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Effect of Metal Ions on Peptide Folding

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ABSTRACT: Metal sulfides and other minerals were present on early Earth and most likely contributed to the origins of life as we know it. At some stage in prebiotic chemical evolution, random coil polypeptides obtained alpha helices and beta sheet structures to create enzymes. Many early proteins contain transition metal sulfide clusters at their core active sites. Here, I examined the effects of Zns, FeS and CdS, three metal sulfides that existed in early Earth environmental settings, on structure of random coil polyglutamic acid under neutral pH conditions. These minerals did not induce a conformational change in a polyglutamic acid because the negative surface charge on both the minerals and peptide inhibited their interaction. Goethite also does not induce a conformational change of the polypeptide.

Introduction

One of the major questions in Origins of Life research is to understand how proteins formed in Early Earth environments. This question is crucial to understanding life because the folding determines many proteins' ability to act as enzymes, which are essential for life. Many ancient enzymes contain transition metals or metal sulfide nanoclusters at their core active sites (Maeda et al., 1986) Hence, it was proposed that transition metals and sulfide minerals, which would have been present in the early Earth environment, may have played a role in inducing the change from unstructured polypeptides to folded enzymes (Bertrand and Brack, 2000).

Bertrand and Brack (2000), determined that polypeptides change from random coiling to beta-sheets upon binding to cadmium sulfide or solid molybdenum. The effect was ascribed to cadmium or molybdenum ions leached from the solid surfaces. However, these are very insoluble solids at neutral pH. In a related study, a simple peptide made of polyglutamic acid structure changes from random coil to alpha helix upon binding transition metals (Maeda et al., 1986). Furthermore, the addition of Na^+ to the system destroyed the folding, and the polyglutamic acid reverted to random coil structure. It would also be interesting to examine whether a polycationic peptide, such as poly-Lysine, which has a negative random coil structure, can adsorb to the sulfide particles, and if the surface induces a conformational change.

The goal of this study was to analyze the effects of metal sulfide nanoparticles on structure of a random coil polypeptide and to determine if the deleterious effect of Na^+ ions can be "rescued" by interaction with sulfide mineral nanoparticles (NPs). An iron oxyhydroxide mineral, goethite (FeOOH), was also used. Goethite was not present on early Earth because of a reducing atmosphere on early Earth where no O_2 was present. However, goethite was used for exploring the effects of mineral surface charge compared to the metal sulfide minerals. The metal sulfides are negatively charged (Isoelectric Points, IEPs < 7) whereas goethite (IEP ~ 9) has a positive surface charge at neutral pH. The results of the present research may aide understanding of protein formation in the early Earth environment and give insight to whether aided in enzyme formation.

Materials and Methods

Materials

The polypeptide used to carry out this experiment was Poly-L-Glutamic acid (PLE50, average molecular weight = 7500Da) (Alamanda Polymers, Huntsville, AL). PLE50 contains 50 glutamic acid residues and is negatively charged at pH 7, which is the pH of the conditions during the experiment. All other chemicals were purchased from Sigma-Aldrich unless otherwise specified. Ultrapure water (resistivity = 18.2 MΩ•cm) (Barnstead™ GenPure™ xCAD Plus, Rockford, IN) was used for all experiments. The CdS NPs were ordered from Strem Chemicals (Newburyport, MA).

Metal Sulfide (FeS and ZnS) NP Synthesis

The following NPs were used in this experiment: Iron sulfide (FeS), Zinc sulfide (ZnS), Cadmium sulfide (CdS) and Goethite, which is an iron oxide mineral. Iron sulfide NPs were synthesized following the methods described by Chandrawat et al. (2019). In detail, 1.0 g (3.699 mmol) of Iron(III) chloride hexahydrate (Sigma-Aldrich) and 0.25 g (3.2842mmol) of thiourea (Sigma-Aldrich) were dissolved in ethylene glycol and heated at 180 °C for 3 hours. The solution and black precipitates products were then cooled to 27 °C and vacuum-filtered and washed 4-5 times in 20 mL of ethanol to extract the NPs. The particles were dried at 60 °C and then stored in a container at room temperature for later use (Chandrawat et al., 2019). Zinc sulfide NPs were prepared following the procedure described by Zhang, Rustad and Banfield (2007). Here, 4.089 g (0.03 mol) of Zinc chloride (Sigma-Aldrich) and 7.2054 g (0.03 mol) of Sodium sulfide nonahydrate (Sigma-Aldrich) were each dissolved in two beakers of 200 mL deionized water, respectively. The Sodium sulfide solution was added dropwise into the Zinc chloride solution slowly under magnetic stirring, forming the white zinc sulfide particles. The solution was then separated via gravity filtration and the precipitate was washed until the pH of the filtrate was approximately 7. The NPs were dried at 80 °C for approximately 2.5 hours and then stored in vials for later use (Zhang et al., 2007).

