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Hexametaphosphate, a Common Food Additive, Aggregated the Hen Egg White Lysozyme

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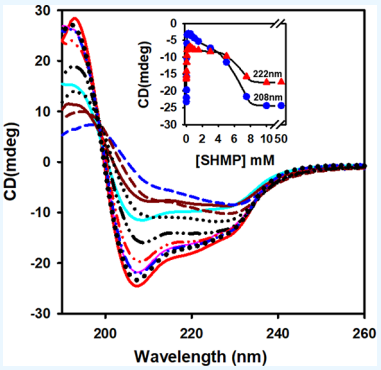
ABSTRACT: Polyphosphate polymers are chains of phosphate monomers chemically bonded together via phosphoanhydride bonds. They are found in all prokaryotic and eukaryotic organisms and are among the earliest, most anionic, and most mysterious molecules known. They are everywhere, from small cellular components to additives in our food. There is a strong association between hyperphosphatemia and mortality. That is why it is crucial to assess how polyphosphates, as food additives, affect the quality of edible proteins. This study investigated the effect of inexpensive and widely used food additives (hexametaphosphate labeled as E452) on bakery items, meat products, fish, and soft drinks. Using various spectroscopic and microscopic techniques, we examined how hexametaphosphate affected the aggregation propensity, structure, and stability of a commonly used food protein: hen egg white lysozyme (HEWL). The solubility of HEWL is affected in a bimodal fashion by the concentration of hexametaphosphate. The bimodal concentration-dependent effect was also observed in the tertiary and secondary structural changes. Hexametaphosphate-induced HEWL aggregates were amorphous, as evidenced by ThT fluorescence, far-UV CD, and TEM imaging. This study showed that the food additive (hexametaphosphate) may denature and aggregate proteins and may lead to undesirable health issues.



1. INTRODUCTION

Naturally, phosphates in the form of organic esters are present in food and cannot be eliminated. They are essential for a number of physiological processes including bone health, energy metabolism, and DNA synthesis. The amount of phosphates in food varies greatly depending on the type of food and how it is processed. The phosphate esters are hydrolyzed in the intestine, absorbed, and enter the bloodstream via the intestinal lining. The intestinal absorption of natural phosphates is low, while free inorganic phosphates added as food additives are quickly absorbed.[1,2]

Polyphosphates are a class of food additives that are used to improve food quality. These additives, which are made up of phosphate chains, are frequently added to processed meats, seafood, and other food products.[3−5] Polyphosphate additives are used for various purposes, including increasing moisture retention, improving texture and tenderness, and preventing microorganism growth.[3] They are commonly used in processed meats such as sausages and deli meats to improve texture and increase water-holding capacity, preventing drying out during processing and storage. However, there are some concerns about the safety of polyphosphate food additives. According to some studies, these additives may have negative health effects, such as encouraging the growth of harmful bacteria in the gut and increasing the risk of cardiovascular disease.[3,6] Furthermore, due to safety concerns, some countries have restricted using polyphosphate additives in certain foods.[3]

Food and safety authorities allow the use of several inorganic phosphates for various purposes (taste enhancers, preservatives, antiglycation agents, acidifying agents, stabilizers, etc.). Different phosphates, such as mono-, di-, tri-, and polyphosphates, are legally used in the food industry. Their E-numbers on food packets are E-339, E-340, E-341, E-450, E-451, and E-452.[7,8] Hexametaphosphate is a food additive that is commonly used as a sequestrant, an emulsifier, and a thickening agent. Its E number is 452. A commonly used hexametaphosphate is sodium hexametaphosphate. There are many different names for sodium hexametaphosphate ($Na_6[(PO_3)_6]$). Sodium polymetaphosphate, Hex, or Graham's Salt are some of them.

