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Conformational Effects of Polyelectrolyte-Coated gold Nanoparticle Exposure on Creatine Phosphokinase Protein

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Abstract

Interactions between engineered nanoparticles and biomolecules are central to understanding the biological and environmental implications of nanomaterial exposure. Proteins, due to their essential physiological roles, are particularly important at the nano–bio interface. In this study, we investigate the effects of polyelectrolyte-coated gold nanoparticles (PAH-AuNPs) on the structure of creatine phosphokinase (CPK), an enzyme widely used as a biomarker for myocardial infarction. PAH-wrapped gold nanoparticles (~4 nm) were synthesized and characterized using UV–Vis spectroscopy, ζ-potential measurements, dynamic light scattering, and transmission electron microscopy. Incubation experiments were conducted to assess protein adsorption and concentration changes following nanoparticle exposure. Circular dichroism spectroscopy, combined with secondary structure analysis, was employed to evaluate conformational changes in CPK after interaction with the nanoparticles. While protein concentration changes following incubation were inconsistent across conditions, circular dichroism analysis revealed generally minor and variable alterations in secondary structure. Notably, significant conformational differences were observed between freshly prepared CPK and protein stocks stored at room temperature, independent of nanoparticle exposure. These findings suggest that protein handling and storage conditions may play a critical role in observed structural changes at the nano–bio interface. The results highlight the complexity of nanoparticle–protein interactions and provide insight for future studies on the structural and functional impacts of nanoparticle exposure on biologically relevant proteins.

Keywords: Gold Nanoparticles, Polyelectrolyte Coating, Protein–Nanoparticle Interactions, Creatine Phosphokinase, Nano–Bio Interface.

General Introduction

Understanding the implications of the interaction that takes place between nanoparticles and biomolecules is essential to inform the development of emerging technologies that involve a release of nanoparticles. The Center for Sustainable Nanotechnology (CSN) emphasizes how relatively little is known about this phenomenon and how it impacts our environment and the organisms in it. Biomolecules such as proteins are especially relevant to study in this context, given their importance in the human body to carry out functions. One such protein that enables cellular function by serving as an enzyme for certain biochemical reactions is creatine phosphokinase (CPK). Found in our heart, brain, and skeletal muscles, CPK is the oldest marker to identify myocardial infarctions [1]. These important properties of CPK make it interesting to study at the nano-bio interface. A possible consequence of the interaction between this protein and nanoparticles is a conformational change observed in the protein structure. This could dictate changes in the functions carried out by the protein. This is especially relevant in a physiological environment where changes to protein function could either be detrimental or beneficial to cellular function [2]. In this study, the secondary structure of the protein is analyzed after nanoparticle exposure to determine any deviation from the original confirmation.

Materials and Methods

Materials

We acquired gold(III) chloride trihydrate (HAuCl4□3H2O),

sodium citrate, and sodium borohydride from Sigma-Aldrich. Phosphate Buffered Saline (PBS) was procured from Lonza. Sodium chloride and poly(allylamine) hydrochloride (PAH) were obtained from Sigma-Aldrich. We purchased Creatine Phosphokinase (CPK) from Millipore-Sigma. We used nanopure water for all experiments.

Methods

Synthesis and purification of gold nanoparticles

The synthesis of PAH-wrapped nanoparticles (PAH-AuNPs) was performed using a flow-reactor method described by published protocols [3,4] with a few modifications. First, 1626 mL of growth solution containing 0.01 M HAuCl4 and 0.1 M sodium citrate, and 1626 mL of 0.1 M sodium borohydride solution was made. Then, the solutions were allowed a residence time of ~2-3 min in the flow reactor to generate 4 nm diameter citrate-capped nanoparticles. The ~3.2 L combined solution was adjusted to 1 mM NaCl, followed by an addition of excess PAH to the solution. For purification, the reaction mixture was subjected to double-centrifugation at 13,000 xg for 55 minutes each wash. The pellets obtained from the centrifugation were combined and subsequently diluted to 1000 nM. The PAH-AuNPs generated were aliquoted for later use in experiments.

Characterization of Gold Nanoparticles

The nanoparticles were subjected to characterization at the citrate-stabilized stage as well as after the polyelectrolyte coating was performed. The concentration of the PAH-AuNPs was measured using UV-Vis spectroscopy. The surface charge of the NPs was determined through ζ-potential measurements. The mean diameter of the NPs was measured through dynamic light scattering (DLS). The diameter was also determined through an Image-J analysis of transmission electron microscopy (TEM) images taken of the NPs.

