Influence of nanoparticles on thermal stability of aspartate aminotransferase

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Abstract: For the first time, the complex study of influence of gold, titan dioxide and magnetite nanoparticles on the catalytic properties, thermo-inactivation and aggregation of oligomeric enzyme was performed on the example of aspartate aminotransferase. It has been established that coating of nanoparticles with dextran sulphate contributed to the increase of thermostability of mAspAT, which was observed at 60 ^{0}C and higher. The antiaggregation strength of nanoparticles can be ranged as follows: TiO_2 NP > Au NPs > Fe_3O_4 NPs. The aim of the research - comparative study of the kinetic of thermal inactivation of mitochondrial aspartate aminotransferase (mAspAT) in the presence of native and dextran sulfate-modified TiO_2 and Fe_3O_4 nanoparticles (NP). Both, native and dextran sulphate-modified NPs showed the strongest thermal protection at 60 ^{0}C and above. The thermal inactivation rate constant (k_{in}) of mAspAT was significantly decreased in the presence of NP-TiO_2. Modification of NP surface with dextran sulphate enhanced that effect. Magnetite NP had revealed lower thermal protecting properties. Structural stability of mAspAT in the presence of NPs was characterized by the following thermodynamic parameters: E_a^{in} (inactivation energy), ΔH (enthalpy), and ΔS (entropy) and ΔG (Gibbs free energy). In conclusion, interaction between mAspAT and NPs leads to increase of conformational rigidity of the enzyme and depends mainly on the nature of NP. Stability of gold colloid nanoparticles (Au NPs) is dependent on many factors like buffer concentration and pH values of medium, as well the recombinant AspAT can protect gold colloid nanoparticles from aggregation caused by influence of acidity of buffer or medium.

Keywords: Au, TiO_2, Fe_3O_4, nanoparticles, aspartate aminotransferase, thermoinactivation

Introduction

Nanomaterials have widely been used in biotechnology and medicine as wrote (1). A contact between nanomaterials and a protein, major component of biological environment, is inevitable. There are only few papers devoted to study the interaction of TiO_2 and Fe_3O_4 nanoparticles (NPs) with enzymes. Majority of them has been conducted with relatively simply proteins consisting of one polypeptide chain (1-4). Gold nanoparticles (GNPs) that couple with biomolecules are of great current interest because of their biomedical applications. Protein interaction with GNPs depends on various factors such as the shape of the nanoparticles, temperature, salt concentration, surface coating layer and others (5). The aim of this work is to study the thermal stability of enzyme comprising two polypeptide chains and two molecules of vitamin B_6 as coenzyme in the presence of TiO_2 and Fe_3O_4 NPs. Mitochondrial aspartate aminotransferase (mAspAT) was chosen as a model object. To investigate the influence of pH on aspartate aminotransferase (AspAT) adsorption on the surfaces of citrate modified gold nanoparticles (6 - 8).

Materials and methods

The mAspAT was isolated from a pig heart by column chromatography (9). Solution of native TiO_2 and Fe_3O_4 nanoparticles (NP TiO_2 and NP Fe_3O_4) and TiO_2 and Fe_3O_4 NPs modified with dextran sulfate (NP TiO_2-DS and NP Fe_3O_4-DS) was prepared as described. To study the influence of NPs on the thermal inactivation of mAspAT, enzyme was incubated with or without NPs at 50, 55, 60, 65 and 70 °C. Samples were taken after 1, 2, 3, 5, 10, 20, 30 and 40 min of incubation and placed into ice bath. Then, the residual activity of mAspAT was measured according Karmen (10). All measurements of enzyme activity were carried out in the 50 mM phosphate buffer at pH 6.8. Thermal inactivation of mAspAT was characterized by inactivation rate constant (k_{in}) of pseudo first order in min^{-1} (11-13). That was determined from the time dependence of A_i/A_0, where A_0 is activity of the initial enzyme sample, and A_i is activity of the same sample after heat treatment. From the data obtained, we calculated the values of activation energy for the inactivation process (E_a^{in}), Gibs free energy (ΔG), entropy (ΔS) and enthalpy (ΔH).

Results and discusson

It is known, that protein sensitivity to heating is one of the most efficient tests of their conformational stability. Analysis of time course of AspAT thermal inactivation shows that at the temperature below 65 °C this process occurs in one stage, whereas at 65 °C and higher, thermal inactivation has already two phases, slow and fast as shown in Figures 1 and 2.

Figure 1: Thermal stability of mAspAT at 65 °C in presence of NPs Fe_3O_4

1= mAspAT (control), 2 = mAspAT +NPs Fe_3O_4, 3 = mAspAT+NPsFe_3O_4 –CD.

