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Effect of Gamma Irradiation on the Activities of Glucose and Cholestrol Oxidases

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ABSTRACT

The effect of radiation on efficiency, retention of activities and stability of the activity during different exposure duration of radiation were studied. In the present work the effectiveness of gamma rays on the glucose and cholesterol oxidases are studied. We find that the last three dose (20, 30 and 45) minute of gamma ray have potential to cause global changes in the reducing enzyme activity. While the cholesterol oxidase activity was reduced after exposure to radiation at 10, 20,30 and 45 min. To investigate the stability of Glucose oxidase and cholesterol oxidase to radiation , different exposure time were preform, and the results show the tolerance capability of glucose oxidase was more than cholesterol oxidase when exposure to radiation for 10 min, the results shown that the activity of glucose oxidase was 0.506 U/ml, while the activity of cholesterol oxidase was o.472 U /ml.

1. Introduction

Glucose oxidase and cholesterol Oxidase belongs to the family of oxidoreductases, such enzymes acting on the CH-OH group of donor with oxygen as acceptor. These enzymes have been mostly used in many analytical tools.such as biosensor, biocatalyst, and bile acid biosynthesis or drug-delivery systems (Salama *et al* ., 2012). Ionizing radiation is a type of electromagnetic wave that carries enough energy to remove electrons from an atom. Ionizing radiation is produced by unstable atoms because they have an excess of mass or energy or may be both of them (Liebel *et al*., 2012). Unstable atoms are said to be radioactive and these emissions are called radiation. There are three basic type of ionize radiation these types include alpha particle, which include two protons and two neutrons, beta particle which include electrons , gamma rays and x-rays, which include photons and Neutrons which include uncharged protons (Metz-Flamant *et al*., 2012). Effects of radiation o the Biological system is basically results in the immediate formation of free radicals and causes oxidative stress damage of important cell components due to interaction with water molecules. The indirect effect of radiation typically divided into two categories. The first category represents exposure to high doses of radiation over short periods of time to produce acute term effects. The second category includes exposure to low radiation doses over a long time producing chronic term effects. The cell membrane and other cellular organelles are considerable the main targets for free radical attack (Sreedhar *et al*., 2013). The Gamma rays are ionizing radiation belongs to the electromagnetic group of radiations and are extremely high frequency waves, and carry a large amount of energy (Richard S. and Dianella H., 2012). Visualize any changes in the activity of glucose oxidase enzymes, confer sensitivity to the radiation stress after exposure to gamma radiation.

2. Experimental Work

Enzymes ad reagents

Glucose oxidase (GOD) (Sigma); Peroxidase (POD) 1000U/L (Sigma); 4-Aminoantipyrine A reagent for glucose determination in the presence of phenol and peroxidase (Sigma); R1 buffer for cholesterol determination (Tris pH 7.4 92 mmol/L ad Phenol 0.3 mmol/L)(bio system);Glucose aqueous primary standard 100 mg/dL (bio system); o-Dianisidine Solution (ODA) ; KOH ; Potassium Phosphate; Peroxidase Enzyme Solution (POD) ; and Cholesterol Oxidase Solution (Sigma-Aldrich) ; Cholesterol standard 5.17 mmol/L (bio system).

Source of radiation

The source of irradiation was located at the collage Science of Al-Nahrin University. We have used gamma-rays produced in a model Gamma cell 220 60Co irradiator as described by (Beauregard & Potier, 1982; Beauregard *et al*., 1983), except that the temperature of the irradiated samples was maintained at 25 °C. The irradiation was conducted at a dose rate of approx. 2 Mrad/h in a specially designed tube rack allowing iso dose exposure of the samples.