Creatine Phosphokinase (CPK) adsorption onto PAH-wrapped nanoparticles

An interaction between CPK protein and PAH-AuNPs was established through incubation experiments, followed by purification. First, the NPs were sonicated to obtain a uniform dispersion [5]. CPK stock was prepared beforehand by diluting lyrophysized CPK powder in PBS buffer to obtain a final concentration of 22 uM. The protein stock was aliquoted, stored in the freezer, and thawed at room temperature as needed. CPK protein was dispensed in low-protein binding microcentrifuge tubes containing PBS buffer. PAH-AuNPs were then suspended in this solution, bringing the final volume of the mixture to 400 uL. Multiple tubes of this reaction mixture were prepared with each tube containing varying ratios of CPK protein to PAH-AuNPs. This was done to obtain the optimal concentration ratio of CPK-AuNPs. Negative controls for protein concentration change due to nanoparticle exposure were also prepared for each tube by replacement of AuNPs with nanopure water in the same amounts. The incubation experiment was carried out by placing the tubes in a Belly Dancer shaker.

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The incubation time was varied between each round of experiments to obtain the optimal length. The tubes containing the reaction mixture were then purified using double-centrifugation at 10,000 xg for 10 minutes. The supernatant was removed and transferred to another set of low-binding microcentrifuge tubes. The supernatants were subjected to UV-Vis spectroscopy to determine any loss in protein concentration due to nanoparticle exposure. This was done through a comparison between CPK concentrations in the control and treatment tubes. The following equation (Beer-Lambert Law) was used to calculate the concentrations [6] of protein after incubation experiments and extinction coefficients (Table 1) derived from publications were utilized.

Table 1: Extinction coefficients of various substances tested for concentration in incubation study

Sample	Extinction coefficient (M-1 cm-1)
Creatine Phosphokinase (CPK)	70956 [5]
4 nm AuNP	7144443.1
20 nm AuNP	4 x 10^8

Circular Dichroism (CD)

The secondary structure level of the soft corona in the supernatant was characterized using circular dichroism (CD). The data derived from CD was also inputted into a website called BestSel, which fitted the graphs to determine the percentages of secondary structure components found in the soft corona. The secondary structure percentages of the protein in the treatment tubes were compared to those of the control tubes to determine any conformational change in the CPK protein as a result of nanoparticle exposure.

Results and Discussion

Characterization of Gold Nanoparticles

The PAH wrapping of the AuNPs was confirmed through characterization techniques at the citrate-stabilized stage and after the coating was attempted. Techniques including UV-Vi's spectroscopy and ζ potential were utilized to accomplish this. The UV-Vis spectra (Figure 1a) revealed a shift of the plasmon band toward a longer wavelength from citrate-capped AuNPs (~522 nm) to PAH AuNPs (~528 nm), pointing towards the existence of a PAH coating on the NP surface [7]. The ζ potential measurements (Figure 1b) revealed the surface charge of the AuNPs. Prior to the PAH-wrapping, the AuNPs exhibit a negative surface charge. After the wrapping, this value became positive, confirming the presence of a polyelectrolyte layer [8]. TEM images (Figure 1c) of the PAH-AuNPs were analyzed through software ImageJ, which determined the mean diameter of the NPs to be close to 4 nm.

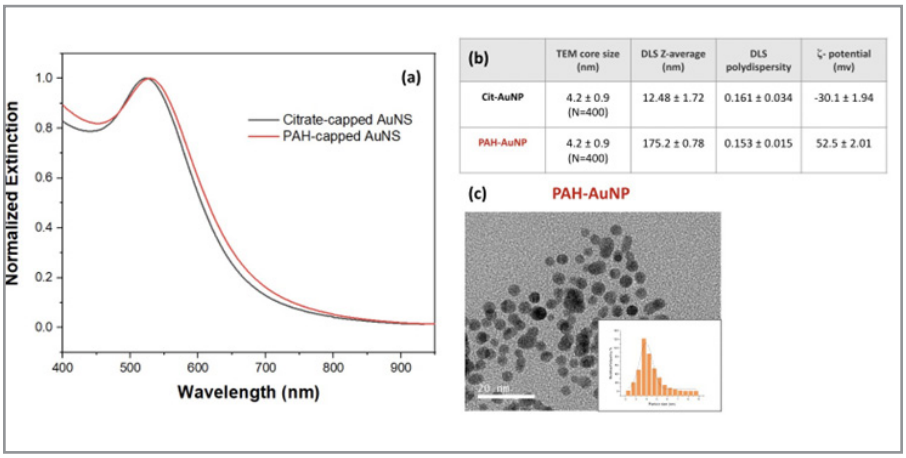


Figure 1: PAH-AuNP Characterization (a) UV-VIS spectra of AuNPs at citrate-capped stage and after PAH-wrapping; (b) TEM core size (nm), DLS Z-Average (nm), DLS polydispersity, and ζ potential (mv) of the AuNPs at citrate-capped stage and after PAH-wrapping. DLS Z-Average reflects the average diameter for the NPs.

