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Probing the interaction induced conformation transitions in acid phosphatase with cobalt ferrite nanoparticles: Relation to inhibition and bio-activity of *Chlorella vulgaris* acid phosphatase

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ABSTRACT

The present study explored the interaction and kinetics of cobalt ferrite nanoparticles (NPs) with acid phosphatase (ACP) by utilizing diverse range of spectroscopic techniques. The results corroborate, the $CoFe_2O_4$ NPs cause fluorescence quenching in ACP by static quenching mechanism. The negative values of van't Hoff thermodynamic expressions ($\Delta H = -0.3293\,J\,mol^{-1}K^{-1}$ and $\Delta G = -3.960\,kJ\,mol^{-1}K^{-1}$) corroborate the spontaneity and exothermic nature of static quenching. The positive value of ΔS ($13.2893\,J\,mol^{-1}K^{-1}$) corroborate that major contributors of higher and stronger binding affinity among $CoFe_2O_4$ NPs with ACP were electrostatic. In addition, FTIR, UV-CD, UV–vis spectroscopy and three dimensional fluorescence (3D) techniques confirmed that $CoFe_2O_4$ NPs binding induces microenvironment perturbations leading to secondary and tertiary conformation changes in ACP to a great extent. Furthermore, synchronous fluorescence spectroscopy (SFS) affirmed the comparatively significant changes in microenvironment around tryptophan (Trp) residue by $CoFe_2O_4$ NPs. The effect of $CoFe_2O_4$ NPs on the activation kinetics of ACP was further examined in *Chlorella vulgaris*. Apparent Michaelis constant (Km) values of 0.57 and 26.5 mM with activation energy values of 0.538 and 3.428 kJ mol^{-1} were determined without and with 200 μM $CoFe_2O_4$ NPs. Apparent Vmax value of $-7\,Umml^{-1}$ corroborate that enzyme active sites were completely captured by the NPs leaving no space for the substrate. The results confirmed that $CoFe_2O_4$ NPs ceased the activity by unfolding of ACP enzyme. This suggests $CoFe_2O_4$ NPs perturbed the enzyme activity by transitions in conformation and hence the metabolic activity of ACP. This study provides the pavement for novel and simple approach of using sensitive biomarkers for sensing NPs in environment.

1. Introduction

Cobalt ferrite ($CoFe_2O_4$) magnetic NPs are used extensively in modern nano-based biomedical therapies and electronic devices [1,2]. This extensive use of $CoFe_2O_4$ NPs results in their dispersal and accumulation into the environment [3], disturbing the ecosystem and inducing toxicity in aquatic organisms by biochemical perturbations [4,5]. The interaction of metal NPs with proteins and enzymes induces biochemical changes by altering their structure. Physicochemical properties like purity, inertness, size, shape and charge etc. also determine their interactions with proteins and enzymes [6,7]. The interactions and alterations in enzyme activity by metallic NPs and ions have been used as an important index of metal toxicity [8,9]. When NPs come in physical contact with bio(macro)molecules, these bio-molecules undergo competitive binding on the surface of the NPs forming the Protein corona, results in alteration of fate and mechanism of nanomedicine and nanotoxicology [10]. Previous studies also showed that corona formation depends on chemical formulation, size, culture conditions, serum concentration, surface decoration and charge of the NPs [11,12].

ACPs have been studied extensively because their dysregulation is associated with various patho-physiological conditions, development of diagnostic and therapeutic methods (e.g., metastatic prostate cancer diagnosis and abnormal bone desorption linked

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to osteoporosis) [13]. ACPs control the metabolism of xenobiotics especially organic phosphates. Besides metabolism, ACPs also control the digestive processes, hydrolysis of phospholipids [14], fertilization [15], assimilation of inorganic phosphate [16] and spore differentiation [17]. Metal NPs (CuO, Ag, ZnO, Cr_2O_3 and Ni) are also deemed have high influence on the proper catalytic activity of ACPs, in turn changes the activities of enzymes i.e.; esterase, β-D-galactosidase, nitrate reductase, urease, ATPase [18,19]. As in mammals, direct attachment of phosphorous to one of both irons of ferritin found in oxidized ACPs [20].

Most importantly, mammalian ACPs share the structural similarities with non-mammalian species such as bacteria, fungi, parasites, and plants [13]. Algae have inherited the ability to purify the aquatic ecosystem by bio-concentration and metabolizing aquatic pollutants i-e; insecticides and PAH's [21,22]. Therefore, an alga is one of the best model organisms used for the toxicity assessment of xenobiotics and NPs as well [1]. In addition, enzyme activity is a sensitive tool extensively used for indicating the quality of microbiological life at different trophic levels [23,24]. Enzyme activity also changes with presence of xenobiotics in the environmental [23]. Physicochemical properties of nanomaterials such as size, shape, coating material and impurities are principle contributing factors in determining the interactions with biomolecules e.g., proteins and enzymes [8,25]. These interactions results in changed conformation, hence the functionality, Nano-Fe_2O_3 higher affinity for biological molecules [26]. There are only few reports about NPs also have an effect on the activity of enzymes e.g., Ureases, dehydrogenases and phosphatases. Acid phosphatase is very sensitive towards CuO, Ni and ZnO NPs [23], comparatively ZnO NPs are less inhibiting than CuO NPs [27].

