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# Review and Comparison of Electrochemical Effect of Denaturation Material on the Structure of Cytochrome c

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**Abstract.** So far most protein denaturation studies are done by spectroscopy methods. This study is for review of structural and functional of protein three dimensional. With all the precision with which these methods have many limitations. So find a way such as electrochemical methods to follow changes in protein structure can be useful. This paper has been examines the impact of denaturation material, such as urea and SDS on the structure of cytochrome c, While cytochrome c which has a metalloproteinase which is a redox property and the results of the method vltamtry have been compared by spectrometry method. All chemicals and salt was prepared from Merck Co. Electrochemical Measurement was by potential acetate/Galvan acetate device. Cytochrome c was used in 20 mM phosphate buffer with pH=7/0. All spectra related to natural and denatured proteins by denaturation material were recorded by spectrophotometer Cary 50 in range 700-200 nm. CD spectra recorded by spectrophotometry model AVIV-215. Structure of the protein has been insecurity in the 5-8 M urea; this insecurity cans the range of 3-9/5 M in spectrum device. Cytochrome c was active structure in high concentration (26 mM) of SDS. 20 mM of SDS is sufficient for insecurity of 0/5 mM of cytochrome c. In this study it was found that Vltamtry are independent method for detect protein structural changes. And proved, Vltamtry method is more sensitive to small changes in protein structure compared with the methods of spectroscopy.

**Keywords:** Cytochrome c, Electrochemistry, SDS, Urea

## 1. Introduction

Today, different methods and techniques used to study interaction for material, drugs and effectors upon proteins. Some of these techniques are spectroscopy, circular dichroic (CD) and voltammetry [1-2]. It is important to find rules that under which a protein molecule obtain three-dimensional structure and the study materials that is the ability to interaction and influence on protein. Although much work has been performed in this field, the final and decisive method has yet to solve this problem. Since Most studies protein denaturation has been done by spectroscopy method such as optical spectroscopy, uv-visible spectroscopy, Fluorescence spectroscopy and et al. This method is used of behavior of proteins in front of light for the prosecution of protein structure [3-5]. With all the precision are these methods, there are several limitations. For example, a sample survey of spectroscopic methods should be clear, are free of solvents and other contaminants (such as nucleic acid) and usually sample should be only one protein. The most Spectroscopic methods have not high efficiency for the natural samples [6-7]. According to reports, Find a way to follow the structural changes in proteins seems necessary that are not that some of these restrictions. Seems to be voltammetry is method that lacking this limitation; the sample is not necessary to be clear. . In this method, the presence of different proteins within the electrolyte does not generally interfere because standard potential of the proteins appear in different voltages. Possible contamination of the sample does not create a

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nuisance; because this is less likely that that Contamination of samples is exactly the same response voltammetry of redox potential of protein [8]. Buffers used in molecular biology are usually no response voltammetry. The only parts that can cause nuisance are presence of oxygen within the electrolyte that it is also easy to control. One of the aspects of three-dimensional structure of proteins is protein folding process and structural changes. Process control and regulation of protein activity is performed Interfere with the effectors. Ability to exchange electrons in redox proteins are results of biological activities that depend on the three-dimensional structure of protein and folding process [9]. Since the redox proteins are ability of to exchange electrons with an oxidation state to another state; in this study is compared the effect on two denaturation material on the structure of cytochrome c that Basis of these reactions is redox. Cytochrome c is an electron carrier in the respiratory chain between cytochrome c reductase and cytochrome c oxidase complexes and is an activator of programmed cell death. Very extensive studies done on the structure of this small protein showed that this protein is included of 104 amino acid, four alpha helix, lacks the structure of beta and there is a non-protein group called hem prosthetic group [8-9]. The electro-chemical methods are new methods in the biological sciences. The widespread use of this method is the ability of these methods in study of thermodynamic and kinetic issues. As a simple Voltammetry cyclic differential includes information such as formal potential restoration of electroactive materials, number of electrons exchanged and the rate constants in electrode reactions, Chemical stability of modified and the mechanism of information such as the catalytic processes [10]. In this article, reviewed and compared of electrochemical effect of two denaturation material on the structure of cytochrome c with the spectrophotometer and electrochemical method. In addition to the material effect on the structure of cytochrome c, this determines the efficiency of electrochemical methods as new methods.

# 2. Materials and Methods

## 2.1. Preparation of Buffer Solution

In all experiments performed on cytochrome c was used 20 mM phosphate buffer with pH=0.7. To prepare 1 liter of buffer solution, 3.5 g of K2HPO4 and 1.3 g of KH2PO in a balloon volume has been a 1 liter. The mode of solution pH equaled to 7. If necessary to precise regulation of pH=7 was used a hydrochloric acid solution of 2 M or NaOH.

