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Original Article

Thermodynamics of Mitochondrial Aspartate Aminotransferases in the Presence of Nanoparticles

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ABSTRACT

Background and objectives. *The nanoparticles are extensively used in the fields of biotechnology, the medical biochemistry and nanomedicne for enhancement and improvement drugs targeting in the chemotherapy. This study was conducted firstly, to understand the underlying mechanisms and potential applications of the thermo-inactivation kinetics of mitochondrial aspartate aminotransferase (mAspAT) in the presence of various nanoparticles, specifically TiO2 and Fe3O4, as well as gold nanoparticles (AuNP), secondly, to determine the impacts of nanoparticle concentration on the aggregation process and the influence the conformational stability and thermal behavior of mAspAT.* ***Methods***. *The mAspAT was isolated from the pig heart, and SDS electrophoresis was used to determine the level of the protein's purity. A solution of native and modified dextran sulfate nanoparticles was prepared and incubated with mAspAT at various temperatures. The thermo-Inactivation was carried out at different temperatures (45, 50, 55, 60, 65, and 70 °C) and the aggregation of mAspAT was studied at 320 nm using spectrophotometry. The output data was manipulated (Calculations) and analyzed by Software Stadia 6.0.* ***Results***. *TiO2 and Fe3O4 nanoparticles, both native and dextran sulfate-coated, demonstrated thermoprotective effects on mAspAT at temperatures above 60 °C. The interaction of mAspAT with TiO2 nanoparticles resulted in a decrease in thermoinactivation energy (Ea), indicating increased conformational stability. Therefore, constant thermoinactivation rate (kin) of mAspAT was significantly decreased in the presence of TiO2 nanoparticles. The dextran sulfate modification further enhanced this effect.* ***Conclusion***. *The study concludes that the interaction of mAspAT with NP results in an increase in the conformation rigidity of the enzyme, which is primarily determined by the nature of NP and can be increased by coating the surface of NP with a polymer dextran sulfate. Also, Gold nanoparticles could potentially be used to increase the stability of mAspAT, preventing it from thermoaggregation.*

Keywords: Aspartate Aminotransferase, Thermostability, Nanoparticles, Thermoinactivation.

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INTRODUCTION

Nanomaterials and nanotechnology have seen extensive use in a variety of fields in recent years. They have applications in agriculture, biology, and medicine [1]. Among their many uses are in the cosmetics and medicine industries, in diagnosis, in the immobilization of proteins, enzymes, and antibodies, in drug delivery systems, in magnetic

biosensors, and in the collection of biological images [2]. Nanoparticles are becoming increasingly commonplace in technological settings. If this trend continues, more and more biological systems will be put at risk from exposure to nanoparticles. Therefore, it is crucial to understand the impact of nanoparticles on oligomeric proteins and cellular metabolism, in addition to how biological objects like proteins interact with nanoparticles [3,4]. Few studies have investigated the effect of nanoparticles on the thermostability of proteins other than the more complex ones, such as bovine serum albumin (BSA) [5,6], ribonuclease and trypsin [6,7]. We have previously shown that adsorption interactions underlie the possibility of interaction between the oligomeric protein of the mitochondrial isoenzyme AspAT (L-aspartate 2-oxoglutarate aminotransferase mAspAT) and TiO_2, Fe_3O_4, and colloidal gold (AuNP) [8]. Proteins' sensitivity to heat is well known to be one of the most reliable techniques for detecting conformational changes in molecules. We opted to take a thermodynamic approach to study mAspAT's thermo-inactivation in the presence of NP. The purpose of this study was to compare the kinetics of thermo-inactivation of mAspAT when treated with native (TiO_2 NP, Fe_3O_4 NP) and modified (dextran sulfate TiO_2, dextran sulfate Fe_3O_4) nanoparticles.

METHODS

mAspAT was extracted from the pig heart by with modification [9] and its purity was evaluated using SDS electrophoresis on a 12 percent polyacrylamide gel. After converting to 50 mmol/l phosphate buffer with pH 7.5, 0.5 mmol/l EDTA, 20% glycerol, 0.05% thimerosal, and sterilizing with ultrasound, the enzyme was anchored into sterile bottles and stored at +2 degrees Celsius.