According to the best of our knowledge, it is the first report describing the detailed mechanism of pristine $CoFe_2O_4$ NPs interaction, adsorption and conformation changes in Acid phosphatase (ACP) by quenching mechanisms, binding parameters, thermodynamic parameters and binding mode. In addition, conformation transformation by protein corona formation was also confirmed by various analytical techniques like TGA, VSM and DLS. The biochemical inactivity of ACP as result of adsorption on NPs and conformation transformation was further confirmed by inhibition of *Chlorella vulgaris* acid phosphatase (CV-ACP) activity. The inhibition of CV-ACP activity triggered by $CoFe_2O_4$ NPs was advanced by enzyme stability, determination of Michaelis constant (Km) and activation energy.

2. Material and method

2.1. Materials

Wheat germ acid phosphatase (ACP), *p*-nitrophenylphosphate (pNPP) and HEPES were purchased from Aladdin industrial Inc., Shanghai. All ACP solutions were prepared in the 0.1 M HBS buffer solution (HEPES = 0.01 M, NaCl = 0.15 M, pH 7.4). ACP solutions were stored in the dark at 4 °C until further use. $CoFe_2O_4$ NPs was purchased from Shanghai future technology, china. Milli-Q water was used to prepare all the suspensions. All the other reagents were at least of AR grade.

2.2. Physicochemical characterization of $CoFe_2O_4$ NPs

The detailed method for determination of physicochemical characterization of $CoFe_2O_4$ NPs have provided in Supplementary method SI#1.

2.3. Steady state fluorescence spectroscopy

All steady state fluorescence spectra were recorded with a SpectraMax M5 Microplate Reader (Molecular Devices, USA) by excitation of ACP at 295 nm and emission was scanned from 300 to 450 nm. Steady state fluorescence spectra were recorded at 298, 303 and 308 K in the range of 300–420 nm. The spectrum bandwidths of excitation and emission slits were both kept at 5.0 nm. The fluorescence intensities of ACP and ACP-NPs conjugate suspensions were corrected for inner filter effect according to the following equation [28].

$$F_{Cor} = F_{obs} Antilog\left(\frac{A_{ex} + A_{em}}{2}\right) \quad (1)$$

F_{cor} and F_{obs} are the corrected and observed fluorescence intensities at the emission wavelength (A_{em}), respectively. A_{ex} and A_{em} are the sum of the absorbance of all components at the excitation and the emission wavelength, respectively.

2.4. Circular dichroism measurement

Circular dichroism (CD) measurements for ACP (2 μM) with $CoFe_2O_4$ NPs concentrations (0, 12.5, 25, 50, 100 and 200 μM) were recorded on a JASCO (J-810) spectropolarimeter with scanning range of 190–260 nm with cell path length was 0.1 cm. The CD results were expressed in terms of the mean residue ellipticity (MRE) in deg cm^2 dem^{-1} according to the following equation:

$$\text{MRE} = \text{CD}_{obs}(\text{m deg})/C_p\text{nl} \times 10 \quad (2)$$

where Cp = molar concentration of the protein, n = amino acid residues (521 for ACP) and *l* = path-length (0.1 cm). The α-helical contents of free and combined BSA were calculated from MRE values at 208 nm using the following equation:

$$\alpha\text{-helix}\,(\%) = -\text{MRE}_{208} - 4000/33000 - 4000 \quad (3)$$

2.5. Synchronous and three dimensional (3D) fluorescence spectroscopy

The synchronous fluorescence spectra of all ACP suspensions were recorded on a F97Pro Spectrofluorimeter with 1 cm quartz cell (Shanghai LengGuang Industrial Co., Ltd., Shanghai, China) at different scanning intervals of Δl ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$) at room temperature. The Δλ values were set at 15 nm and 60 nm, respectively. Three-dimensional fluorescence spectroscopy was conducted with setting excitation 250–330 nm and emission wavelengths from 250 to 450 nm with an increment of 5 nm.

2.6. UV-spectrophotometry

Absorbance spectra were recorded in the range of 190–350 nm, using a double beam spectrophotometer (JASSCO-V550, Japan). Absorption spectra were recorded by keeping the concentration of ACP constant (2 μM) while varying the $CoFe_2O_4$ NPs concentration from 12.5 to 200 μM. Absorbance values were recorded after each successive addition of $CoFe_2O_4$ NPs.

2.7. Fourier transforms infrared (FTIR) spectroscopy

The FTIR spectra of ACP in the presence and absence of $CoFe_2O_4$ NPs were recorded in the range of 600–1800 cm^{-1}. ACP concentration was fixed at 2.0 μM while that of $CoFe_2O_4$ NPs was 2.0 μM in the presence of HBS buffer.