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Kinetic study of the effect of Titanium Dioxide Nanoparticles on salivary peroxidase activity

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Abstract
Recently, understanding of the interaction of proteins with various nanomaterials has grown. The protein corona can detect not only how nanoparticles interact with cells but their biological effects and toxicity too. Structural and optical properties of the titanium dioxide nanoparticles (TiO_2NPs) have been investigated using (UV-Vis) spectroscopy and Scanning Electron Microscope (SEM). The effect of TiO_2 NPs on salivary peroxidase activity was studied kinetically. The results in this study indicated that salivary peroxidase activated by TiO_2 NPs. the Vmax and Km for enzyme activity without nanoparticles were (133.33)U/L, and (0.133)mol/L respectively, and (714.3)U/L, (0.357)mol/L in the presence of TiO2NPs.

Keywords: Peroxidase activity, TiO_2 NPs, Saliva, and Kinetic study.

1. Introduction

Over the past decade, nanotechnology has experienced rapid growth with its broad application in drug delivery, imaging and diagnosis [1-3]. Nanoparticles (NPs) are materials at sub micrometer scales (1-100 nm), so they may produce other advantages to the biomedical field through increased biocompatibility [4]. Metal oxide nanoparticles are safe for applications because they are more stable and have salient properties [5]. Titanium dioxide (TiO_2) and ZnO are widely used as UV blockers in cosmetic lotions. TiO_2 nanoparticles are produced universal for multiplicity of bioengineering uses. They are also consumed as a material for orthopedic implants, and as a powder TiO_2 is commonly used as a whitener in toothpastes [6, 7]. Near 4.6 million tons of pigmentary TiO_2 are used yearly, this number is increasing as application continues to rise [8]. TiO_2 NPs produced oxidative stress and apoptosis in animal cells [9]. Most of biological effects of NPs seem due to their interactions with proteins. The interaction of lysozyme with TiO_2 NPs has been described [10], the effect of TiO_2 NPs on salivary ALP activity on gingivitis has been studied too [11].
Peroxidase (EC: 1.11.1.7; hydrogen peroxide oxidoreductase) is widely distributed in the living world and participated in several physiological processes. It's a hemeprotien catalyzing the oxidation by hydrogen peroxide of large number of compounds, including phenols, aromatic amines, thiosanisoles, halide and thyocianate ions, and fatty acids [12]. In human body, peroxidase founds in several body fluids; plasma, tears, and saliva in addition to other parts of cells acting as scavenger of free radicals in presence of hydrogen peroxide. The most important enzyme in the salivary antioxidant system, peroxidase, is represented in two peroxidase enzymes in saliva: salivary peroxidase secreted from the major salivary glands, mainly the parotid gland contributes 80% of oral peroxidase activity, and myeloperoxidase produced by leukocytes in inflammatory regions of the oral cavity contributes the remaining 20%of the oral peroxidase activity [13, 14].
Although the function of peroxidases is seen mainly in terms of causing the conversion of toxic H2O2 to H2O, their participation in other reactions, such as cell wall formation, lignification, the protection of tissues from pathogenic microorganisms, suberization, axing catabolism, defense, stress, etc., should be noted [12].
Until nowadays, there is no study about the effect of TiO2 NPs on salivary peroxidase activity kinetically. Then, it is very important to investigate the effect of these nanoparticles on salivary total peroxidase activity.

Materials and Methods

1. Nanoparticles solution

Titanium dioxide nanoparticles have been obtained from Hongwunanmter, china. TiO2 NPs solution was prepared by dissolving TiO2 Nano powder in 25% ethanol and 75% deionized water as a solvent. Absorbance spectra of NPs stock solution were measured by UV- VIS spectrophotometer. Structure and Nano size measurement of ZnO NPs powder were identified by the Scanning Electron Microscope SEM (Electronic Microscope Centre- College of applied Science, University of Technology, Iraq).

2. Salivary Total peroxidase assay

Peroxidase activity was determined colorimetrically. Wide variety of hydrogen donors have been utilized in peroxidase assay systems. In this study an improved assay was adopted using 4- aminoantipyrine as hydrogen donor [15]. The activity is determined by measuring the increase in absorbance at nμ= 510 nm resulting from the decomposition of hydrogen peroxide per time of incubation. After adding 1.4 ml of (4—aminoantipyrine (2.5 mM) with phenol (0.17 M)) solution to 1.5 ml of (hydrogen peroxide (1.7Mm) in phosphate buffer (0.2 M) pH 7.0) solution, the reaction was initiated by addition of (100μl) of saliva with mixing. The increasing in the absorbance at 510nm, was calculated for 5 minutes, to obtain (ΔA/min). One unit of enzyme activity represent the

decomposition of one µmole of hydrogen peroxide per min. at pH = 7.0 under the specified conditions.