Determination of glucose oxidase (GOD) activity

A colorimetric method using 4-aminoantipyrine- in a glucose oxidase-peroxidase system is proposed to determine the glucose level in biological system. The principle involved in the estimation of glucose level, firstly the β-D glucose is oxidized into gluconic acid and hydrogen peroxide in the presence of glucose oxidase (GOD). The hydrogen peroxides the reacts with phenol and 4-amino antipyrine by the action of peroxidase to form a pink colored quinoamine dye complex. In the present work, the level of β-D glucose is measured before and after exposure the glucose oxidase to radiation, the absorbance of samples and standard are read against the blank at 500 nm, all working step was doe according to the procedure of Sigma Company.

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Sample preparation:

Reagent 1 (Buffer solution): 50 mM Sodium Acetate Buffer, then Adjust to pH 5.1 at 35ºC with 1 M HCL.)

Reagent 2: O-Dianisidine Dihydrochloride (ODD*)*: This Reagent was Prepared immediately by Dissolve 10 mg in 4 ml Distilled water to obtained 0.21 mM final concentration .

Reagent 3: Peroxidase Enzyme Solution (POD)

Reagent 4: Glucose Oxidase Enzyme Solution (GOD): This Solution was prepared immediately before use, Reagent 5 (Standard solution): Glucose solution (10% (w/v). Standard solution was prepared y dissolved 10 mg of glucose in 100 ml distilled water.

Reagent 6 (working reagent): Working reagent was prepared by pipetting 192 ml of Reagent 3 and 40 ml of Reagent 5 ,the Mix well and adjust to pH 5.1 at 25°C. Pipette the following reagents into test tube as follow

Reagent	**Volume (ml) Test /**	**Volume (ml) /blank**
Reagent 1	-----	0.04
Reagent 4	0.04	-----
Reagent 5	0.04	0.04
working reagent (Reagent 6)	0.97	0.97
Final Volume (ml)	1	1

All samples are mixed then incubated for 5 mn at 37°C. The increasing in A_{500nm} were recorded them obtained the A500/time (minute) by use the maximum linear rate for both test Δ7.5 unit /ml ΔA500mm The colour is stable for 30 mn. Then results were recorded immediately (Bergmeyer *et al*., 1974).

International Unit (IU) :One unit of will convert 1.0 µmole of cholesterol to 4-cholesten 3 one per minute at pH 7.5 at 25 C. Calculations of enzyme activity unit/ml A500/time (minute)test- A500/time (minute) blank (1.0)(df)/(7.5)(0.10).

where

Number 1: total volume

Df : dilution factor

7.5 : milli molar extinction coefficient of o-Dianisidine oxidaze at 500nm

0.10 : volume in milliliters of enzyme used

Determination of Cholesterol oxidase Activity:

The activity of cholesterol oxidase activity was determined based on estimate the level of cholesterol .This can perform using the colorimetric method depending on a quinonimine as the indicator formed from hydroge peroxide ad 4-aminoantipyrine in the presence of phenol and peroxidase.

Sample preparation

Reagent 1 (Buffer solution): 50 mM Potassium Phosphate Buffer (6.8 mg/ml) with with 1M KOH , Adjust pH to 7.5 at 25°C.

Reagent 2: % (w/v) o-Dianisidine Solution (ODA*)*: This Reagent was Prepared immediately by mix 10.0 mg/ml solution of o-Dianisidine and Reagent 1.

Reagent 3: Peroxidase Enzyme Solution (POD)

Reagent 4: Cholesterol Oxidase Solution: This Solution was prepared immediately before use, prepare a solution containing 0.1 – 0.2 unit/mL of cholesterol oxidase in cold Reagent 1.

Reagent 5 (Standard solution): cholesterol solution (200 mg/dl).