A solution of native nanoparticles (TiO2 NP and Fe3O4 NP) and modified dextran sulfate nanoparticles (TiO2DS NP and Fe3O4DS NP) was prepared as previously described by us, taking into consideration the recommendations of [10]. This is how the effect of nanoparticles on the thermostability of mAspAT activity was investigated:

1500 μl of a 50 mg/l solution of native and coated dextran sulfate nanoparticles was centrifuged at 12000 g for 5 minutes, and the supernatant liquid was discarded. The precipitation was added to 1500 μl of mAspAT (5 μg/ml), suspended by pipetting, mixed on a vortex, and incubated at room temperature for 40 minutes. The enzyme was then incubated for 10 minutes in a water bath at temperatures of 50, 55, 60, 65, and 70 0C in closed Eppendorf probes. The control samples were enzymes devoid of nanoparticles. After the incubation, the samples were placed in an ice bath. The residual activity was expressed as a proportion of the unheated sample's activity.

To determine the effect of native or modified sulfate dextran NP on the thermo-inactivation of mAspAT, the enzyme was incubated at 50, 55, 60, 65, and 70 °C with NP. After 1, 2, 3, 5, 10, 20, 30, and 40 minutes of incubation, the samples were collected and placed in an ice bath. The residual activity was then identified. All mAspAT activity measurements were performed at room temperature and 50 mmol/l phosphate buffer solution, pH 6.8 concentration. The rate of thermo-inactivation of mAspAT was characterized by the constant speed (kin) of the pseudo-first-order reaction. in min-1, which was determined based on the dependence of AI/A0 on time, where A0 is the activity of the original enzyme sample and AI is the activity of the identical sample after thermal treatment. According to [11], the activation energy (Ea), free energy, entropy, and enthalpy values of the thermo-inactivation process were calculated from the obtained data.

The protein concentration was determined by Peterson [12] and 280 nm absorption. For the mAspAT, use the A1 percent value of 280 = 14.0 [9]. The activity of mAspAT was measured using the spectrophotometric method [13], as described previously, at room temperature and concentrations of 50 mmol/l and 20 mmol/l of phosphate buffer solution, pH 6.8 and 20.

The following formulas are used to calculate thermodynamic parameters [14]:

$Ea_{in} = - R * tg\alpha$ (1)

$\Delta G = -2{,}303*R*T*[lg(k_{in}/T)-lg(R/N*h)]$ (2);

$\Delta H = Eain-R*T$ (3)

$\Delta S=(\Delta H-\Delta G)/T$ (4)

where:

Eain - activation energy of the thermo-inactivation process; ΔG - Gibbs' free energy;

ΔH - enthalpy of the thermo-inactivation process; ΔS - entropy of the thermostabilization process;

*tgα - the tangent of the angle of inclination of the direct Arrhenius dependence; T - temperature in degrees Kelvin; R - universal gas constant - 8,314 J / K * mol;*

N – Avogadro constant $6{,}02*10^{23}$; h – Planck constant, $6{,}6252*10^{-34}$.

k_{in} is the constant of the speed of the thermo-inactivation reaction at the temperature of Ti.

The equations (1–4) are utilized to calculate the constant of the initial thermos-inactivation rate. The results are processed statistically and represent the mean of five independent experiments. Excel was utilized for calculations and graph construction.

The constant thermoinactivation of aspartate aminotransferase (mAsAT) is the rate of irreversible thermal inactivation of the enzyme, which depends on the temperature and the activation energy for the inactivation reaction. The higher the temperature and the lower the activation energy, the faster the enzyme will lose its activity due to denaturation.

The activity of an enzyme is also affected by other thermodynamic parameters, such as the catalytic activation energy, the enthalpy, entropy, and Gibbs energy of the reaction, and the equilibrium constant of the active-inactive transition. These parameters determine how fast and how efficiently the enzyme can catalyze its substrate into products, and how sensitive the enzyme is to changes in temperature and pH. For example, a lower catalytic activation energy means that the enzyme can overcome the energy barrier for the reaction more easily, resulting in a higher rate of activity. A higher enthalpy of reaction means that the reaction is more endothermic and requires more heat input, resulting in a lower rate of activity. A higher entropy of reaction means that the reaction is more disorderly and spontaneous, resulting in a higher rate of activity. A higher Gibbs energy of reaction means that the reaction is less favorable and requires more work input, resulting in a lower rate of activity. A higher equilibrium constant of the active-inactive transition means that the enzyme is more likely to be in its active form and less likely to be inactivated by temperature or pH changes, resulting in a higher rate of activity [15].