Incubation Experiments

The binding of CPK protein onto the nanoparticle surface was induced through incubation. The duration, type of AuNP used, and the solvent of the samples were varied to obtain optimal conditions to carry out incubation. The concentration measurements obtained from UV-Vi's spectroscopy (Figure 2) point towards some concentration changes in a few samples. However, these changes were largely inconsistent across replicates of the same incubation conditions as well as across varied conditions.

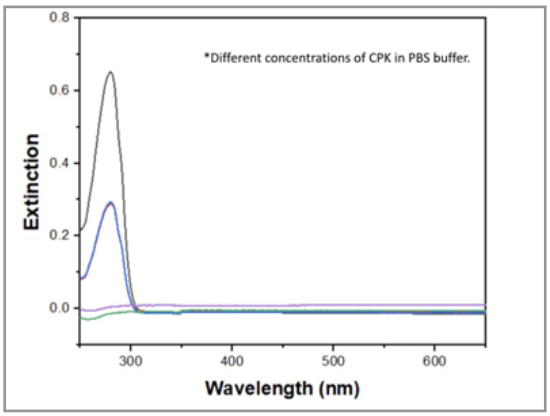


Figure 2: Example of UV- Vis Spectra taken after incubation experiment to determine CPK protein content in supernatant.

Table 2: Summary of results obtained from CPK-AuNP incubation studies along with relevant observations and procedural notes (TA1 and TA2 represent the first and second batch of PAH-AuNPs synthesized, respectively.)

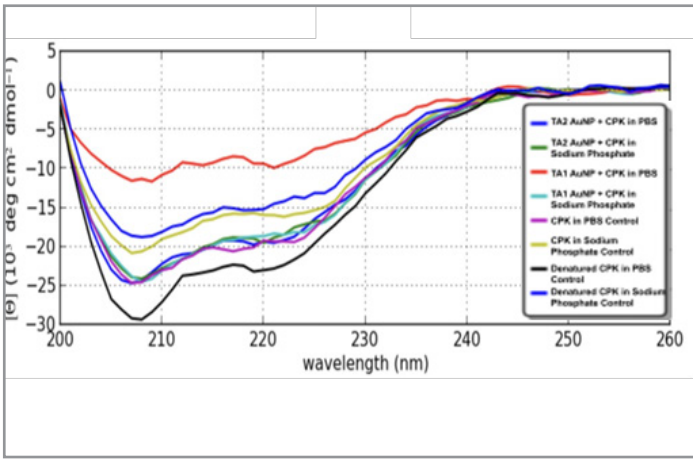
Date of Incubation	Sample	Protein Concentration Before (uM)	Protein Concentration After (uM)	Nanoparticle Concentration Added (nM)	Observations	Buffer	AuNP	Notes
7/12/2022	1	10.0	9.4	100	Aggregates, film along tube walls	PBS	TA1	Used regular micro centrifuge tubes.
	2	5.0	4.2	100	Aggregates, film along tube wall	PBS	TA1	
	3	5.0	4.3	50	Aggregates, film along tube wall	PBS	TA1	
	4	0.0	0.0	100	Colorless, aggregates	PBS	TA1	

	5	0.0	0.0	10	Colorless, aggregates	PBS	TA1	
	1	15.0	13.5	100	Pink	PBS	TA1	
	2	15.0	14.9	10	Colorless	PBS	TA1	
7/14/2022	3	5.0	3.8	200	Colorless, aggregates	PBS	TA1	Used low protein binding micro centrifuge tubes
	4	5.0	4.8	300	Colorless, aggregates	PBS	TA1	
	5	5.0	4.7	0	-	PBS	-	
	1	5.0	4.1	200	Pink, aggregates	PBS	TA1	
7/15/2022	2	5.0	4.4	200	Pink, aggregates	PBS	TA1	Used low protein binding micro centrifuge tubes
	3	5.0	3.9	0	-	PBS	-	
	4	5.0	3.5	0	-	PBS	-	
	1	17.7	16.4	200	Pink, aggregates	PBS	TA1	
	2	17.7	16.9	100	Pink, film	PBS	TA1	
	3	17.7	17.0	50	Pink	PBS	TA1	
7/19/2022	4	17.7	16.9	10	Pale pink	PBS	TA1	Used low protein binding micro centrifuge tubes
	5	5.0	4.4	200	Colorless, film	PBS	TA1	
	6	5.0	4.6	100	Colorless, clusters	PBS	TA1	
	7	5.0	4.7	50	Colorless	PBS	TA1	
	8	5.0	4.6	10	Pale Pink	PBS	TA1	
7/20/2022	1	17.7	15.0	50	Pink	PBS	TA1	Used low protein binding tubes
	2	17.7	13.9	50	Pale Pink	PBS	Old MUk NPs	