The permissible limits of hexametaphosphate in different types of foods are regulated by various governmental organizations.[7] However, due to the absence of phosphate

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content labeling in processed foods, inorganic phosphates are frequently used in substantial quantities as an additive in processed fish, sausages, bakery items, and soft drinks. The phosphate content of industrially processed food is significantly higher than that of natural food.[9] This is because inorganic phosphates are inexpensive and improve the functionality of processed food. Consuming an excessive amount of phosphate, particularly in the form of additives in processed foods, has been associated with a wide range of adverse health effects such as kidney disease, osteoporosis, and cardiovascular disease. The accumulation of phosphate in the blood can result in calcification of soft tissue, which can lead to cardiovascular disease and other complications.

Not all phosphate additives are equal, and their safety and efficacy may vary depending on their specific properties and how they are used in various food products. As with any food additive, it is critical to carefully consider the potential risks and benefits of phosphate additives and ensure that they are used in the food industry in a safe and responsible manner. Polyphosphates, in general, benefit the functional qualities of processed foods; however, it is not known how inorganic polyphosphates influence the solubility, structural integrity, and stability of food proteins. According to our knowledge, no studies have been done on the effects of hexametaphosphate on the structure and function of a common food protein called hen egg white lysozyme (HEWL). Through the use of a variety of spectroscopic and microscopic techniques, the purpose of this study was to evaluate the effects of sodium hexametaphosphate (E452a) on the solubility, structure, and stability of HEWL in the interest of gaining a deeper understanding of the impact polyphosphates have on the protein content of food.

HEWL has a small molecular weight of 14.3 kDa and has a compact, globular structure. It is commonly used as a model protein in biochemistry and biophysics.[10] HEWL has a catalytic function in breaking down bacterial cell walls by cleaving the β-1,4-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine. In the food industry, HEWL is used as a preservative to prevent spoilage caused by bacterial growth. It is particularly effective in preserving cheese and other dairy products. Due to its wide range of applications and its abundance in egg white, lysozyme has been extensively studied and is a valuable tool in many fields of science and technology. The presented research is novel because it explains how hexametaphosphate causes HEWL aggregation, which is a cause for concern in terms of food safety. The findings of this study suggest that hexametaphosphate can influence the tertiary and secondary structures of food proteins as well as enzymes involved in food digestion, providing insights into how food additives may negatively affect digestion and highlighting its biological significance in the context of human health and nutrition.

2. MATERIALS AND METHODS

HEWL, sodium hexametaphosphate (hexametaphosphate), and thioflavin T (ThT) were all supplied by Sigma Chemical Co. Milli-Q water (Millipore Corp., USA) was used to make all solutions, and then, the solutions were filtered through 0.22 μm. For this experiment, all other reagents were of analytical quality.

2.1. Stock Preparation and Quantification for HEWL. A stock solution of HEWL was made by dissolving it in 20 mM phosphate buffer at pH 7.5 and filtering the resulting solution through 0.22 μm. Spectrophotometric analysis at 280 nm and the molar extinction coefficient of 37,970 M^{-1} cm^{-1} were used to calculate the HEWL concentration.

2.2. Change of HEWL Solubility by Hexametaphosphate. Deionized water was used to make a fresh 100 mM hexametaphosphate stock solution. HEWL (0.2 mg/mL) was mixed with hexametaphosphate in 20 mM phosphate buffers at pH 7.0. HEWL samples were treated with hexametaphosphate and then allowed to equilibrate to pH 7.0 at room temperature overnight.

2.2.1. Turbidity of Hexametaphosphate-Treated HEWL Samples. The effect of different hexametaphosphate concentrations (from 0 to 50 mM) on the turbidity of HEWL (0.2 mg/mL) was measured by scanning UV−vis absorption from 250 to 400 nm. In a 1.0 cm path length cuvette, absorbance readings were taken from HEWL samples treated with hexametaphosphate using a Carry 60 UV−visible spectrophotometer (Agilent Technologies). The changes in the chromophoric region (280 nm) and nonchromophoric region (360 nm) were plotted with respect to the increase in hexametaphosphate concentrations.