## 2.2. Preparation of Protein Samples

Basically, the study of effect of denaturation material on the structure of protein, there are two methods for preparing protein samples. A protein sample can be prepared and then at each stage of value added denaturation solutions to its. Gradually increases as the denaturation concentration. This method is known as the titration. This method is not accurate because does not have enough time to samples reach equilibrium and protein concentration gradually decreases. In other method, Protein samples are prepared as separate in per concentration of denaturation material. Then are given them 1 hour to reach equilibrium. This paper we used of second method. Soluble protein cytochrome c is the specific light spectrum in the visible region that maximum absorption was at 409 nm (absorption-line sorting). Molar absorption coefficient of horse heart cytochrome c at this wavelength is 1/06×105 M/cm. Using the Beer-Lambert, was calculated (Absorbance of protein solution in 409 nm) concentration of cytochrome c. Cytochrome c was purchased commercially was both the oxide and the reduction and usually it is 98% as oxide. To fully oxidize the protein molecules, potassium cyanide is added to the sample volume of 10% protein content. Cytochrome c protein was stable protein and its solution to be used for several days at room temperature and several weeks in the refrigerator; but in the present work all samples were prepared daily.

## 2.3. Spectroscopy Studies

Spectra of natural and denatured proteins by urea were recorded in the 700-200 nm by spectrophotometer model Cary 50. CD spectra were recorded in the far UV for protein samples (0/2 mg/ml) by spectropolarimeter AVIV-215.

## 2.4. Preparation of Working Electrode

To investigate the direct electron transfer reaction between the electrode and cytochrome c, Gold was chosen as the working electrode and was prepared as follows:

1- Gold electrode surface polished by suspension of aluminum oxide 0.6 µ.
2- To remove alumina particles, using of d. d. water and placing the electrode in ultrasonic for 10 min.
3- Insert the electrode into sulfuric acid for 10 min.
4- Clean the electrode will be close to the solution of 0.1 M sulfuric acid and 0.01 M potassium chloride and 10 Cycles were performed between 0.2 and 0.75 V with 100 mV/s speed and a starting point and ending 0.4 V (All relative to the reference electrode of silver - silver chloride).
5- More, 3 cycles were performed, but upper limit potential was chosen as 1, 1.25 and 1.5 V.
6- Now electrode is ready for further work.

## 2.5. Electrochemical Measurements

The electrochemical measurements were performed by the potassium acetate/acetate Galvan A263 model (EG & GC of USA). All experiments were performed using a three electrode system that the electrodes of silver/silver chloride were used as reference electrode. A Platinum rod Electrode is used as the counter electrode. In this study, gold electrode modified by mercaptoethanol used as a working electrode. All electrochemical measurements were performed using a Voltammetry cycle method. Also, All experiments have been conducted in the Faraday cage to noise levels (Noise) is minimized. Before testing, Solution for 30 min was anaerobic with pure nitrogen.

# 3. Results and Discussion

Since most of the studies of denaturant and effects on protein structure has been done by spectroscopy methods; such as visible spectroscopy – UV spectroscopy, fluorescence emission, absorption and heat-sensing methods. This method is used behavior of proteins in the light for the prosecution of protein structure. In this work was used Voltammetry cycle method to follow structural changes of cytochrome c in the presence of two denaturant of urea and SDS and results were compared with spectroscopic methods.

## 3.1. The effect of Urea on the Structure of Cytochrome c

To study the effect of urea on the structure of cytochrome c, was used for the gold electrode modified with 2 - mercaptoethanol at exposed to concentrations of 30 µM of cytochrome c. In the presence of various concentrations from urea (0-8 M) was a Voltammetry cycle (Figure 1). As can be observed with increasing urea concentration, peak flow decreased (characterized by electrochemical activity of the immobilized protein). As shown in Figure 1 is consistent with reduction rate constant, decreased peak current.

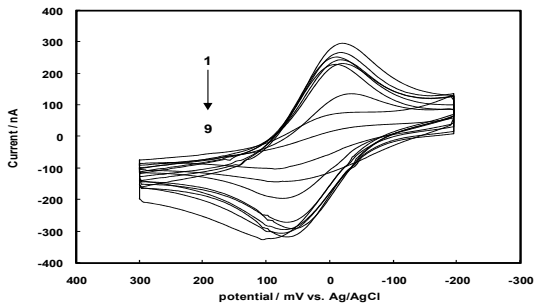


Fig. 1: Voltammogram cycle of cytochrome c. Voltammogram were obtained to the concentrations of 0, 1, 2, 3, 4, 5, 6, 7 and 8 M urea, orderly. 20 mM phosphate buffer and 100 mV/s probe speed.

In Figure 2, is challenged by the effects of various concentrations of urea on Voltammetry cycle of cytochrome c electrode. As is clear in this figure, Cathode and anodic peak currents are reduced to the sigmoid. This behavior is common in denaturation of cytochrome c.

