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Kinetic studies for some enzyme catalyzed reactions

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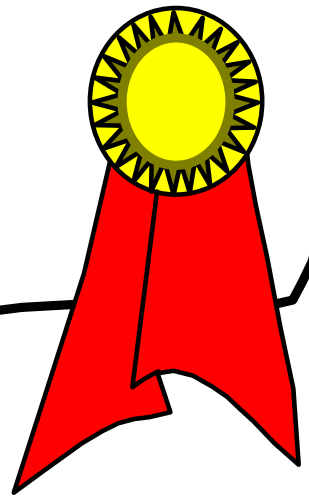
Dedication

*To my **father** and my **mother** with my deep and sincere appreciation for their great efforts during my life and my studies.*

*To my **brothers and my sister** with my great thanks for their help.*

*To my **friends** and **colleagues**.*

Usama A. Bahaa



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Candidate

Usama Ahmed Bahaa Eldin

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(E_a)	activation energy
(NAD)	nicotinamide adenine dinucleotide
(ATP)	Adenine tri phosphate
(Asp)	Aspartic acid
(POD)	Peroxidases
(HRP)	Horse radish peroxides
(CHRP)	Conjugated horse radish peroxides
(k_m)	Michaelis menten constant
(V_{max})	maximum velocity
(k_{cat})	the rate velocity constant
(His)	histidine residue
(S)	Substrate
(I)	Inhibitor
(E)	Enzyme
(EIA)	Enzyme Immunoassays
(ELISA)	Enzyme Linked Immunosorbent Aassay
ΔH^*)	Enthalpy of activation
(ΔS^*)	entropy of activation
(ΔG^*)	free energy of activation
(PABA)	para aminobenzoic acid
(LCTN)	L-cystine
(T_m)	denaturation temperature
(O.D)	optical density
(IgG)	Immunoglobulin

Summary

In this study, we have chosen for study the Horse Radish Peroxidase conjugated to a specific mouse antibody against human hepatitis B virus. This conjugated Horse radish peroxisdase (CHRP) is commonly used as an important component in enzyme Immunoassays (EIA) for detection of hepatitis B (surface) antigen in human serum or plasma.[34] We have studied the characteristics of this CHRP enzymatic reaction, factors affecting its catalytic behavior, and how some substances could inhibit its action, determination of some important catalytic and thermodynamic parameters. We can classify our study into three main Parts:

Part 1: Introduction

In this part, important information have been mentioned about the enzyme properties generally, and specially about the native Horse radish peroxidase (HRP). Moreover, the introduction includes literature survey on the studies that had been done on Horse radish peroxidase and also the aim of the work.

Part 2: Experimental design:

This chapter includes description of the experiments carried out in this study for CHRP characterization and also for determination of the kinetic and thermodynamic parameters. The inhibitors of the CHRP were included in the experimental design for to study their effect on the CHRP.

Part 3: Results and discussion

Which may be classified into three chapters as shown:

Chapter I) characterization of the enzymatic reaction of the conjugated HRP: in this chapter , the different factors that should affect the reaction were studied such as: the effect of each conjugated HRP and substrate concentrations, the effect of pH of the reaction meduim, and the effect of the temperature on the reaction.

chapter II) Determination of some important kinetic and thermodynamic parameters: In this chapter, Michaelis menten constant k_m, the maximum velocity of the reaction V_{max}, and the rate velocity constant or k_2 (or k_{cat}) were calculated, where k_2 at different temperatures in order to obtain the activation energy of the reaction E_a, and so, other activation thermodynamic parameters: enthalpy ΔH^*, entropy ΔS^* and the free energy ΔG^* were also evaluated.

chapter III)) Inhibition study on the conjugate HRP reaction: in this chapter, two important biochemical substances were chosen for their important biological functions: (i) The inhibition effect of para-aminobenzoic acid (PABA), which has many important biological properties, it was used to improve the protein used in the body and also relates to red blood cell formation as well as assisting the manufacture of folic acid in the intestines. Para-aminobenzoic acid is used in sunscreen preparations since it can protect the skin against ultra-violet radiation. (ii) second inhibitor used is l-cystien (LCTN) which considered as an important source of sulfide in human metabolism and many other bilolgical functions.[21] . We studied each enzyme separately and compared there results with those of uninhibited reactions to determine the inhibition functions.

Part 1: Introduction

Enzymes are protein molecules that catalyze chemical reactions. where the molecules at the beginning of the process, called substrates, are converted into different molecules, called products. [1,2]

The existence of enzymes has been known for well over a century. Some of the earliest studies were performed in 1835 by the Swedish chemist Jon Jakob Berzelius who termed their chemical action catalysis . It was not until 1926, however, that the first enzyme was obtained in pure form, a feat accomplished by James B. Sumner of Cornell University. Sumner was able to isolate and crystallize the enzyme urease from the jack bean. His work was to earn him the 1947 Nobel Prize. [3]

John H. Northrop and Wendell M. Stanley of the Rockefeller Institute for Medical Research shared the 1947 Nobel Prize with Sumner. They discovered a complex procedure for isolating pepsin. This precipitation technique devised by Northrop and Stanley has been used to crystallize several enzymes. [4]

All known enzymes are proteins (A few ribonucleoprotein enzymes have been discovered and, for some of these, the catalytic activity is in the RNA part rather than the protein part. Link to discussion of these ribozymes). They are high molecular weight compounds made up principally of chains of amino acids linked together through peptide bonds linkage.

Enzymes can be denatured and precipitated with salts, solvents and other reagents. They have molecular weights ranging from 10,000 to 2,000,000.

Many enzymes require the presence of other compounds - cofactors (co –enzymes)- before their catalytic activity can be exerted. This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion-activator) is called the holoenzyme.

According to Holum [4], the cofactor may be:

- A coenzyme - a non-protein organic substance which is dialyzable, thermostable and loosely attached to the protein part.

- A prosthetic group - an organic substance which is dialyzable and thermostable which is firmly attached to the protein or apoenzyme portion.
- A metal-ion-activator - these include many metals such as: K^{+}, Fe^{++}, Fe^{+++}, Cu^{++}, Co^{++}, Zn^{++}, Mn^{++}, Mg^{++}, Ca^{++}, and Mo^{+++}.

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group.

In general, there are four distinct types of specificity[5]:

- Absolute specificity - the enzyme will catalyze only one reaction.
- Group specificity - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.