2.2.2. Hexametaphosphate-Induced HEWL Aggregation: Rayleigh Scattering (RLS) Kinetics. The rate of hexametaphosphate-induced HEWL aggregation at pH 7.0 was determined by using RLS measurements. Using a stirrer-connected Carry Eclipse fluorometer, we monitored the RLS kinetics in the presence of up to 15 mM hexametaphosphate. As a function of time (s), the RLS intensity was recorded in a cuvette with a 1.0 cm path length having a 3 mL sample and a magnetic bar at 350 nm. The wavelengths of both excitation and emission were maintained at 350 nm. Slits 1.5 and 2.5 nm in width were used for excitation and emission, respectively. After recording RLS kinetics for 100 s without hexametaphosphate, data were collected for another 100 s after varying concentrations of hexametaphosphate were added.

2.2.3. Hexametaphosphate-Induced Tertiary Structure Changes in HEWL Measured by Intrinsic Fluorescence Measurements. We used a Carry Eclipse spectrofluorometer to measure the intrinsic tryptophan fluorescence in HEWL samples treated with hexametaphosphate at room temperature. Hexametaphosphate (pH 7.0; 0−50 mM) was applied to HEWL (0.2 mg/mL). Excitation at 295 nm was used to track environmental shifts near the Trp fluorophore in the HEWL samples. Emission spectra were collected from 300 to 400 nm. The excitation slit width was held at 5 nm, and the emission slit width was held at 5 nm as well.

2.2.4. Hexametaphosphate-Induced Secondary Structure Changes in HEWL Measured by Far-UV Circular Dichroism. In this study, a ChirascanPlus spectropolarimeter (Applied Photophysics, UK) was used to determine the far-UV CD of HEWL samples that had been treated with hexametaphosphate. Far-UV CD measurements were taken between 190 and 260 nm at room temperature in a 0.1 cm path-length cuvette. All samples had the same HEWL concentration of 0.2 mg/mL. The spectrum of the test samples was subtracted from the spectrum of hexametaphosphate blanks of varying concentrations.

2.2.5. ThT Fluorescence Measurement of Hexametaphosphate-Induced HEWL. ThT was dissolved in deionized water and then filtered using a 0.22 μm syringe filter to produce the stock solution. The ThT concentration was determined using an extinction coefficient of 36,000 M^{-1} cm^{-1} at 412 nm (M. S. Khan et al., 2016). HEWL samples (0.2 mg/mL) were incubated in the dark with 10 μM ThT for 15 min to detect

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the formation of amyloid fibrils after being treated with varying concentrations of hexametaphosphate. ThT fluorescence was detected in HEWL samples treated with hexametaphosphate at 440 nm. The emission spectrum was measured from 450 to 600 nm in wavelength. Both the excitation and emission slits were set to a width of 5.0 nm in this experiment. Hexametaphosphate-induced HEWL experiments using Th-T Kinetics were also conducted at a pH of 7.0.

2.2.6. Hexametaphosphate-Induced HEWL Aggregation Study by Transmission Electron Microscopy. TEM images of hexametaphosphate-induced HEWL aggregation were captured by a JEOL-1400 instrument at 120 kV. HEWL samples (10 μL) treated with 0.5 mM hexametaphosphate at pH 7.0 were examined by transmission electron microscopy. The sample was applied to the copper grid with a 200 mesh. Negative staining with 2% uranyl acetate was performed after washing the grids for 2 min. The samples were air-dried before TEM imaging.

3. RESULTS

3.1. Effect of Hexametaphosphate on HEWL Solubility. The HEWL aggregate formation experiment with hexametaphosphate was performed at pH of 7.0. The aggregation behavior of HEWL in the presence of hexametaphosphate was measured by using changes in turbidity at 350 nm. Figure 1 depicts the relationship between