Reagent 6 (working reagent): Working reagent was prepared by pipetting 40 ml of Reagent 1 and 0.50 ml of Reagent 2 ,the Mix well and adjust to pH 7.5 at 25°C with 0.1 M HCl or KOH if necessary. Add Reagent 1 to a final volume of 50 ml, and stand for approximately 10 minutes before use. Pipette the following reagents into test tube as follow:

Reagent	Volume (ml) Test /	Volume(ml) /blank
Reagent 1	-----	0.04
Reagent 3	0.04	0.04
Reagent 4	0.04	-----
Reagent 5	0.04	0.04
working reagent (Reagent 6)	0.9	0.9
Final Volume (ml)	1	1

All samples are mixed then incubated for 5 mn at 37°C. The increasing in A_{500nm} were recorded them obtained the A500/time (minute) by use the maximum linear rate for both test Δ7.5 unit /ml ΔA500mm The colour is stable for 30 mn. Then results were recorded immediately

International Unit (IU) :One unit of will convert 1.0 µmole of cholesterol to 4-cholesten 3 one per minute at pH 7.5 at 25 C. Calculations :7.5.1 unit/ml A500/time (minute)test- A500/time (minute) blank (1.0)(df)/(7.5)(0.10)

where

Number o1: total volume

Df : dilution factor

7.5 : milli molar extinction coefficient of o-Dianisidine oxidaze at 500nm

0.10 : volume in milliliters of enzyme used

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of concentration in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

3. Result ad discussion

The main target of the present study was to stimulate cholesterol oxidase and glucose oxidase increasing activities by irradiation after pretreatment with gamma ray at different exposure times. However, the effect of gamma radiation has been studied on the glucose oxidase and cholesterol oxidase activities and is documented in figure 1 and 2, respectively

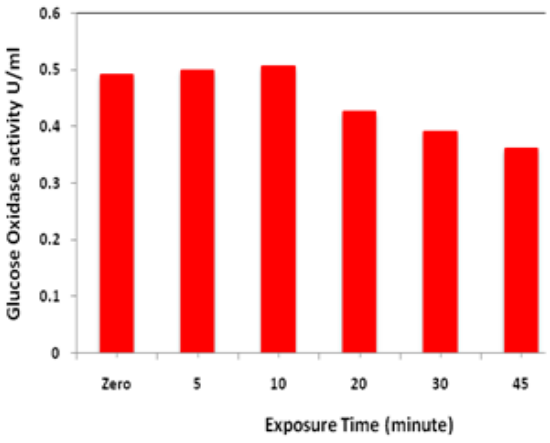

Figure 1. Glucose oxidase activities of different fractions from irradiated Sesame with gamma rays.

Results in figure one shown that glucose oxidase activity was increase when exposure to 5 and 10 mn of gamma ray, the statistical analysis showed a significant difference ($p > 0.05$) in the enzyme activity at different exposure time. Otherwise, there are significant difference ($p>0.05$) in the reduced the

activities of the cholesterol oxidase when enzyme pre incubated to gamma radiation at 10,20,30,45 min as show in figure two.

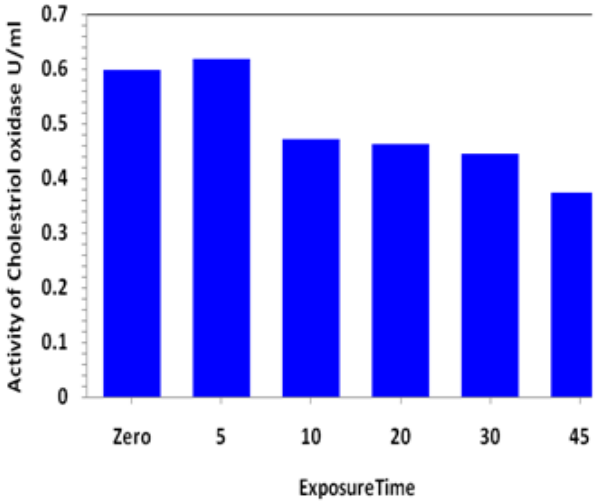

Figure 2. Cholesterol oxidase activities of different fractions from irradiated Sesame with gamma rays.