	1	5.0	5.5	100	Colorless with aggregates	PBS	TA2	Used low protein binding micro centrifuge tubes
8/01/2022	2	5.0	5.4	100	Bright pink	Sodium Phosphate	TA2	
	3	5.0	4.8	100	Colorless with aggregates	PBS	TA1	
	4	5.0	5.2	0	-	PBS	-	
	5	2.5	2.7	0	-	PBS	-	
	6	5.0	4.9	0	-	Sodium Phosphate	-	
8/03/2022	1	5.0	5.2	100	Colorless, aggregates	PBS	TA2	Used low protein binding micro centrifuge tubes
	2	5.0	4.9	100	Colorless, aggregates	Sodium Phosphate	TA2	
	3	5.0	5.6	0	-	PBS	-	
	4	5.0	5.2	0	-	Sodium Phosphate	-	
	5	5.0	5.8	200	Pink, aggregates	PBS	TA2	
	6	5.0	4.1	200	Pink, aggregates	Sodium Phosphate	TA2	
	7	5.0	5.3	0	-	PBS	-	
	8	5.0	5.3	0	0	Sodium Phosphate	-	

Circular Dichroism (CD)

CD spectroscopy (Figure 3) was utilized as a method to determine the secondary structure of protein left in the supernatant after incubation studies. The spectrum of the buffer was subtracted from the sample spectrum before analysis using BestSel. This enabled a conformational comparison between treatment and control samples, as well as between multiple treatment samples. We observed that although slight deviations from the original structure were observed across most samples, these changes were largely inconsistent even across samples subjected to similar incubation conditions. However, a significant change in conformation was observed between CPK stock that was stored in the freezer and stock that had been left at room temperature (Figure 3b row 5-8)



	helix	anti -parallel	parallel	turn	others
1	49.5	2.7	0.0	10.5	37.3
2	43.9	2.2	0.0	6.6	47.3
3	21.6	26.3	1.9	11.1	39.1
4	49.9	2.3	0.0	7.8	40.1
5	48.1	4.1	0.0	9.0	38.9
6	37.6	3.9	0.0	10.0	48.5
7	52.0	5.9	0.0	9.9	32.0
8	49.2	17.3	0.0	14.8	18.7

Figure 3: Example of circular dichroism data of AuNP-CPK samples, CPK controls, and denatured CPK controls.

Incubation conditions shown in Table 2 for 7/26/22. (a) Graph obtained from CD (b) Table showing secondary structure components of soft corona protein present in supernatant. All the secondary structure data is reported as percentages.

Concluding Remarks

The effects of polyelectrolyte-wrapped nanoparticles on protein creatine phosphokinase were studied through a series of experiments. In addition, the concentration of protein left in the solution after NP exposure was also measured. In this part of the study, no significant results were obtained as the concentration change was inconsistent across replicate samples. We also believe that these results are specific to incubation conditions and could vary as the conditions are changed. Next, the secondary structure of the protein was analyzed after interaction with NPs. Again, there was no significant change evident in most of the samples. However, some interesting deviations in the secondary structure were observed between protein stock that was stored in the freezer and stock that was left at room temperature for a few days. This interesting result could inform future work to study the structural effects of NPs on CPK, or proteins with similar properties, as the denatured structure of creatine phosphokinase can exhibit different levels of binding capabilities to the nanoparticle that can further affect its structural profile [9].

References

1. Aujla R.S.; Patel R. Creatine Phosphokinase. StatPearls 2022.
2. Satzer P.; Svec F.; Sekot G.; Jungbauer A. Eng. Life Sci. 2016. 16(3):238-246.
3. Lohse, S. E.; Eller, J. R.; Sivapalan, S. T.; Plews, M. R.; Murphy, C. J. ACS Nano 2013, 7, 4135−4150.
4. Qiu, T. A.; Torelli, M. D.; Vartanian, A. M.; Rackstraw, N. B.; Buchman, J. T.; Jacob, L. M.; Murphy, C. J.; Hamers, R. J.; Haynes, C. L. Anal. Chem. 2017, 89(3), 1823–1830.
5. Nandhakumar, S.; Dhanaraju, M. D.; Sundar, V. D.; Heera, B. Bulletin of Faculty of Pharmacy, Cairo University 2017, 55(2), 249–258.
6. Grimsley, G. R.; Pace, C. N. Current protocols in protein science 2004, Chapter 3.
7. Worthington Enzyme Manual. Creatine Kinase.
8. Dorris, A.; Rucareanu, S.; Reven, L.; Barrett, C. J.; Lennox, R. B. Langmuir 2008, 2532–2538.
9. Volodina, K.V.; Avnir, D.; Vinogradov, V.V. Sci. Rep. 2017, 7, 1458.