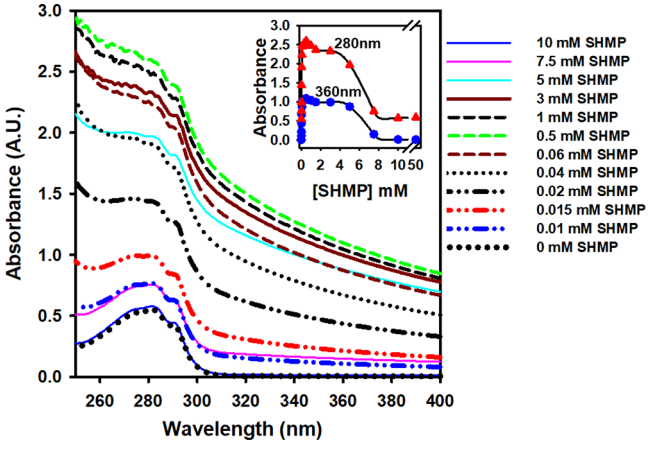

Figure 1. Effect of hexametaphosphate on HEWL turbidity at pH 7.5. The turbidity of the HEWL solution (0.2 mg/mL) was measured at 360 nm in the presence of 0−50 mM hexametaphosphate. The inset shows two wavelengths (280 nm; red triangle; and 360 nm; blue circles) at different hexametaphosphate concentrations.

turbidity changes at 360 nm and hexametaphosphate concentrations. The turbidity of HEWL solutions increased linearly in samples containing 0 to 0.1 mM hexametaphosphate, with a plateau observed between 0.1 and 3.0 mM hexametaphosphate. However, increasing the concentration of hexametaphosphate results in the gradual solubilization of HEWL aggregates that were nearly solubilized above 10 mM hexametaphosphate. The absorbance at the 280 nm (chromogenic) and 350 nm (nonchromogenic region) of HEWL treated with 10 mM hexametaphosphate were identical to the native HEWL, indicating the gain of native-like conformation and solubility.

3.2. Hexametaphosphate-Induced Changes in the Tertiary Structure of HEWL. The intrinsic fluorescence of HEWL is primarily due to its six tryptophan residues. Evaluation of the wavelength maximum and intrinsic fluorescence intensity revealed changes in the tryptophan microenvironment.[11,12] Fluorescence spectra of HEWL in the presence of 0−50 mM hexametaphosphate were recorded between 300 and 400 nm after the irradiation at 295 nm (Figure 2). At a pH of 7.0, the peak wavelength of HEWL was

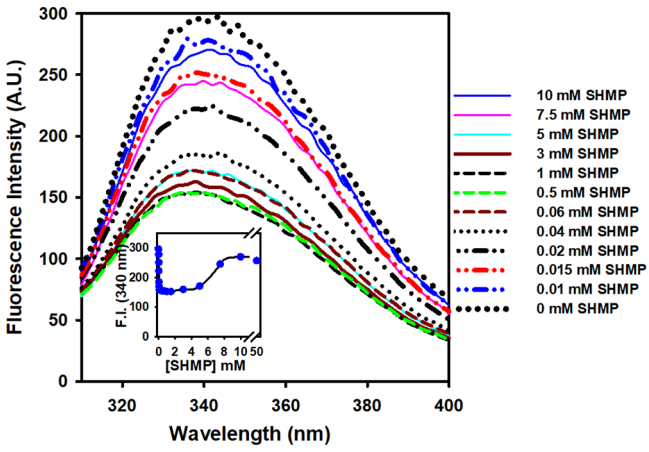

Figure 2. Change of the tertiary structure of HEWL by hexametaphosphate. At pH 7.0 and in the presence of 0−50 mM hexametaphosphate, the intrinsic fluorescence spectra of HEWL were measured. The HEWL concentration was 0.2 mg/mL in all samples. The fluorescence intensity at 340 nm versus hexametaphosphate concentration is shown in the inset figure.

340 nm. The fluorescence intensity gradually decreased with increasing concentrations of hexametaphosphate (0 to 0.1 mM). Aggregation of HEWL was found to be dose-dependent in this hexametaphosphate concentration range. The aggregated samples showed a slight blue shift (2 to 3 nm) in the wavelength maxima. Aggregation of HEWL was linked to a decrease in fluorescent intensity. The fluorescence intensity of HEWL was found to increase above 3 mM hexametaphosphate and to return to near-native levels at 10 mM hexametaphosphate. The changes in the intrinsic fluorescence intensity corresponded to the HEWL solubility state, as shown in Figure 1.