Aggregation of aspartate aminotransferase

The effect of AuNP on the thermoaggregation of mAspAT was studied at 320 nm and carried out as follows: 500 µl of the AuNP solution (50 mg/l) centrifugated at 16000g * 27 min. The supernatant was removed and 500 µl of mAspAT (0.5 mg/ml) was added to the NP sediment in a 5 mmol/l sodium phosphate buffer with a pH of 6.8 and incubated for 40 min to adsorb mAspat on the NP surface. The control sample was tested without NP. Experimental samples of the enzyme without and with AuNP s were incubated at 55, 60, 65, 73 °C. The degree of aggregation of mAspAT was measured by changing the absorption at 320 nm every 2 min for 20 min [16].

The dependency of the thermoaggregation of mAspAT on the concentration of NP has studied on the Carry 50 Bio (Varian, Australia) spectrophotometer at 320 nm according to the following scheme: 500 µl of NPs solution with a concentration 6,25, 12,5 25, 50 mg/l centrifugated in plastic tubes of the Eppendorf tube at 16000g 27 min for the deposition of nanoparticles. The supernatant was removed and 500 µl of mAspAT solution at a concentration of 0.5 mg/ml in 5.0 mmol/l phosphate buffer, pH 6.8 was added to the sediment.

The samples were incubated for 40 minutes at room temperature. The dependency of the aggregation on the concentration of NPs was studied at 55 °C and the following Au NPs concentrations: 1) control – contained no NPs, 2) 6.25 mg/l, 3) 12.5 mg/L, 4) 25 mg/ l, 5) 50 mg/ L Au NPs.

Incubation duration 20 min. The constant of the speed of aggregation was calculated by the method proposed by Kurganov [17]. Protein content was measured using the Peterson method [12] or by absorption at 280 nm, using A1% value 280 = 14,0 and determined for mAspAT [18].

The results are presented as an arithmetic average plus/minus standard error of the average (X ± Sx). The results were processed using the software Excel or Stadia 6.0. All experiments were conducted at least five times.

RESULTS AND DISCUSSION

The value t1/2, which indicates the temperature at which 50 percent of an enzyme's activity is lost, is one of the most widely accepted parameters for describing the thermal stability of biopolymers. The value of t1/2 is determined by the protein's activation-free energy. Changes in t1/2 are directly related to changes in the resistance of the adsorbed protein to conformation shifts that occur with it on nanoparticles [19].

In the initial phase of our research, t1/2 mAspAT was evaluated in the presence of native and modified dextran sulfate TiO2 NP sulfate (Figure 1). Consequently, for the control (enzyme free from NPs) mAspAT t1/2 = 62 0C, which is consistent with the data [20], whereas for mAspAT adsorbed to TiO2 NP and DS TiO2 NP, t1/2 increased to 65 0C and 67,5 0C, respectively, indicating an increase in the thermal stability of the enzyme in interaction with the nanoparticles.

mAspAT activity at 60 0C was compared to determine the contribution of native and modified DS nanoparticles to the preservation of enzyme activity during heating. In the absence of TiO2 NP and after 20 minutes of incubation at this temperature, mAspAT retains 55.4% of its activity.

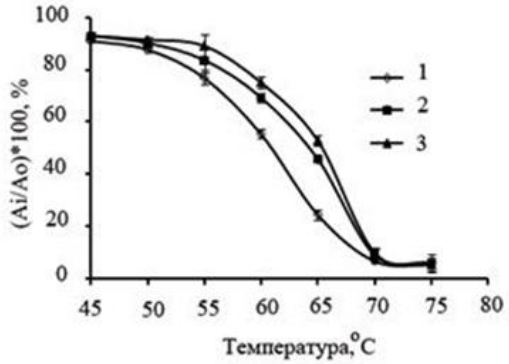

Figure 1: The influence of intact and modified dextran sulfate TiO2 NP on the thermoinactivation of mAspAT. 1) Control - mAspat without NP; 2) mAspAT with TiO2 NP; 3) mAspAT with dextran sulfate coated TiO2 NP. Twenty-minute incubation time.

