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Investigation of the CuO nanoparticles interaction on activity and structure of *Lepidium draba* peroxidase enzyme

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Introduction

Nowadays, the use of nanoparticles due to their unique properties, which are caused by their size and dimensions, is drastically increased (Ball, 2002; Brandes *et al*., 2006). Metal nanoparticles with high specific surface area and high surface reactivity can not only be absorbed by normal physical contact, but also can interact with cells and proteins (Wang *et al*., 2005). Among metal oxide nanoparticles, copper oxide nanoparticles have attracted a lot of attention due to their attractive properties such as high stability, reactivity and high crystal temperature (Chang *et al.*, 2012; Dai *et al.*, 2000).

Peroxidase enzyme is widely distributed in nature and it can be easily extracted from most plant cells, some organs and animal tissues. Plants peroxidase is belongs to oxidoreductases enzyme and contains a heme as a prosthetic group (Welinder, 1976). Due to the importance of peroxidases in biotechnology, recently, the gene encoding the peroxidase enzyme (LDP) from the *Lepidium draba* plant was isolated and after being identified and sequenced, it was cloned and expressed in the *E.coli* host. This enzyme is a polypeptide with a length of 308 amino acids and has the highest similarity with neutral HRP isoenzymes (Fattahian *et al.*, 2017). In this study, alterations in activity and structure of recombinant *Lepidium draba* peroxidase were analyzed in the presence of CuO nanoparticles (nCuO).

Methods and Materials

In order to purify the recombinant LDP enzyme, first the bacteria containing LDP peroxidase gene were cultured in LB culture medium containing kanamycin at 37°C and after OD_{600} reached 0.7, overexpression was done by the use of 1 mM IPTG for a period of 7 hours at a temperature of 18 ºC. The recombinant enzyme was purified by sepharose nickel column (Fattahian *et al.,* 2017). SDS-PAGE gel was used to confirm protein purity and the Bradford's method was used to determine protein concentration (Bradford, 1976). Enzyme activity was measured by the method of Krainer and ko-workers (Krainer *et al.,* 2014).

To investigate the enzyme activity alteration in the presence of CuO nanoparticles, reaction mixture was prepared with a final volume of 1 ml containing of 1 mM H_2O_2, 0.6 mM TMB, 0.071 mg/ml of enzyme and different concentrations of 0, 35, 80, 130, 180, and 210 nM CuO nanoparticles. The reaction mixture was incubated for 4 hours at room temperature. Then the enzyme activity was measured according to the mentioned method. The results were analyzed using SPSS software at a significant level of 5%.

In order to investigate the effects of nanoparticles on enzyme structur fluorescence studies were used. To avoid disturbing the fluorescence spectrum, imidazole and salts in the protein solution were removed using a 10 kDa Amicon filter (Fattahian *et al.*, 2017). The relevant spectra were recorded using a Cary Elips spectrophotometer made in Australia.

The intrinsic fluorescence spectrum of the protein was recorded at the excitation wavelength of 280 nm after 5 minutes of incubation at 298, 303, 308 and 313 ºK in the presence of different concentrations of the nanoparticle. Determination of thermodynamic parameters of enzyme-nanoparticle complex were deduced of intrinsic fluorescence spectrum at different temperature. ANS fluorophore was used to investigate changes in enzyme hydrophobicity in the presence of nanoparticles (Riahi-Madvar *et al.*, 2021).

Results & Discussion

High purity of the enzyme was selected for other experiments as the purified enzymes was separated on SDS PAGE gel. Enzyme activity was affected in the presence of different concentrations of CuO nanoparticles in a process dependent on the concentration, and the activity of the enzyme increased significantly compared to the control sample. Alteration in enzyme activity is probably due to the enzyme structural changes upon interaction with nanoparticles. So intrinsic and extrinsic fluorescence studies were used to investigate the possible changes in enzyme structure in the presence of copper nanoparticles.

Based on the results, the intrinsic fluorescence of the enzyme has decreased in the presence of CuO. The corresponding Stern-Volmer diagram showed that the relationship between the emission reduction rate and nanoparticle concentration is linear. Similar results were reported by Huang and coworkers in the interaction of HSA with CdTe:Zn^{2+} quantum dots (Huang *et al*., 2015), which is consistent with the results obtained in this research. Similar results have also been reported in the interaction of nCuO with HSA (Riahi-Madvar & Ghaseminesab, 2018).

External fluorescence studies was shown the ANS emission decreased after binding to the nan—enzyme complex in compared to the enzyme. These observations are consistent with the results published by Sen and coworkers. They reported that the emission spectrum of ANS fluorescence in albumin bound to gold nanoparticles is lower than that of protein (Sen *et al*., 2011). The decrease in the fluorescence emission of this fluorophore indicates the fact that the surface hydrophobicity of the protein has decreased due to the interaction with this particle.

According to the results, changes in the enzyme activity as well as in intrinsic and extrinsic fluorescence indicated a kind of interaction between enzyme and nCuO. In order to investigate the type of interaction between nCuO and enzyme, thermodynamic parameters were analyzed based on the intrinsic fluorescence studies in the presence of different concentrations of nanoparticles at different temperatures (298, 303, 308 and 313 ºK). Results similar to those observed at 298 ºK (Figure 5) were also observed at higher temperatures (303, 308 and 313 degrees Kelvin). Based on the Stern-Volmer diagram related to fluorescence quenching at different temperatures, it can be concluded that the slope of the corresponding diagram increases with increasing temperature. In other words, with the increase in temperature, the quenching rate of the intrinsic fluorescence related to aromatic amino acids in the presence of different nanoparticle concentrations increases as a function of the nanoparticle concentration. These results are contrary to the observations on the interaction of copper oxide nanoparticles with HSA, which showed that the slope of the Stern-Volmer diagram related to fluorescence quenching decreases with increasing temperature (Riahi-Madvar & Ghaseminesab, 2018). Thermodynamic analysis showed enthalpy and entropy are positive and free energy is negative.

Conclusion

According to the results, it can be concluded that the interaction between nCuO and LDP enzyme proceeds spontaneously and by using hydrophobic interactions. This interaction, which is associated with the structural changes of the enzyme that can be the main reason for the change in enzyme activity.

Keywords*: Fluorescence, Hydrophobic interactions, Thermodynamic parameters.*

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Declaration of conflict of interest

The authors declare that they have no conflicts of interest.