3.3. Hexametaphosphate-Induced Changes in the Secondary Structure of HEWL. HEWL is a small protein with a predominance of alpha-helices. As a result, the far-ultraviolet CD spectra of the HEWL showed a single minimum at 208 nm. The HEWL at pH 7.0 exhibited a far-UV CD minima at 208 nm (Figure 3). The ellipticity at 208 nm decreased in the presence of 0−0.1 mM hexametaphosphate, indicating a loss of secondary structure in the HEWL (Figure 3). Within this concentration range, the intrinsic fluorescence of tryptophan was also observed to decrease (Figure 2). In the presence of increased concentrations of hexametaphosphate (ranging from 0.1 to 3.0 mM), there is no discernible change in ellipticity. At this concentration range, the shapes of the far-UV CD spectra and the negative ellipticities were nearly identical. Over 3.0 mM hexametaphosphate, HEWL gained a secondary structure, and over 10 mM hexametaphosphate, a native-like secondary structure was gained. The tendency of HEWL to aggregate is linked to the loss of its secondary structure (Figure 1).

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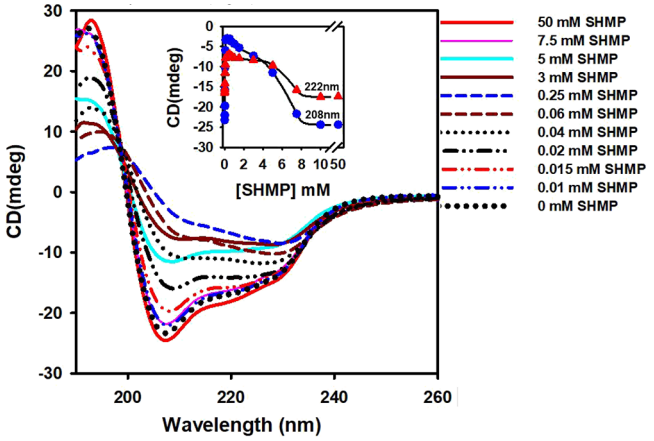

Figure 3. Effect of hexametaphosphate on the secondary structure of HEWL. The far UV CD spectra of HEWL that was treated with 0−50 mM hexametaphosphate were recorded between 190 and 260 nm. Throughout the course of the experiment, the concentration of HEWL was maintained at 0.2 mg/mL. In the inset figure, the changes in ellipticity at 222 and 208 nm were plotted with respect to the concentration of hexametaphosphate.

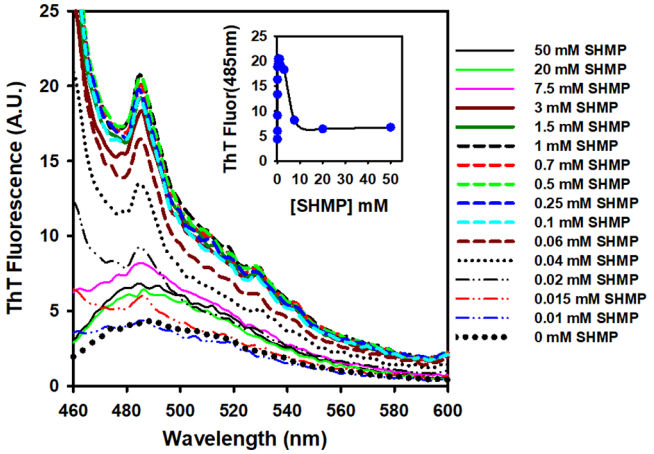

Figure 4. Effect of hexametaphosphate on the ThT fluorescence of HEWL measured at a pH of 7.0. In each of the experiments, 10 μM ThT was added to the samples of HEWL that had been treated with hexametaphosphate. After 15 min of incubation in the dark, the samples were excited at 440 nm, keeping ex and em slits set of 5 nm. The plot of the ThT fluorescence intensity at 485 nm versus the increase in the hexametaphosphate concentration was shown in the inset figure.