Transmission Electron Microscopy (TEM)

The NPs were characterized by Transmission Electron Microscopy. The samples were suspended in 70% (v/v) ethanol and sonicated for 10 minutes to produce a final concentration 0.5 mg/mL. Then 2.5 μL of the suspended nanoparticles was dropped via pipette onto grid (Formvar/carbon supported copper grids, 300 mesh, Ted Pella, Redding, CA) and then air-dried overnight. After drying, the grids were visualized via TEM by the JSM-1230 instrument (JEOL, Peabody, MS).

The NPS and were suspended in 10 mM Tris buffer, pH of 7.4 to obtain a concentration 1mg/mL in the suspension. The PLE50 was dissolved in 10 mM Tris buffer, pH of 7.4 to obtain a concentration of 100μM. The aqueous solutions were sonicated before each analysis to ensure particles were suspended in solution.

UV-Vis Absorbance Spectroscopy

Ultra-Violet spectroscopy (UV-1800, Shimadzu, Japan) was conducted on peptide in solution, mineral NPS in solution to observe the possible interactions between the PLE50 and NPS. UV spectroscopy was run in a 1 cm quartz cuvette from 220-600nm. A 50/50 volumetric ratio solution of PLE50 and each nanoparticle individually was made containing 1 mL of each solution. The concentration of the goethite suspension was reduced 5-fold due to excessive

interference in the original 1 mg/mL suspension. PLE50 and the NPS were also run individually with a 50/50 solution of 1 mL of the aqueous solution combined with 1 mL of 10 mM Tris buffer, pH 7.4.

CD Spectroscopy

Conformations of the PLE50 in solution and in the presence of various mineral suspensions was determined by CD J-1500 CD spectrometer, (Jasco, Easton, MD). CD spectroscopy followed the same protocol as UV spectroscopy with the following modifications: 0.1 mL of the aqueous solution or suspensions were used with a 0.1 cm quartz cuvette pathlength, wavelengths were scanned from 190-260 nm with 2 nm bandwidth, and a scan speed of 100nm/min at 25 °C. The CD spectra were analyzed by CDPro Analysis to obtain secondary structure of the peptide in solution and in each mineral suspension.

Results

Nanoparticle Characterization

The TEM brightfield analysis revealed the shape and size of the NPS. The FeS NPs show two size populations. At low magnification, large (~ 1 μm) dark (electron-dense) particles are seen (Figure 1a). Smaller particles (~ 50 nm or smaller) that are less electron dense are revealed at higher magnification. These particles seem to aggregate to form the 1 μm sized particles. In contrast, the ZnS NPs form a less electron dense “network” of tiny NPs that are not well-resolved by the TEM instrument (Figure 2). This mesh-like network with “gaps” in between the particles is formed because the electron beam destroys the water molecules that remain associated with the NPs. These results is consistent with the original ZnS synthesis work of Zhang et al. (2007), who reported particles of ~2-3 nm size that are ~ 14% hydrated. NPs. The CdS nanoparticles are large (2-3 μm) and electron dense with a cuboidal shape (Figure 3). As seen in Figure 4, goethite has a needle-like structure and the particles are ~ 100-300 nm long.