3. Saliva samples collection

Un-stimulated whole saliva samples of twenty –one normal volunteers from Baghdad city were collected with age range of (30-45) years. The sample was collected after volunteer was asked to rinse his mouth thoroughly with water to insure the removal of any possible debris or contaminating materials and waiting for 1-2 min for water clearance. The samples were collected 2-3 hours after the volunteer usual breakfast time. Saliva was collected between 9-12 a.m. Then the collected saliva was separated by centrifuge at 1500 xg for 15 minutes and then the clear supernatant store at -20˚C (freeze) until biochemical analysis

4. Effect of TiO2 nanoparticles on salivary Total Peroxidase activity

Stock solution of (300 µg/ml) concentration of TiO2 NPs was prepared and then the following concentrations (2, 5, 10, 15, 20, 25, 50, and 100) µg/ml were prepared by diluting with the same solvent. Salivary total peroxidase activity was measured by using 100µl of saliva in the same method with replace 100µl of the solvent (3:1, water: ethanol) with 100µl of TiO2 NPs solution. The percentage effect on activity was calculated by comparing the activity with and without TiO2 NPs and under the same conditions of assay according to the following equation:

٪ Activation = 100 x (Activity in the presence of nanoparticles / Activity without the nanoparticles) -100

A constant final concentration of TiO2NPs (1.7 µg/ml) was used to measure the enzyme activity in saliva samples on kinetic studies.

5. Effect of different concentration of substrate on peroxidase activity with and without TiO2 nanoparticles:

Enzymatic reaction was carried out under the same reaction condition using different concentrations of substrate H2O2 [1, 2, 4, 6, 8, 10] mm with Nano and without nanoparticles. The relationship between each substrate concentration and the enzyme activity was plotted in order to determine the optimum substrate concentration for each enzyme activity. Then, by using Line weaver-Burk equation [16]. The relationship between 1\ [V] and 1\[S] was plotted. Apparent Vmax, Km and type of inhibition were evaluated.

6. Effect of pH values on salivary peroxidase activity.

Different buffer solutions with pH values (5.5, 6.0, 7.0, 8.0, and 9.0) were prepared. Peroxidase activity was measured in presence of TiO2 NPs and without NPs in each pH values.

7. Statistical analysis

ata were analyzed using SPSS (statistical package of social science) software version 14. Descriptive statistics: including means, standard deviations.

Results and Discussion

Titanium dioxide nanoparticles solution was absorbed in a peak around 240 nm, as shown in figure [1] which characterized the UV-Vis. Absorption spectra of TiO2NPs solution. And this is similar to another study in 2012 which was indicated the absorbance intensity of GalA-TiO2 NPs dispersion in the UV region < 300 nm.

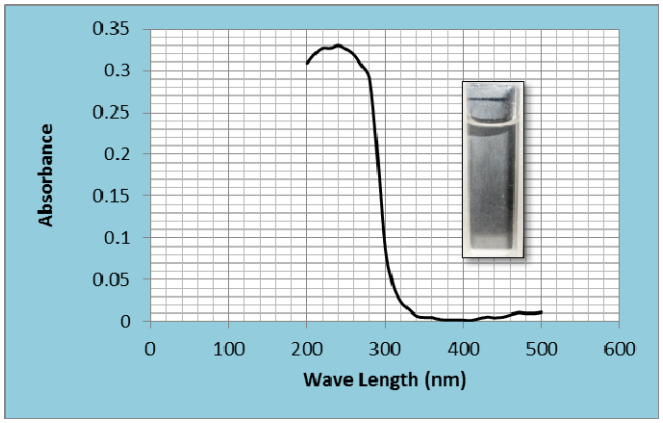

Fig 1: Absorbance spectra of the titanium dioxide nanoparticles

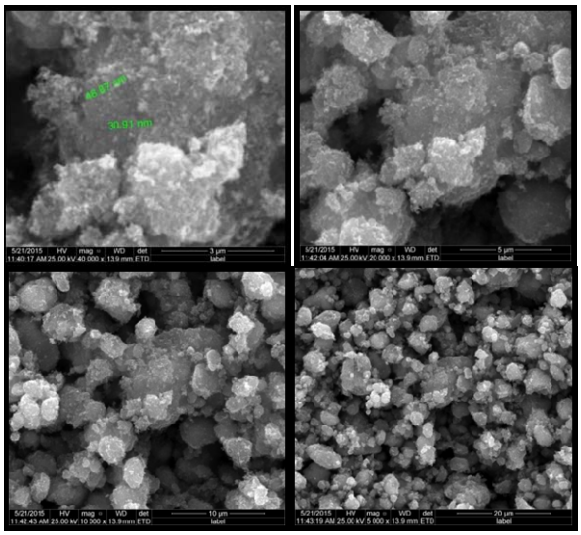

Fig 2: shows SEM pictures and size distributions of titanium dioxide nanoparticles TiO2NPs using in this research, which have the average diameters of 30 nm.