3.4. Effect of Hexametaphosphate on the ThT Fluorescence of HEWL.

In its free state (in an aqueous solution), the fluorescence emitted by the thioflavin T (ThT) dye is virtually undetectable. When ThT binds to the stacked b-sheets of amyloid fibrils, a significant amount (over a 5-fold increase) of fluorescence is produced. ThT can also bind to aggregated protein, albeit with a much lower quantum yield (an increase of 3−5 fold). As a result, the ThT assay is the preferred method for determining whether aggregated proteins contain amyloid fibrils. The aggregation of HEWL that was induced by hexametaphosphate was characterized through the use of ThT fluorescence in this study. The presence of amorphous structures in the aggregates is indicated by the fact that increasing the concentration of hexametaphosphate from 0 to 3 mM leads to a minor increase in ThT fluorescence (Figure 4). Also, the presence of the cross-beta sheeted structure that is typical of amyloid fibrils was not observed in the far-UV CD experiment (Figure 3).

3.5. RLS Kinetics of HEWL Induced by Hexametaphosphate.

Using Rayleigh light scattering (RLS) at a neutral pH of 7.0, we investigated the kinetics of hexametaphosphate-induced HEWL aggregation and solubilization. The RLS kinetics were measured at a wavelength of 350 nm. Figure 5 shows the kinetic traces of hexametaphosphate-induced aggregation and solubilization of HEWL at various hexametaphosphate concentrations. The absence of scattering of HEWL in the absence of hexametaphosphate indicates that it is soluble. After 0.05 mM hexametaphosphate was added, the amount of light scattered rapidly increased before reaching a plateau quickly. The subsequent addition of hexametaphosphate causes an increase in RLS, albeit to a lesser degree of intensity. There was little difference in RLS intensity in the presence of 0.4−3.0 mM hexametaphosphate. The solution becomes nearly transparent above 10 mM concentrations of hexametaphosphate, indicating that further increases in hexametaphosphate concentration result in rapid solubilization of HEWL aggregates. These findings indicate that neither the aggregation nor the solubilization processes involved a lag phase.

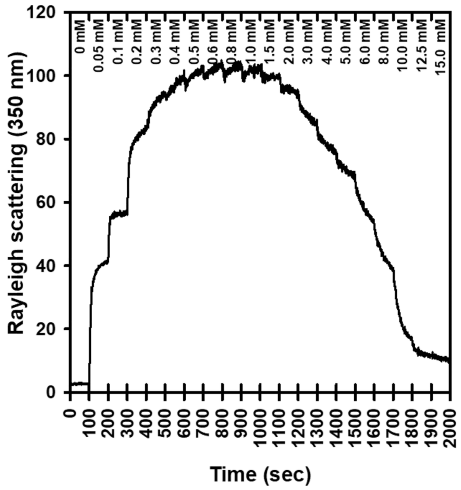

Figure 5. Hexametaphosphate-induced HEWL aggregation kinetics at pH 7.0. Rayleigh scattering was measured at 350 nm with HEWL samples (0.2 mg/mL) that did not contain hexametaphosphate. RLS was continuously monitored while different concentrations of hexametaphosphate (labeled on the top *x*-axis) were added every 100 s.

3.6. ThT Kinetics of HEWL Induced by Hexametaphosphate.

Using the dye thioflavin T, we investigated the binding kinetics of thioflavin T in a hexametaphosphate-induced HEWL (Figure 6). The HEWL ThT fluorescence at pH 7.0 without hexametaphosphate was used as a reference point. Monitoring the increase in ThT fluorescence kinetics at 485 nm after adding different concentrations of hexametaphosphate (labeled on the inside *x*-axis) revealed that the ThT fluorescence increased, but only slightly. The increase in ThT was not very significant (less than three folds) because no amyloid fibrils were present.