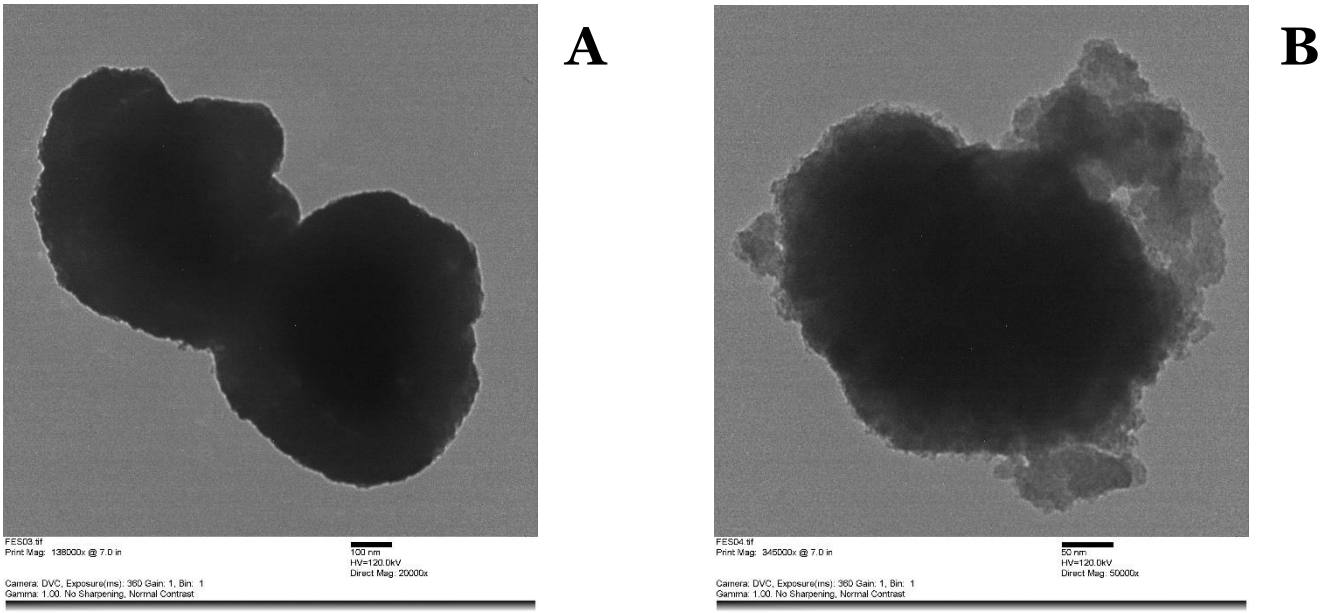

Figure 1. A: TEM image of FeS NPS at 138000x @ 7.0 in. Scale is set to 100nm. B: TEM image of FeS NPS at 345000x @ 7.0 in. Scale is 50 nm.