The addition of native or DS TiO2 NP increases the enzyme's thermal stability. Therefore, the activity of mAspAT is retained by 69.2% and 75.8%, respectively, when compared to the enzyme activity with TiO2 NP and no thermal treatment (Figure 1). Thus, the sulfate coating of dextran sulfate-coated nanoparticles contributes to a 20% increase in the thermal stability of mAspAT. Due to the enzyme's multi-point binding to the nanoparticles, this increase in the stability of the adsorbed mAspAT is most likely accompanied by an increase in conformational rigidity. According to [21], electrostatic and hydrophobic interactions, hydrogen gluing, and other types of interactions serve as the primary stabilizing forces for the multi-point binding of nanoparticles to proteins.

Based on a thermodynamic approach, the impact of the interaction of native and modified dextran sulfate TiO2 NP and Fe3O4 sulfate with AspAT on enzyme thermoactivation was evaluated. Previously, we have demonstrated that the catalytic and kinetic properties of mAspAT change upon interaction with TiO2 NP, but the nature of these changes has not been determined.

Because the sensitivity of proteins to heat is one of the most effective tests for conformational stability [11], the kinetics of the thermo-inactivation process of mAspAT in the presence of native and coated with

dextran sulfate TiO2 NP, Fe3O4 NP, and the corresponding thermodynamic parameters were investigated to determine the effects of nanoparticles on this parameter. mAspAT was thermoinactivated at 45, 50, 55, 60, 65, and 70 0C, with a pH of 6.8 and a phosphate buffer solution concentration of 5 mmol/l. As previously demonstrated, when measuring mAspAT activity, the concentration of the phosphate buffer was increased to 50 mmol/l to dissociate mAspAT from the surface of NP. The outcomes are depicted in Figure 2, where the thermo-inactivation rate is a function of temperature and time.

Analysis of the dependence of thermo-inactivation of mAspAT on exposure time reveals that at temperatures up to 65 °C, the process is uni-stage, whereas at temperatures above 65 0C, the process occurs in two stages - fast and slow.

Moreover, as the temperature rises, the time of appearance of the fracture on the straight, which characterizes the dependence of ln(Ai/A0) on the duration of incubation, decreases (Figure 2). The two-phase process indicates the transition of the enzyme to a more stable but less catalytically active state [22], which is consistent with Tsou's theory that the active center of the enzyme is more conformationally labile than the rest of the protein molecule [23].

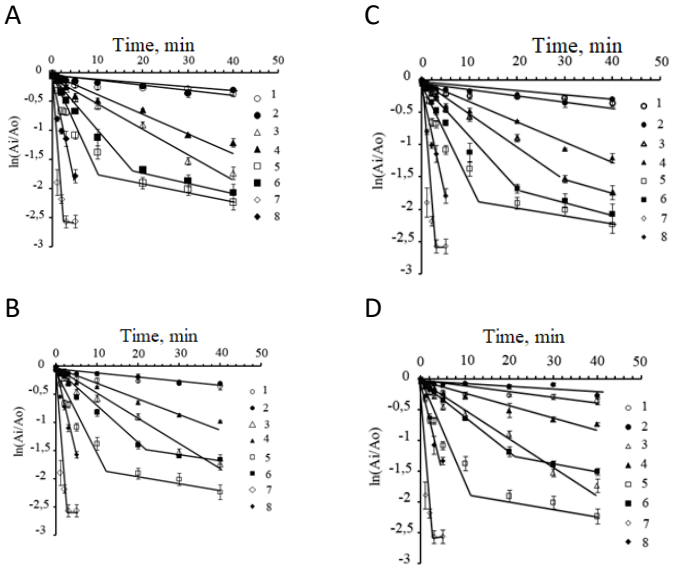

Figure 2. The effects of TiO2 NP and Fe3O4 on the thermo-inactivation kinetics of mAspAT. ***A) mAspat + indigenous TiO2 NP; B) mAspatAT + DS TiO2 NP; C) mAspatAT + Fe3O4 NP; D) mAspat + indigenous DS Fe3O4 NP; 1,3,5,7 enzymes devoid of nanoparticles; 2,4,6,8 enzymes containing nanoparticles. mAspAT samples were heated to the following temperatures: 1,2 – 55 °C; 3,4 – 60 °C; 5,6 – 65 °C; and 7,8 – 70 °C. A0 represents the activity of the original enzyme sample; Ai represents the activity after thermal treatment.***

Comparing the rate of inactivation of free and adsorbed mAspAT on the surface of NP demonstrates conclusively that NP stabilizes mAspAT. As the temperature rises, the distinction between the inactivation rates of free and nanoparticle-associated mAspAT becomes evident.

