Polarographic Analyzer Model No. 384-B equipped with cell assembly Model No. 303A-SMDE from EG & G PARC, USA. A platinum wire (0.5 mm diameter), Ag-AgCl / KCl (saturated) and HMDE were used as counter, reference and working electrodes respectively.
All voltammetric measurements were carried out under nitrogen atmosphere. The scan rates were adjusted in the range from 0.1 $V.s^{-1}$ to 0.5 $V.s^{-1}$. For irreversible reactions the conventional equations were used to calculate α_{ns}, D_{ox} and k^0_{fh}.

Discussion of the Results

The behaviour of two active binding sites of lysozyme in the presence of hard metal ion, we have selected pH 2.0, 4.0 and 6.0. Eu (III) undergoes a single electron transfer reduction at -0.70 V in the absence of lignad. The nature of cyclic voltammogram indicates that the reaction at electrode surface is quasi reversible. At pH 2.0 carboxylic group may undergoes deprotonation therefore binding of Eu (III) preferentially takes place towards the carboxylate group.

A significant cathodic shift in reduction peak potential of Eu (III) is observed, which indicates the interaction of lysozyme with Eu (III) ion in the solution. The current height is found increased due to the catalytic behaviour of an enzyme in presence of reducible species like Eu (III).

The above observation is supported from the plot of is i_{pc} vs. $v^{1/2}$ and the plot of i_{pc} vs. v. The straight-line passes through-the origin in the plot of i_{pc} vs. $v^{1/2}$ indicate that the process is diffusion controlled at the electrode surface.

It independent of appearance of adsorption current because the second plot (i_{pc} vs. v) is parabolic in nature particularly for the Eu (III) Eu (II) couple system in the presence of lysozyme in solution at pH 2.0. Dissociation of carboxylic groups from Glu-35 and Asp-52 is found predominant at pH 4.0, but in weak acidic conditions binding of Eu (III) with carboxylic groups is found labile in the presence of aqua ions. The dissociation of sulfhydryl group may be started at pH 6.0, but the effect of the dissociation on Eu (III) is negligible because it is a hard metal ion. The current height is found decreased with cathodic peak and it sifts more cathodically, indicates the interaction of lysozyme with metal ion in the solution as shown in table–1

Table 1: Cyclic-Voltammetric data for Eu-Lysozyme (HEW) (1:1) in pH 4.0±0.1 at 298°K 1°K at different scan rate

Sr. No.	Scan Rate ($V.S^{-1}$)	Peak-Potential (-Ep, Volt)	Peak-current (i_{pc},μA)
1	0.1	0.900	0.166
2	0.2	0.930	0.265
3	0.3	1.010	0365
4	0.4	1.020	0.494
5	0.5	1.020	0.540

The cyclic-voltammograms of Eu (III)–lysozyme clearly suggest that the system at the electrode surface is irreversible in nature as shown in figure 1.

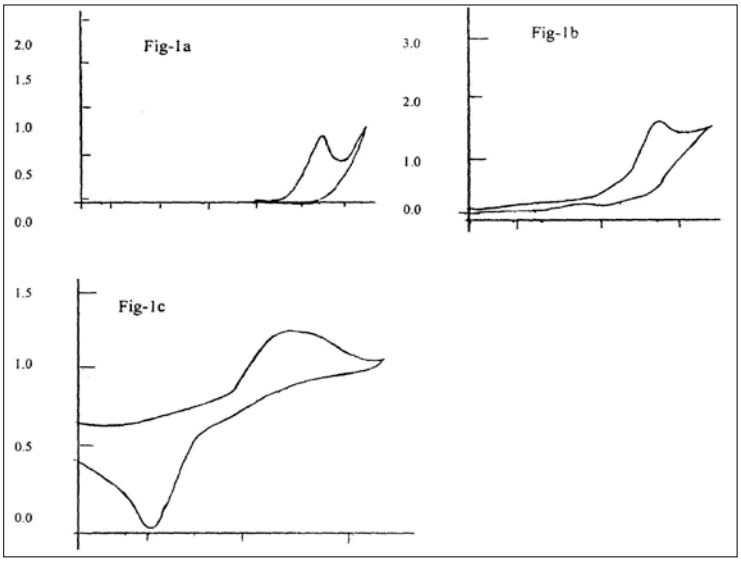

Fig 1:(a) Cyclic-Voltammogram of Lysozyme (b) Cyclic-Voltammogram of Eu (III)-Lysozyme (1:1) (c) Cyclic-voltammogram of Eu (III)-Lysozyme-Zn (II) (1:1:1) in pH 4.05 at 0.1 V. s^{-1} scan rate

The electrode kinetic parameters such as transfer coefficient (α_{na}) diffusion coefficient (D_{ox}) and heterogeneous forward rate constants (k^0_{fh}) are also calculated as shown in table-2

Table 2: Electrode kinetic parameters for Eu-Lysozyme (HEW) in pH 4.0±0.1 at 298°K±1°K

Sr. No.	Transfer coefficient (α_{ns})	Diffusion coefficient (D_{ex} $Cm^2.s^{-1}$)	Forward Rate constant ($k°_{fn}$) $cm.S^{-1}$
1	0.397	1.57×10^{-7}	2.37×10^{-9}

The irreversible interaction of Eu (III) with lysozyme is found very stable in the solution because the values of all electrode kinetic parameters suggest the irreversibility of the system and highly stable nature of the complex.

Conclusion

The introduction of Zn (II) in the binary mixture of Eu (III)-lysozyme system, enhanced the complex formation of Eu (III) with lysozyme at pH 6.0 because Zn (II) being soft metal ion, it has tendency to coordinate with deprotonated sulfhydryl group in the solution.

The unique behaviour of the ternary system in cyclic-voltammogram is appeared as the single well-defined peak is obtained suggests that both Eu (III) and Zn (II) are participating in the complex formation with equal affinity for coordinating binding sites of lysozyme.

References

1. Owusu RK. Food Chemistry. 2012;43:41.
2. Proctar VA, Cunningham FF. CRC Review in Food Science and Nutrition. 2018;26:359.
3. Canfield RE. J. Biol. Chem. 2013;238:2698.
4. Bolenand DW, Santoro MM. Biochemistry. 2018;27:8069.
5. Safanova TB, Afanasyeva TI, Kravchenks N, Cherkasov A, Antibiotiki. 2021;26:264.