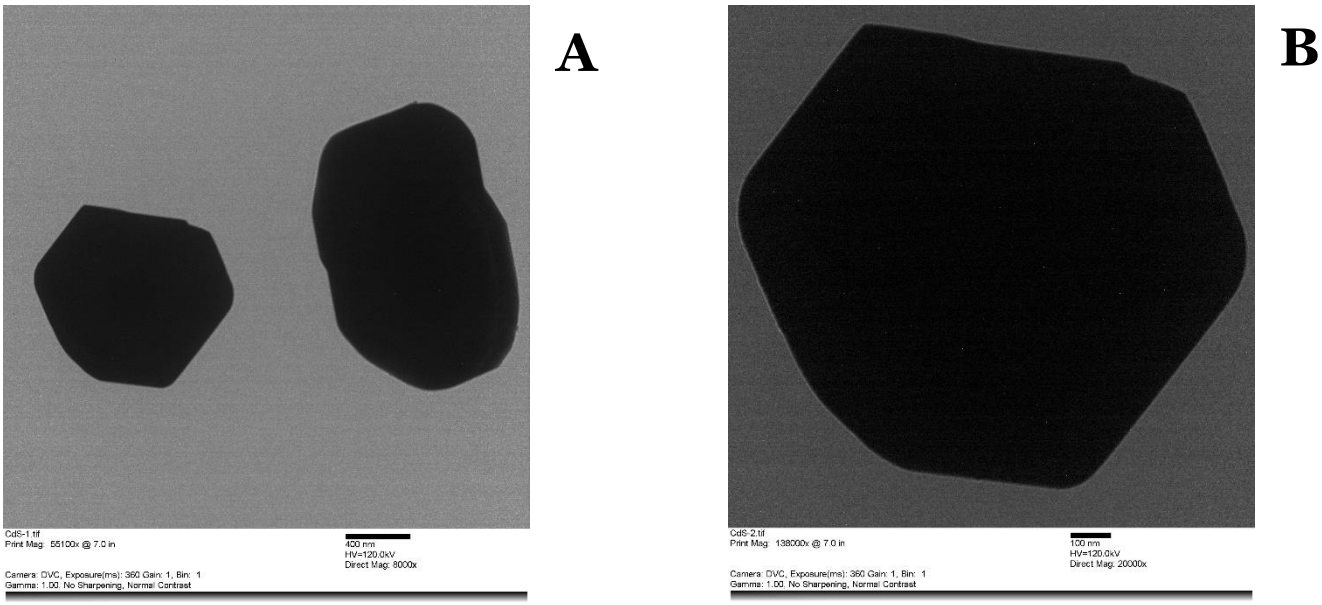

Figure 2. A: TEM image of ZnS NPs at 172000x @ 7.0 in. Scale is set to 100nm. B: TEM image of ZnS NPS at 689000x @ 7.0 in. Scale is set to 20 nm.

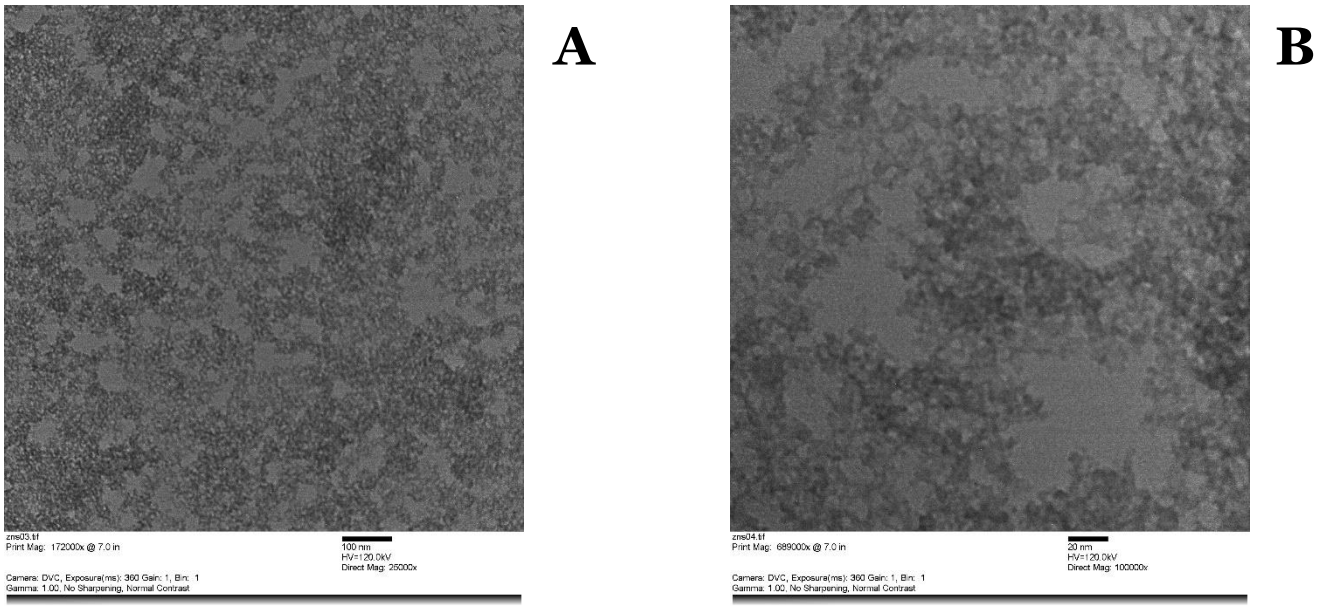

Figure 3. A: TEM images showing CdS NPs at 55100x @ 7.0 in. Scale is set to 400 nm. B: TEM image of CdS NPs at 138000x @ 7.0 in. Scale is set to 100 nm.