To assess quantitatively the effect of NP on the thermo-inactivation of mAspAT from semi-logarithmic anamorphosis (Figure 2), the constant of the speed of the first stage of thermo-inactivation was calculated.

(kin). The outcomes are shown in Table 1. It is noted that both native and modified dextran sulfate NP demonstrate their thermoprotective properties most effectively at temperatures above 60 °C. Moreover, NPs based on TiO2 exhibit the greatest resistance to mAspAT thermoinactivation.

This effect is significantly enhanced by the addition of sulfate to dextran, which cannot be said for magnetite nanoparticles. Their dextran sulfate coating did not affect kin throughout the vast majority of the temperature range (Table 1).

Table 1: Influence of TiO2 and Fe3O4 nanoparticles on mAspAT thermo-inactivation rate constants

Parameters	Value of k_{in} in sec^{-1} ($x10^{-5}$)			
	55 ^{0}C	60 ^{0}C	65 ^{0}C	70 ^{0}C
mAspAT–control	22±1,8	98±4,5	360±22	1430±131
mAspAT+ TiO_2 NP	15±0,6	61±3	183±15	610±41
mAspAT+ TiO_2DS NP	9,7 ±0,3	48±7,7	115±9,5	596±58
mAspAT+ Fe_3O_4 NP	19,5±0,34	80,5±1,5	221±15,6	634±69
mAspAT+ Fe_3O_4DS NP	20±1,3	72±5,1	204±19	634±51,3

In the presence of DS TiO2NP, the thermo-inactivation rate of mAspAT at 60, 65, and 70 ° C decreased by 2.04, 3.13, and 2.3 times, relative to the control, at 60, 65, and 70 ° C, respectively. Notably, the thermoprotective effect of TiO2 DS NP was most pronounced at temperatures close to t1/2.

To comprehend the mechanism of action of nanoparticles on the thermo-inactivation of mAspAT, the thermodynamic parameters of the rapid stage of this process were calculated [22] based on the Arrhenius dependence of the logarithm of the constant of thermos-inactivation speed on temperature (Figure 3). The results of the calculations are shown in Table 2. It has been demonstrated that the enthalpy of the activation of the mAspAT thermo-inactivation process is independent of the type of NP and the modification of their surface by dextran sulfate and that its value falls within the range typical of the majority of proteins.

In addition, it should be noted as shown in Table 2, that the values of Ea and Δ H mAspAT are highly dependent on the type of NP and the dextran sulfate modification of their surface. The thermo-inactivation energy decreased by 25% in the presence of TiO2 NP. The interaction between mAspAT and DS TiO2 NP decreased this value by 40%.

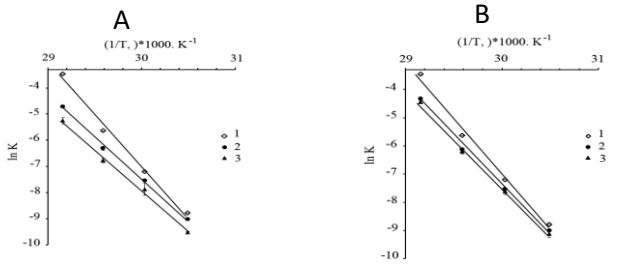

Figure 3: The influence of NP on temperature dependence is the constant of the thermo-inactivation rate of mAspAT. A) TiO2 NP; B) Fe3O4 NP. 1) Standard - mAspAT without NP; 2) mAspAT with indigenous NPs; 3) mAspAT with NP coated with dextran sulfate; (ln k) - the constant of the thermo-inactivation rate of mAspAT in minutes per minute; T is the temperature in Kelvin degrees.

In the dynamics of NP's changes, a very close resemblance emerged. In the presence of Fe3O4 NP and DS Fe3O4 NP, however, the variations in these parameters were significantly less pronounced. Attention is drawn to a significant decrease in the value of S, which, as in the previous case, was most pronounced for TiO2 NP and DS TiO2 NP.

This can be interpreted as evidence of the formation of a more rigid enzyme conformation when interacting with NP based on a large number of weak bonds that are destroyed by exposure to high temperatures.