3.7. TEM Analysis of Hexametaphosphate-Induced HEWL Aggregates.

Through the use of transmission electron microscopy (TEM), a high-resolution image of the morphology of hexametaphosphate-induced HEWL aggregate was

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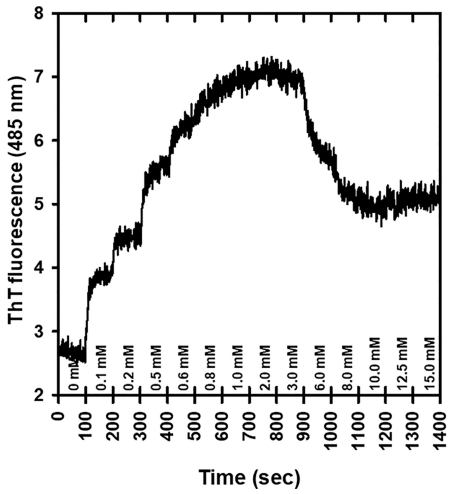

Figure 6. Effect of hexametaphosphate on the ThT fluorescence kinetics of HEWL. At a pH of 7.0, HEWL samples containing 0.2 mg/mL were mixed with 10 μM ThT dye, and the ThT fluorescence kinetics of the reaction were observed at 485 nm. When various concentrations of hexametaphosphate were added (which are labeled on the inside of the *x*-axis), the ThT fluorescence slightly increased without a lag phase and quickly reached a plateau.

obtained (Figure 7). HEWL was soluble at a pH of 7.0; however, in the presence of 1 mM hexametaphosphate, HEWL

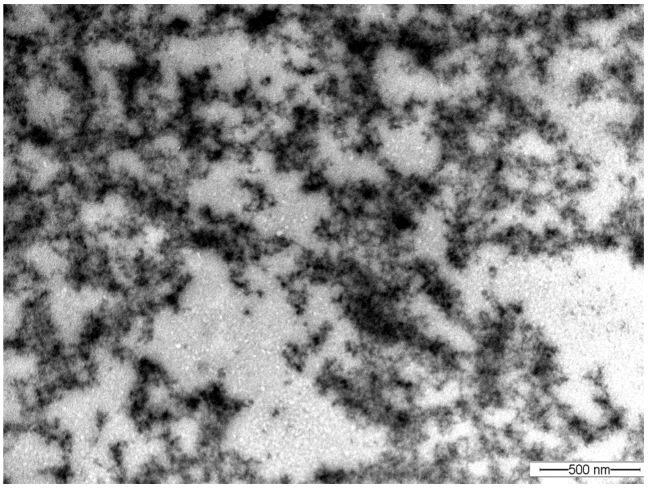

Figure 7. Micrographs of HEWL aggregates induced by hexametaphosphate. HEWL (0.2 mg/mL) was treated with 1 mM hexametaphosphate at a pH of 7.0.

became aggregated. The findings of the far-UV CD spectroscopy (Figure 3) and the ThT fluorescence measurements (Figure 4) were supported by the TEM image, which demonstrated the presence of an amorphous-like aggregate.

4. DISCUSSION

According to large-scale epidemiological research, hyperphosphatemia (excess phosphate in the blood) is associated with an increased risk of death in people with renal disease and cardiac disease.[2,13] This is because high phosphate levels can cause various complications, including cardiovascular disease, calcification of blood vessels and soft tissues, and renal osteodystrophy. Some studies suggest that even young people who are otherwise healthy may be adversely affected by phosphate additives in food.[14−16] When compared to people with higher incomes, people living in poverty in the United States have a hyperphosphatemia incidence rate that is twice as high. This is most likely because low-income populations may have limited access to healthy food options, such as fresh fruits and vegetables, and may rely more heavily on processed foods that are often high in phosphates. Processed foods, such as soda, processed meats, and canned foods, can contain high levels of phosphates and contribute to hyperphosphatemia. As a result, managing hyperphosphatemia is an important aspect of treating people with renal and cardiac diseases. This may include dietary changes to reduce phosphate intake and medications, such as phosphate binders, which can help prevent phosphate absorption from the diet. It is possible to reduce the risk of complications and improve outcomes for people with hyperphosphatemia by managing it effectively.