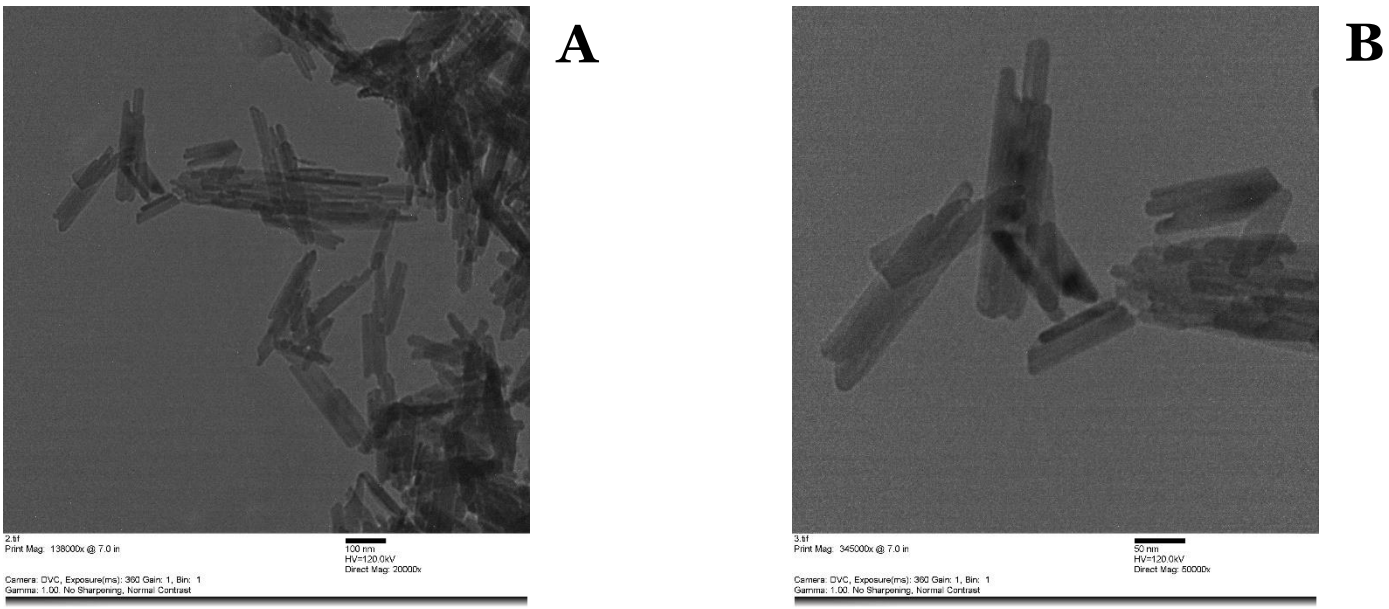

Figure 4. A: TEM image showing Goethite NPS at 138000x @ 7.0 in. Scale is set to 100nm. B: TEM image showing Goethite NPS at 345000x @ 7.0 in. Scale is set to 50nm.

Conformational Analysis

Spectra of PLE50 in buffer, of each mineral suspension in buffer and of the peptide in mineral suspension were obtained. The spectra of the peptide alone and of the peptide with each mineral NP suspension show a strong negative band at ~ 198 nm^{-1} and a small positive band at ~ 218 nm^{-1} both of which are characteristic of random coil structure (Myer, 1969) (Figure 5a-c). An alpha helix is typically characterized by positive bands at ~ 185-187 nm^{-1} and negative bands at

222 nm^{-1} and 208 nm^{-1}, a beta sheet would show a large positive band at ~ 195-197 nm^{-1} and a negative band at ~ 216-218 nm^{-1}. None of these features was observed. The CDPro model deconvolution of the structures is reported in Figure 6. Thus, the PLE50 peptide in buffer at pH 7 showed random coil structure consistent with previous studies (Myer, 1969) and the sulfide minerals had no apparent effect on the peptide structure.