Table (1) shows the difference in mean and standard deviation for total salivary peroxidase activity without NPs and with TiO2 NPs respectively. The results in this study were indicated that TiO2NPs significantly activated salivary peroxidase activity (p=0.00), and the mean and SD of salivary peroxidase activity in absence NPs and in presence of it were (95.38 ± 36.987), and (115.76 ± 37.365) respectively.

Table 1: Total salivary peroxidase activity in absence TiO2 NPs and in presence of NPs.

	N	Means	t	df	p value
Without Nano	21	95.38 ± 36.987	17.938	41	0.000
With Nano	21	115.76 ± 37.365			

In figure (3) the effect of different concentrations of TiO2 NPs on total salivary peroxidase activity were shown. Our results show that with increasing TiO2 NPs concentration, total salivary peroxidase activity was increased.

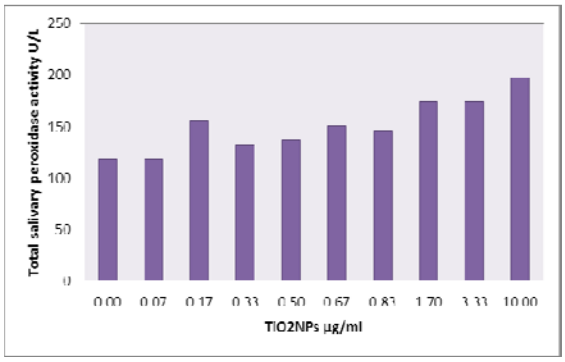

Fig 3: Relationship of total salivary peroxidase activity with different concentrations of TiO2 NPs.

The greater percentage of activation of TiO2 NPs on enzyme activity was 68% at concentration [10] µg/ml as shown in figure (4).

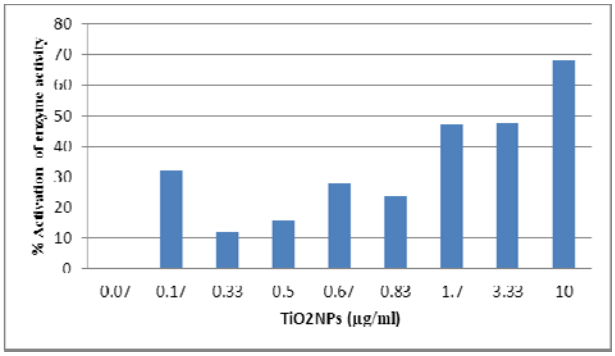

Fig 4: Percentage of activation of total salivary peroxidase activity with different concentrations of TiO2 NPs.

Many studies show an evident conformational change when enzyme interacts with NPs. In one of them, ZnO NPs have been detected to modify the secondary structure of lysozyme, moreover the enzyme keep its catalytic activity and resist to 8M urea denaturation in presence of these NPs [17].

Figure (5) indicated the effect of different concentrations of substrate on total salivary peroxidase activity with and without TiO2NPs.Substrate concentrations (0.17 and 0.25) mole/L were found to be the concentrations that give optimum enzyme activity (141.3) U/L in the presence of TiO2 NPs. (0.25) mol/L of substrate was found to give maximum velocity of enzyme reaction (131.9) U/L without TiO2NPs.

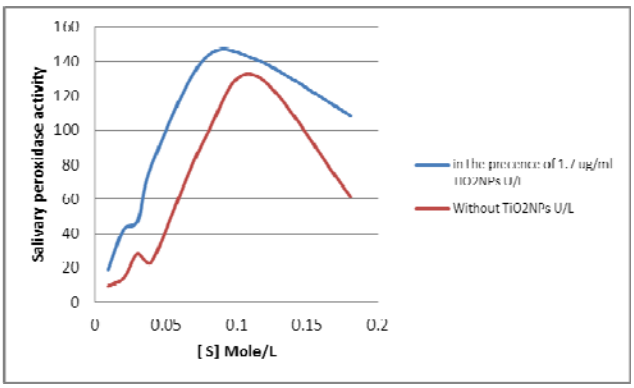

Fig 5: Kinetic profile of salivary peroxidase with and without TiO2NPs.