Partial inactivation in the activity of glucose oxidase and cholesterol oxidase was obtained after exposure to small dose of radiation from gamma rays. Evidence illustrated that the inhibition in the glucose oxidase activity was due to oxidation of the active site of the enzymes. The ionic efficiency of gamma radiations on inhibition of cholestero l oxidase was similar. However, the reduced the activity of glucose oxidase and cholesterol oxidase were significantly increased with increase the radiation doses and exposure duration (Gautam *et al*., 1998). In addition, the activity of cholesterol oxidase was increased after exposure to 5 min of gamma radiation, while glucose oxidase activity was increased after exposure to 5, 10 min of gamma radiation. The exposure to the radiation may be cause, altering in the chemical composition of the liquids so that lead to formation of reactive oxygen species (ROS) such as O_2- (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide)(Lobo *et al*., 2010) ,these radicals can recombine to form gaseous hydrogen, oxygen, hydrogen peroxide, hydroxyl radicals, and peroxide radicals which may be significant enough to cause the damage in biomolecules and reduced in the enzyme activity ((Yosuke *et al* .,2005; Spotheim *et al*., 2008; Vigor *et al*., 2014) . The increasing in the enzyme activity after exposure to the 5 and 10 mins of radiation may be related to the excess in the hydrogen peroxide that formed in the buffer exposure to the radiation, in addition of that release from the action of glucose oxidase and cholesterol oxidase in the buffer as show below:

Cholesterol + O2 Cholesterol Oxidase > H2O2 + 4 -Cholesten-3-one

H2O2 + o-Dianisidine (reduced) POD > 2 H2O + o-Dianisidine (Oxidized)

β -D-Glucose + O2 + H2O GOD > D-Glucono -1,5 – Lactone + H2O2

H2O2+o-Dianisidine(reduced) POD >o-Dianisidine(oxidized) o-Dianisidine(oxidized)

The effect of γ ray on the stability of glucose oxidase and cholesterol oxidase activates were investigated. It was found the stability of glucose oxidase higher than cholesterol oxidase as show in figure 3, because the radiation causes change in the water or any buffer, the changes that occur in buffer lead to form different product thus acts on the solute. However, tended to be effect on the activities of enzymes. Moreover, both enzymes have different in the structure of the active sites or may be related to chemical structure natural of substrates of glucose oxidase and cholesterol oxidase respectively (glucose and cholesterol) (Berg *et al*.,2007) .The fact that cholesterol is o polar while glucose substrate of glucose oxidase is highly polar ,so that may e cause altering in the stability of both enzymes. further related to that , Consequently some solutes are not protecting at all, as, for instance, NaCl; some others, e.g. glucose, fructose and nucleic acids, are 1000 times more effective (Dale, 1942).

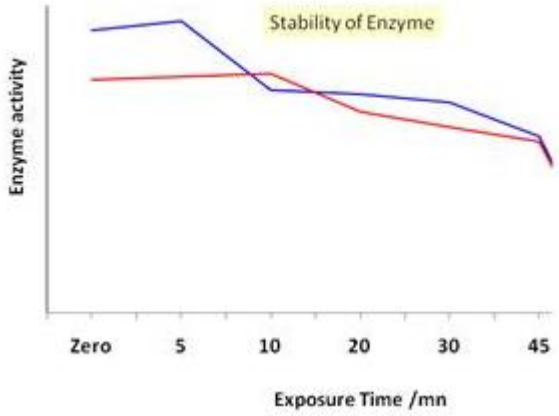

Figure 3. Stability of Glucose oxidase and Cholestrol Oxidase activities.

Conclusions

Cholesterol and Glucose oxidases have received great attention due to their wider use in clinical laboratories (determination of serum glucose or cholesterol glucose in body fluids) and in the biocatalysis;biosensorsand nanotechnologies. Moreover, both enzymes become important tools in several different industries (wine production , bakery, as a food preservative or as additive and color stabiliser) ,and in the wine industry. These applications promoted to development the functions and structures of such enzymes. The results show the tolerance capability of glucose oxidase was more than cholesterol oxidase when exposure to radiation for 10 min and the activity of glucose oxidase was found to be 0.506 U/ml, while the activity of cholesterol oxidase was o.472 U /ml.

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