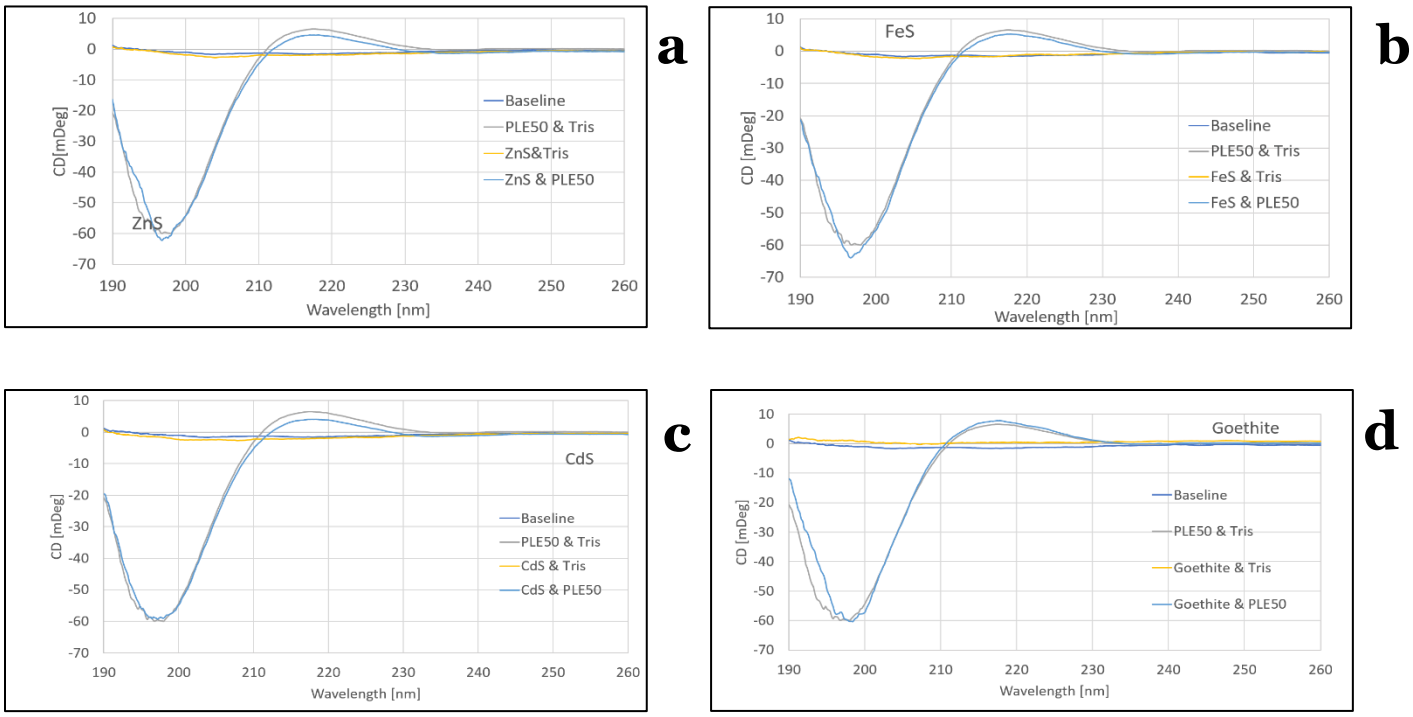

Figure 5. CD spectra of PL50 and mineral NPs alone and together in suspension (a) ZnS, (b) FeS (c) CdS and(d) FeOOH. Baseline is 10mM Tris buffer.