Table 2. The effects of native and modified dextran sulfate on titanium dioxide

Parameter	Eain (kJ/mol)	Δ H (kJ / mol) ΔS	ΔS (J / mol * K)	Δ G (kJ to mol)
mAspAT	328,0	325,9	674,6	104,6
mAspAT+ TiO2 NP	262,2	259,5	470,7	105,1
mAspAT+ TiO2DS NP	234,3	231,6	386,9	102,7
mAspAT+ Fe3O4 NP	288,6	285,8	549,9	103,0
mAspAT+ Fe3O4 DS NP	285,6	285,9	551,1	102,7

Based on the obtained results, it can be concluded that the interaction of mAspAT with NP increases the conformational stability of the enzyme, which is primarily determined by the chemical nature of NP and can be enhanced by the surface coating of NP dextran sulfate.

Variable thermodynamic parameters characterizing the interaction of proteins with NP are determined by the structural characteristics of specific proteins. Thus, it is believed that proteins with label conformation form strong interactions with surfaces due to entropy resulting from conformational changes during adsorption. By modifying the secondary structure, they can form a variety of non-qualifying interactions with the surface. Proteins with a relatively rigid conformation retain a secondary structure, so their adsorption is dependent on hydrophobic or electrostatic interactions, according to [14].

Our experimental results indicate that the conformation of mAspAT molecules adsorbed on native and modified dextran sulfate NP metal oxides is more rigid to minimize structural deformation when heated.

Our findings are consistent with the findings of another research. Thus, [24]. demonstrated that cytochrome c adsorbed on the surface of gold nanoparticles becomes more thermostable due to the temperature-induced special conformation of the heme. Increases significantly the thermal stability of trypsin when combined with nanoparticles. The thermal stability of glucose oxidase adsorbed on the surface of NP colloidal gold [25] or NP based on CoFe2O4/SiO2 [24,26] is greater than that of a free enzyme. Proteins interact with NP at neutral pH due to the formation of hydrogen bonds, hydrophobic interactions, and electrostatic interactions, which, according to [7] should be the primary stabilizing force. Concurrently, conformational changes induced by metal oxides in a secondary structure, such as albumin, are sufficiently profound; α-spirals transform into β-composite layers.

In the first phase, a study was conducted on the influence of fixed concentration of AuNP on the dependency of aggregation development on temperature (Figure 4). It was found that in all the tested temperature regimes, AuNP reduced the aggregation of mAspAT. Thus, already at 55 °C there is a slight inhibition of aggregation. Moreover, the most pronounced effect, reaching 56%, was at 60 °C. Interestingly, at the same temperature, the maximum thermo-protective effect of TiO2 NP was observed [27]. After this temperature limit, the anti-aggregating effect of nanoparticles begins to decrease and at 73 °C it almost completely disappears (Figure 4, D). The time required for the maximum development of the aggregation process when the temperature rises from 55 to 73 °C is significantly reduced.

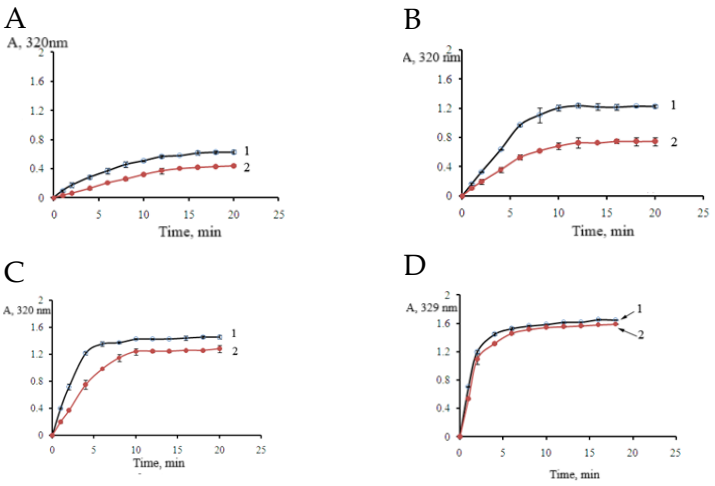

Figure 4. Dependency of mAspAT aggregation on temperature in the presence of AuNP. The aggregation process was recorded at a wavelength of 320 nm and temperatures: a – 55 °C, b – 60 ° C, C – 65 °C and D – 73 ° C. 1 – without gold nanoparticles, 2 – in the presence of gold nanoparticles at a concentration of 50 mg/L

In the presence of NPs, this process is slowed down (Figure 4, B and C), indicating that the NP's have antithermoaggregation effect is manifested in a limited range of temperatures. Apparently, the interaction of mAspAT with NP leads to the fixation of the enzyme on the surface of AuNP and thus the likelihood of protein-protein interactions is reduced.