In this study, denaturation cytochrome c was studied by optical methods. Figure 3 shows the absorption spectrum of cytochrome c in 30 µM in UV region. The peaks of the index are: peptide bonds absorb at 230

nm, aromatic ring absorption at 280 nm, hem dye absorption band at 400 nm and beta absorbance at 520 nm. For denaturation of protein structure increased of urea concentration to 9/5 M.

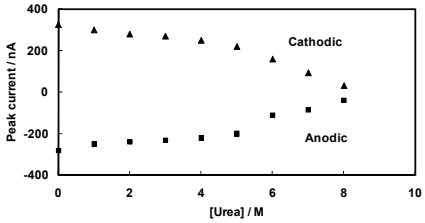


Fig. 2: the cathode and the anodic peak current of effect of different concentrations of urea cycle Voltametry electrode on cytochrome c.

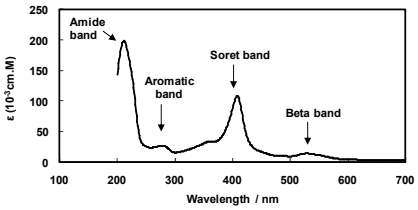


Fig. 3: Absorption spectrum of Uv-Vis of 30 µM cytochrome c in 100 mM phosphate buffer.

Figure 4a, shows an increase in the protein sorting. The maximum absorption is shifted toward the blue wavelengths. In the aromatic absorption is shown in Figure b4 that here it is seen the increasing in absorbance and shifting to blue wavelengths. This shift is due to solvent to aromatic root of protein.

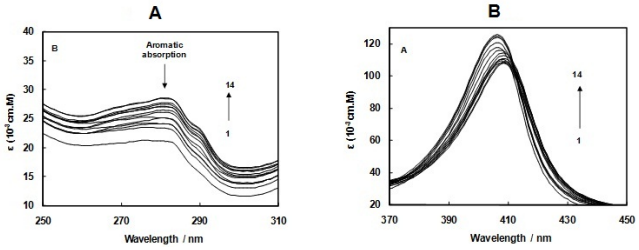


Fig. 4: Effect of urea on (A) surat absorption line, and (B) aromatic absorption 30 µM cytochrome c in 100 mM phosphate buffer, the concentration of urea, respectively 0, 1, 2, 3, 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9 and 9.5 M.

In figure 5a has been shown the absorption spectrum CD of cytochrome c in the far ultraviolet region. This area provides information about the secondly structure of protein. In this chart, two Sink of 208 and 222 nm is important. Figure 5a shows the effect of different concentrations of urea on the 222 nm. As can be seen, increasing urea to 8 M, disappears in the absorption wavelength.

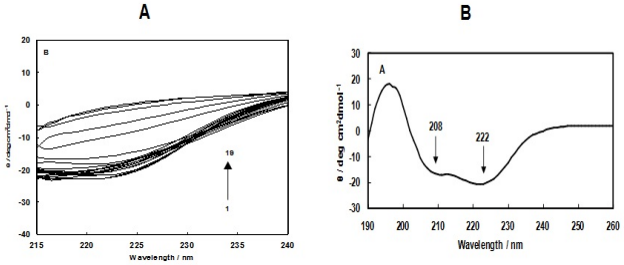


Fig. 5: (a) CD Absorption spectrum of 30 µM cytochrome c; (b) effect of different concentrations of urea on the spectrum of cytochrome c at 222 nm are equal to 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9 M

respectively.

### 3.2. The Effect of SDS on the Structure of Cytochrome c

Figure 6 shows the effect of different concentrations from 0 to 26 mM SDS on cytochrome voltammetry peak. However, the SDS concentration is very high, but protein will maintain Structure and therefore its voltammetry peak, considerably.

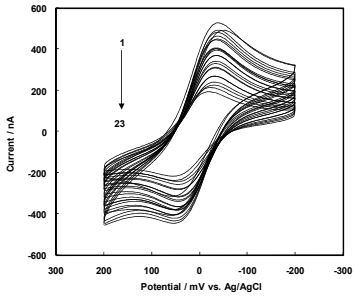


Fig. 6: Effect of different concentrations of SDS on the electrochemical response of cytochrome c; respectively, from top to bottom: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24 and 26 mM. Protein concentration of 100 mM, gold electrode modified with 2-mercaptoethanol, 20 mM of buffer.

## 4. Conclusion

In this study voltammetry of cytochrome c were done in the presence of urea and SDS and found that voltametry as an independent method to detect structural changes of the protein. Denaturation curves from voltametry are different as denaturation curve is obtained by spectroscopy methods. the transition region in voltametry (3-8 M urea) is broader of the phase transition in spectroscopy methods (5-8 M urea). This reflects that voltametry method higher sensitivity to small changes in the structure of the protein. Spectroscopy method based on a static process (the interaction of light with matter) is an electrochemical process but dynamic (reactive transport of dissolved depth of the electrode surface, electrode transfer and product transition from surface to depth).

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