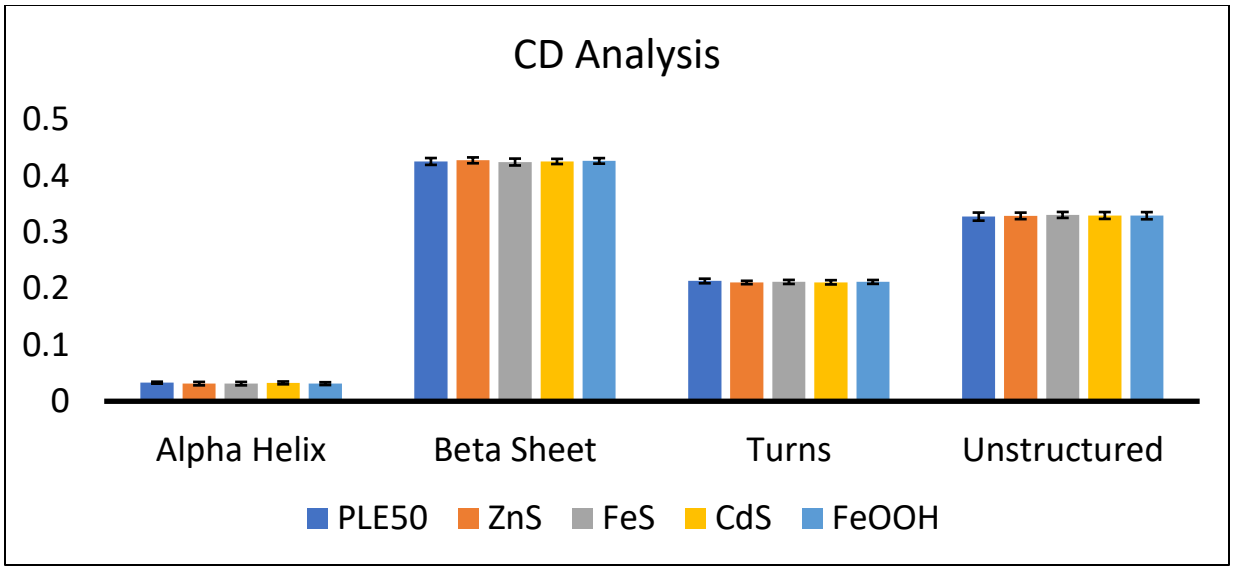

Figure 6. CDPro analysis showing structural characteristics of PLE50 and the mineral NPs with PLE50. Error bars represent standard deviation.

The UV-Vis absorbance spectra were obtained for the peptide in buffer (orange curve), each mineral in buffer suspension (grey curve) and the peptide in mineral NP suspension (yellow curve) systems (Figure 7). The spectra of the individual peptide and mineral suspensions were mathematically added together (light blue curve) and were found to be very similar to that of the peptide in mineral suspension (yellow). If the peptide was interacting with the mineral surface, then the light blue and yellow curves should have been different. These results show a lack of interaction between the peptide and all the mineral NPs.