In the next phase, the dependency of the mAspAT aggregation on the AuNP concentration in the range of 6.25 to 50 g/l was studied. The results shown in (Figure 5) show a clear decrease in the aggregation process with an increase in the concentration of AuNP in the incubation environment.

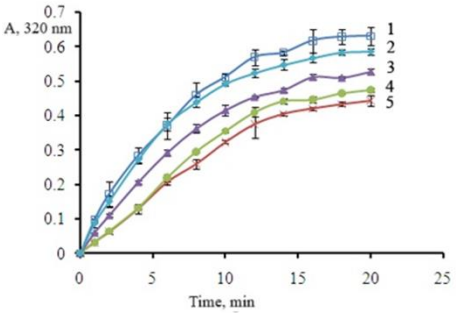

Figure 5. Dependency of aggregation of mAspAT on the concentration of NPs: 1 – control, mAspat without NP; 2 – mAspAT + AuNP 6,25 mg/l; 3 – mAspAT + AuNP 12.5 mg/L; 4 – mAsPAT+ AuNP 25 mg/ l; 5 – mAspAT + AuNP 50 mg/ L.

The enzyme was incubated in the cuvette at the appropriate temperature

In order to explain the cause of this phenomenon, let us refer to the structure of mAspAT and the characteristics of AuNPs. It is known that AspAT consists of two subunits and includes two molecules of the pyridoxal phosphate coenzyme. The active site of the enzyme is organized by two subunits and localized at the site of their interaction. The enzyme is seen as a dimer with a size of 105 × 60 × 50 Å [26]. The AuNPs used in the work are significantly smaller in size. Their hydrodynamic radius is 16–18 nm (1 Å = 10 nm). Taking into account the difference in the size of AuNP and mAspAT, it is possible to assume the possibility of binding several AuNPs to the enzyme molecule. Thus, the concentration-dependent decrease in mAspAT aggregation can be explained by an increase in the number of nanoparticles binding to the enzyme molecule.

This binding can be realized both through negative charges of citrate, which is a component colloidal gold NP, and by the hydrophobic part of the surface of the gold atoms. The formation of links between AuNPs and mAspAT will lead to increased conformational rigidity, reduce the probability of polypeptide chain deployment, and expose on the surface of molecules hydrophobic fragments responsible for intermolecular "fitting" and subsequent formation of protein aggregates.

CONCLUSION

In the presence of native and modified dextran sulfate nanoparticles of TiO2 and Fe3O4, the thermo-inactivation kinetics of mAspAT were compared. mAspAT was thermoinactivated at 45, 50, 55, 60, 65, and 70 0C, with a pH of 6.8 and a phosphate buffer solution concentration of 5 mmol/l. Both native and modified dextran sulfate NP exert their thermoprotective effect most effectively at temperatures above 60 0C. In the presence of TiO2 NP, the constant thermoinactivation rate (kin) of mAspAT is significantly decreased. This effect is enhanced by the modification of the surface of NP with dextran sulfate. At temperatures close to t1/2, the thermoprotective effect of DS TiO2 NP is manifested to the greatest extent. The thermoprotective effect of magnetite nanoparticles was significantly weaker. These parameters characterize the structural stability of mAspAT in the presence of nanoparticles: Ea, H, and S. Based on the obtained results, it can be concluded that the interaction of mAspAT with NP results in an increase in the conformation rigidity of the enzyme, which is primarily determined by the nature of NP and can be increased by coating the surface of NP with a polymer dextran sulfate.

Our study shows that AuNPs decreased the aggregation of mAspAT in the temperature range from 55 to 73 °C. The maximal anti-aggregational effect of AuNP reached 56 % and was observed at 60 °C. We suggest here that interaction between Au-NPs and mAspAT increases the conformational stability of the enzyme molecule. It also reduces the probability of polypeptide chain unfolding, which causes exposure of hydrophobic patches on the protein surface resulting in intra-molecular adhesion followed by protein aggregation.

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