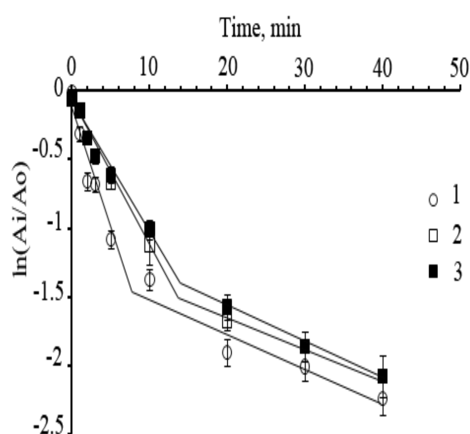

Figure 2: Thermal stability of mAspAT at 65 °C in presence of NPs NPs TiO_2

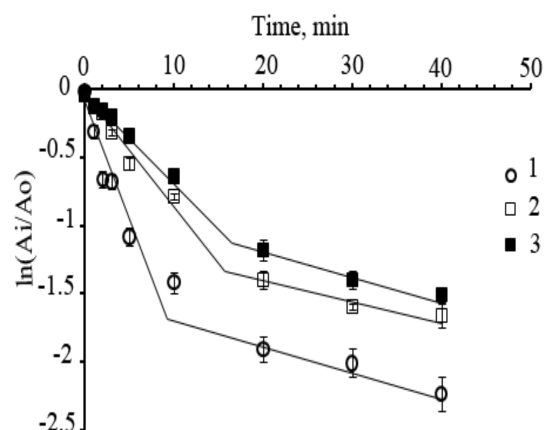

Figure 2: Thermal stability of mAspAT at 65 °C in presence of NPs NPs TiO_2

= mAspAT (control), 2 = mAspAT +NPs TiO_2, 3 = mAspAT+NPs TiO_2 –CD

Biphasic nature of the thermal inactivation suggests transition of enzyme into more stable, but less catalytically active state. Xiue Jiang et al. showed in their work that the adsorbed cytochromes on Gold nanoparticles surfaces became more thermally stable (14), thermal stability of trypsin is more increased in comparison with free trypsin (15), glucose oxidase adsorbed on surface of Gold nanoparticles or $CoFe_2O_4/SiO_2$ (16) is more thermally stable than free glucose oxidase. Comparison of inactivation rate of free mAspAT and adsorbed on the surface of NPs mAspAT clearly demonstrate that NPs stabilize it.

The interaction of proteins with surface modified nanoparticles at neutral pH is based on hydrogen bonds and electrostatic bonds (17). Our results showed that molecules of mAspAT adsorbed on the surface of dextran coated metal oxides nanoparticles have more rigid conformation, this rigidity leads to more thermal stability of mAspAT molecules.

Herewith, TiO_2 NPs provide more pronounced protection of mAspAT against thermal inactivation compared with Fe_3O_4 NPs. Modification of NPs with DS significantly enhance thermal protection effect of TiO_2 NPs, but not of magnetite NPs. Coating of Fe_3O_4 NPs with DS did not affect k_{in} in the wide temperature range whereas, in the presence of NP TiO_2-DS, thermal inactivation rate of mAspAT at 60, 65 and 70 °C was 2.04, 3.13 and 2.3-fold lower compared with control, respectively. At he highest extent, thermal protection effect of NP-TiO_2-DS was observed at the temperature close to $t_{1/2}$. To better understand the mechanism of NPs action on thermal inactivation, we calculated thermodynamic

parameters of the fast stage of this process. We showed, that enthalpy of activation of the mAspAT thermal inactivation does not depend on the NP type and surface

According to our results shown in Table1 both native and DS-treated NPs demonstrated the strongest thermal protective effect at 60 °C and above.

Table 1: Influence of Fe_3O_4 and TiO_2 NPs on thermal inactivation rate of mAspAT

Parameters / Samples	k_{in}, sec^{-1} $(x10^{-5})$	
	60 ^{0}C	65 ^{0}C
mAspAT - control	98±4.5	360±22
mAspAT+NP TiO_2	61±3	183±15
mAspAT+NP TiO_2 DS	48±7.7	115±9.5
mAspAT+NP Fe_3O_4	80.5±1.5	221±15.6
mAspAT+NP Fe_3O_4 DS	72±5.1	204±19

modification with DS. The value of enthalpy of the process activation lies within the range of most proteins. Of note, mAspAT E_a and ΔH values significantly depend on the NP type and surface modification with DS. Similarly, substantial decrease of the ΔS value was observed for NP TiO_2 and NP TiO_2-DS. This fact can indicate the formation of more “rigid” enzyme conformation during interaction with NP-TiO_2. This more “rigid” conformation of mAspAT is determined by the higher number of weak interactions that should be disrupted during heat treatment. In conclusion, NPs provide a “stabilizing effect” for the enzyme molecule that prevents faster thermal inactivation. This results in a longer time of temperature treatment and/or higher temperatures that should be reached to

inactivate NP-TiO_2 and NP-TiO_2-DS treated mAspAT compared with untreated enzyme.
Thus, interaction of mAspAT with NPs leads to increase of conformation stability of the enzyme, which is predetermined by the nature of NP and can be enhanced by the coating of NP surface with DS. Our findings can be useful for the development of stabilizing conditions for enzymes used in high-temperature bioreactors.

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