In a study, Asuri *et al*. 2007 showed that Horsereadish peroxidase and egg white lysozyme retained a high fraction of their activity upon binding to SWNTs. In addition, the SWNT enzymes conjugates being more stable than the non-conjugated enzyme in guanidine hydrochloride and at elevated temperatures, furthermore, a model enzyme, soybean peroxidase, adsorbed onto highly curved surface of C60 fullerenes had an half time 13-fold higher than the free enzyme [18]. Negahdary and Ajdary [19] results demonstrated that moderate concentration of each of gold, silver, and zinc oxide nanoparticles have an increase effect in serum LDH activity as compared with control group in male mice.In other study, an activation effect of gold and silver NPs on serum choline esterase and monoamine oxidase activities were observed [20]. As shown in figure [6], the Vmax and Km for enzyme activity without nanoparticles were (133.33) U/L, and (0.133) mol/L respectively, and (714.3) U/L, (0.357) mol/L in the presence of TiO2NPs.

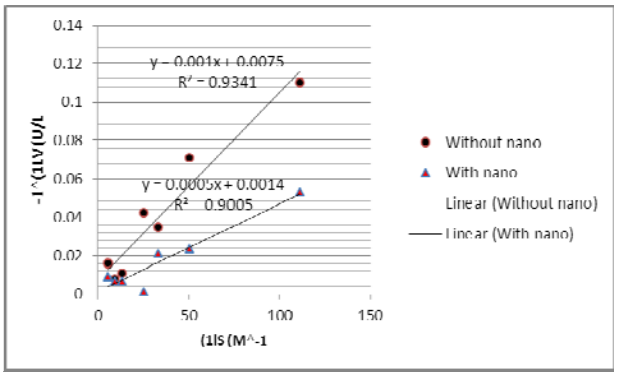

Fig 6: Line weaver-Burk plot of TiO2NPs effect on salivary peroxidase activity.

The effect of pH on enzyme activity in the presence of TiO2NPs was studied in order to realize the nature of binding between the enzyme and TiO2NPs.The results in figure (7) were showed that salivary peroxidase activities were affected in the same way in all pH values in the presence of TiO2NPs except in pH [6], as there was no effect of nanoparticles on enzyme activity. And this is may be related to change in reaction mechanism in the presence of TiO2NPs. So, the activation energy of the complex ES (peroxidase –H202) in the presence of TiO2NPs may be less than this energy without TiO2NPs, at pH [7] 32.3%of activation and at pH [8] 28.6% of activation were observed. From these results it could be concluded that the ionic state of the important groups(on active site of peroxidase) for binding with substrate, is suitable for binding to produce ES complex at pH7and 8.And the enzyme activity reach the optimum value at pH=8 in the presence and absence of TiO2NPs. There was no effect of the presence of TiO2NPs on the enzyme activity at pH= 6 (0% of activation), and we need more kinetic studies in order to explain this effect of pH in addition to protein structure studies to define the salivary peroxidase structure and how it affected by pH value.

In a study, Royal delicious apple peroxidase showed optimum activity at pH 7.0 (Dubey *et al*. 2007) [21]. The pH of peroxidase purified from broccoli (Thongsook and Barrett 2005) [22] and copaifera longolorffii leaves (Maciel *et al*. 2007) [23] was 6.0.

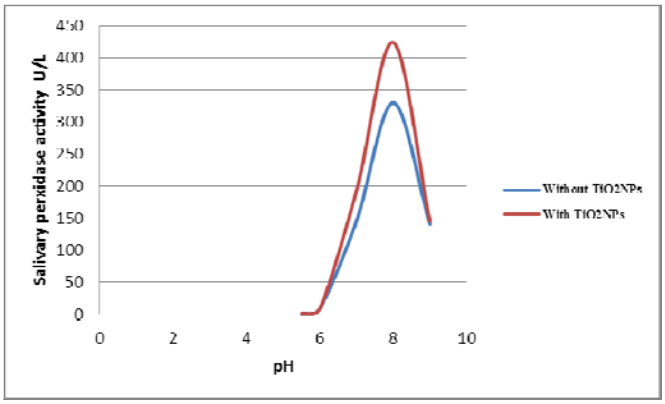

Fig 7: pH effect on total salivary peroxidase activity in absence and presence of TiO2NPs

In summary, the nanoparticles induced protein modification are promising fields for future research. Understanding of such phenomenon is further emphasized by the fact that these materials are used for diagnostic and therapeutic purposes. Total salivary peroxidase activity was activated significantly (p=0.00) by TiO2NPs.

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