
Influence of nanoparticles on thermal stability of aspartate aminotransferase

Abdulati El Salem¹, Waleed RA Abusittah² and Mahmud El Abushhewa^{3*}

¹Department Biochemistry, Faculty of Medicine, Alasmarya Islamic University

²Department of Pediatric, Faculty of Medicine, Azzaytuna University

³Department Biochemistry and Molecular biology, Faculty of Medicine, Azzaytuna University, Libya

Correspondence: alhmroni832004@yahoo.com.au

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 °C and higher. The antiaggregation strength of nanoparticles can be ranged as follows: TiO₂ NP > Au NPs > Fe₃O₄ 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 TiO₂ and Fe₃O₄ nanoparticles (NP). Both, native and dextran sulphate-modified NPs showed the strongest thermal protection at 60 °C and above. The thermal inactivation rate constant (k_{in}) of mAspAT was significantly decreased in the presence of NP-TiO₂. 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, TiO₂, Fe₃O₄, 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}), Gibbs 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

Figure 1: Thermal stability of mAspAT at 65 °C in presence of NPs Fe₃O₄

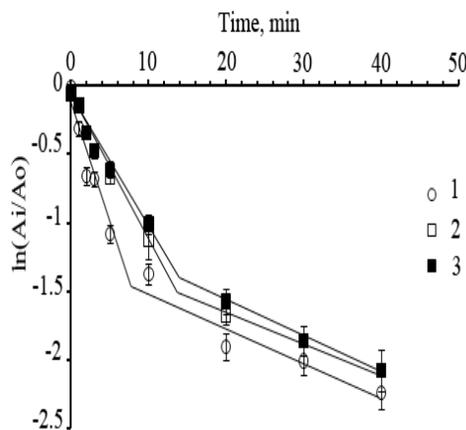


Figure 2: Thermal stability of mAspAT at 65 °C in presence of NPs NPs TiO₂

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

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.

1= mAspAT (control), 2 = mAspAT +NPs Fe₃O₄, 3 = mAspAT+NPsFe₃O₄ –CD.

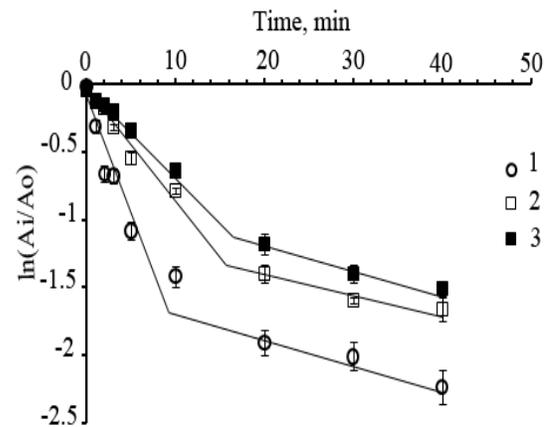


Figure 2: Thermal stability of mAspAT at 65 °C in presence of NPs NPs TiO₂

= mAspAT (control), 2 = mAspAT +NPs TiO₂, 3 = mAspAT+NPs TiO₂ –CD

oxidase adsorbed on surface of Gold nanoparticles or CoFe₂O₄/SiO₂ (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, TiO₂ NPs provide more pronounced protection of mAspAT against thermal inactivation compared with Fe₃O₄ NPs. Modification of NPs with DS significantly enhance thermal protection effect of TiO₂ NPs, but not of magnetite NPs. Coating of Fe₃O₄ NPs with DS did not affect k_{in} in the wide temperature range whereas, in the presence of NP TiO₂-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 the highest extent, thermal protection effect of NP-TiO₂-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 Fe₃O₄ and TiO₂ NPs on thermal inactivation rate of mAspAT

Parameters Samples	$k_{in}, \text{sec}^{-1} (\times 10^{-5})$	
	60 °C	65 °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 ₃ O ₄	80.5±1.5	221±15.6
mAspAT+NP Fe ₃ O 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 TiO₂ and NP TiO₂-DS. This fact can indicate the formation of more “rigid” enzyme conformation during interaction with NP-TiO₂. 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-TiO₂ and NP-TiO₂-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.

References

1. Yu X, et al. (2016) Design of nanoparticle-based carriers for targeted drug delivery. *J Nanomaterials*. 15: 2016.
2. Yin J, et al (2016) Stimuli-responsive block copolymer-based assemblies for cargo delivery and theranostic applications. *Polymers*: 8 (7): 268.
3. Siegler EL, et al (2016) Nanomedicine targeting the tumor microenvironment: therapeutic strategies to inhibit angiogenesis, remodel matrix, and modulate immune responses. *J Cellular Immunother*. 2(2): 69 – 78.
4. Werner M, et al. (2018) Nanomaterial interactions with biomembranes: bridging the gap between soft matter models and biological context. *Biointerphases*. 3(2): 028501.
5. Ventola CL (2017) Progress in nanomedicine: approved and investigational nanodrugs,” *Pharmacy and Therapeutics*. 42(12): 742 – 755.
6. Tran S, et al. (2017) “Cancer nanomedicine: a review of recent success in drug delivery,” *Clinical and Translational Medicine*. 6(1): 44.
7. Souery WN, Bishop CJ (2018) Clinically advancing and promising polymer-based therapeutics. *Acta Biomaterialia*. 67(1): 20.
8. Ma X, Zhao Y (2015) Biomedical applications of supramolecular systems based on host–guest interactions. *Chem Reviews*. 115(15): 7794 – 7839.
9. Barra D. et al. (1976) Large-scale purification and some properties of the mitochondrial aspartate aminotransferase from pig heart. *Eur. J. Biochem*. 64(23):519–526.
10. Karmen A. (1955) A note on spectrometric assay of glutamic oxalacetic transaminase. *J. Clin. Invest*. 34:131-133.

11. Golub N. et al. (2008) Thermal inactivation, denaturation and aggregation of mitochondrial aspartate aminotransferase. *Biophys. Chem.* 135:125-131.
12. Lombardo D (2015) Amphiphiles self-assembly: basic concepts and future perspectives of supramolecular approaches. *Advances in Condensed Matter Physics.* 22:2015.
13. Wang J, Li S, Han Y, et al. (2018) Poly(ethylene glycol)–polylactide micelles for cancer therapy. *Frontiers in Pharmacol.* 9:202.
14. Xiue J, et al. (2005) Cytochrome c superstructure biocomposite nucleated by gold nanoparticle: thermal stability and voltammetric behavior. *Biomacromolecules.* 6: 3030 – 3036.
15. Ding D, Zhu Q (2018) Recent advances of PLGA micro/nanoparticles for the delivery of biomacromolecular therapeutics. *Materials Science and Engineering: C.* 92: 1041–1060.
16. Bodratti A and Alexandridis P (2018) Formulation of poloxamers for drug delivery. *J Functional Biomaterials.* 9(1): 11.
17. Quiñones JP, Peniche H, Peniche C (2018) Chitosan based self-assembled nanoparticles in drug delivery. *Polymers.* 10 (3): 235.