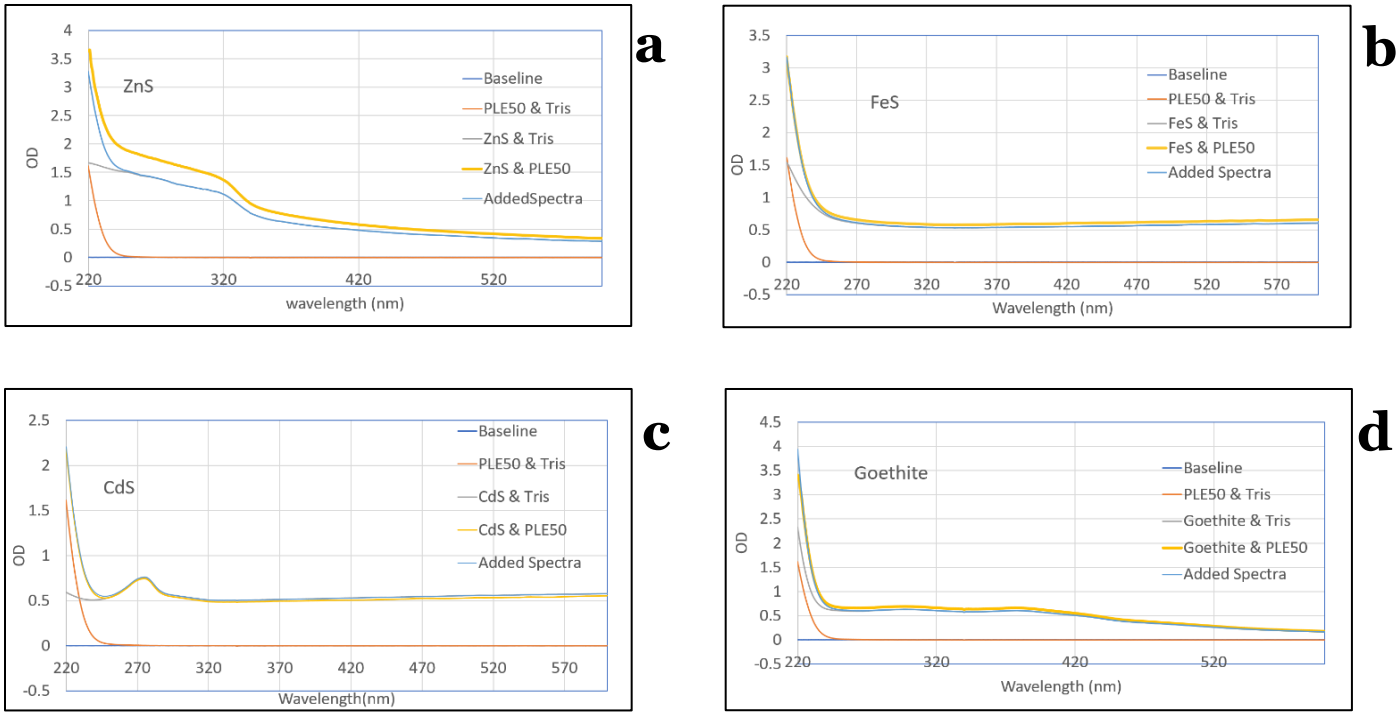

Figure 7. UV analysis of PL50 and mineral NPs alone and together in suspension (a) ZnS, (b) FeS (c) CdS and(d) FeOOH. Baseline is 10mM Tris buffer.

Discussion

In the Tris buffer, PLE50 adopts a random coil conformation. However, when the peptide and each mineral are combined, there is no induced conformational change of the polypeptide. This lack of any effect could be either because the peptide is not adsorbing on to the NP surfaces at all or because peptide adsorption is occurring, but the minerals are having no effect on the conformation. The sulfide minerals and the peptide are both negatively charged, so electrostatic repulsion may prevent adsorption. Goethite's surface is positively charged at neutral pH, therefore the peptide should be adsorbing. However, there seems to be no interaction based on the UV-Vis spectra in *Figure 7*.

The results obtained in the present study are not consistent with those from Bertrand and Brack (2000), where interaction of the peptide was seen with CdS and solid Mo surfaces. Those authors used an alternating (Glu-Leu) polypeptide, which has less negative charge than polyGlu, but repulsion should still have occurred from the CdS surface due to the presence of the Glu side chain residues. If this experiment were to be repeated, I would mimic the protocol of Bertrand and Brack more closely. In particular, Betrand and Brack (2000) used various amounts of crystalline CdS particles in suspension, including 0-500 equivalents of CdS, whereas I only used one concentration of suspended NPs. If more equivalents were tested, a transition of the polypeptide may be seen because their experiment yielded more transitions at 100 equivalents compared to the other equivalents tested (Betrand and Brack, 2000). As seen in *Figures 5c and 6*, the CdS nanoparticles produced no changes in the secondary structure of the polypeptide. It will be important for future experiments to find a concentration of nanoparticle that allows the

polypeptide to be adsorbed onto its surface, therefore allowing the conformational state to be altered. In the case of this experiment, the concentration was assumed to allow for full adsorption, but with more time and resources, it should be tested for full adsorption first.

It would also be interesting for future studies to examine whether adding NaCl to the system would screen the electrostatic repulsion and allow for peptide adsorption on the sulfide minerals. Maeda et al. (1986), confirmed the effects NaCl has on the protein folding, demonstrating the abrogative nature the sodium ion. Because the experiment did not have the protein transitions as anticipated, I was unable to test the negative effects of the electrolyte. However, if repeated, the goal is to find a mineral that defends the protein from the deleterious effects of sodium ions because this would give new insight to the field of Origins of Life and the origin of polypeptides in nature. It would also be instructive to examine a polycationic peptide, such as PolyLysine, which has a native random coil structure, to determine if it adsorbs to the metal sulfide NPs and if the surfaces induce structural organization.

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