Certain inexpensive inorganic phosphates are often added to processed foods to enhance their texture, juiciness, and other characteristics.[17] Also, inorganic phosphates partially replace sodium chloride in processed dairy and meat products.[18,19] The oxidative stability and gel characteristics of beef products are also improved by the addition of phosphates.[20,21] Proteins that have inorganic phosphates added are able to bind to and retain more water because their structure has been relaxed. In order to reduce the level of formation of advanced glycation end products, it is now common practice to add large amounts of inorganic phosphates to soft drinks.

We investigated the effect of hexametaphosphate on a common edible protein (hen egg white lysozyme), which is also used as a model protein in biochemical research and as a food preservative due to its ability to break down bacterial cell walls. As a result, HEWL can help to prevent food spoilage caused by bacterial growth. HEWL has been extensively researched because it is a valuable tool in many fields of science and technology and is commercially available at a low cost with high purity and stability. Lysozyme is found in relatively high concentrations in milk, saliva, blood, and tears,[22] and the pH of milk, saliva, blood, and tears ranges from 6.7 to 7.4. As a result, we chose a pH of 7.0 to study the effect of hexametaphosphate on the structure and solubility of lysozyme. The isoelectric point at the HEWL was determined to be 9.32. At a pH of 7.0, it has 11 protonated arginine residues and 6 protonated lysine residues. Calculations with Protein Calculator v3.4 show that at pH 7.0, HEWL is in a cationic state with a total charge of 7.9. Anionic hexametaphosphate is expected to have a strong electrostatic interaction with cationic HEWL under physiological conditions, which has the potential to damage HEWL's native conformation and solubility by disrupting intramolecular ionic interactions and solvent protein interactions. As shown in Figure 1, hexametaphosphate showed a bimodal concentration-dependent effect on HEWL solubility. At lower concentrations of hexametaphosphate (micromolar range), it leads to a counter-ion-binding effect resulting in charge neutralization of HEWL, thus causing loss of tertiary and secondary structures (Figures 2 and 3) and a decrease in solubility (Figure 1). However, in the presence of higher hexametaphosphate concentrations (millimolar range), HEWL gained native-like tertiary and secondary structures and solubility due to the preferential hydration mechanism.

The bimodal effect on the conformation and solubility of proteins was observed in earlier studies. For example, surfactants like SDS, SDBS, CTAB, nonionic, and zwitterionic

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exhibited loss of structure and solubility of the protein in the micromolar range (generally below their CMC) and gain of native-like structure and solubility in the millimolar range (above their CMC).[23−25] Experiments with the alpha-synuclein and beta2-microglobulin proteins showed that intramolecular hydrogen bonding occurred in the hydrophobic micellar interior environments resulting in the gain of α-helical structures.[26−28] A similar bimodal effect has been observed with heparin[29,30] and salts.[31,32]

HEWL is a protein that is primarily composed of alpha helices in its structure. Figure 3 showed that the peak minima did not shift toward 215 nm, indicating no gain of the beta-sheet structure in the aggregated HEWL samples (i.e., absence of amyloid fibrils). The fact that the ThT fluorescence only marginally increased in samples of aggregated HEWL demonstrates the absence of an amyloid-like structure (Figure 4). In addition, TEM images of aggregated HEWL showed structures that resembled amorphous matter (Figure 7). Aggregation is likely the result of a large number of electrostatic interactions that are not native to the system. At a pH of 7.0, the intramolecular ion pairs and the interactions between HEWL and the solvent are broken because the negatively charged phosphates of hexametaphosphate electrostatically interact with the protonated arginine and lysine residues of HEWL. In the Hofmeister series, the phosphate anion is kosmotropic salt. Various kosmotropes have traditionally been used in protein extraction and purification. At physiological pH, hexaphosphate ions bind with cationic HEWL due to their synergistic kosmotropic activity. Because HEWL is cationic, it binds to it at physiological pH. H-bond interactions between protein and water are broken, resulting in structural destabilization of HEWL driven by hexametaphosphate. This structural destabilization causes HEWL unfolding and decreased solubility, resulting in HEWL aggregation.

The rise in the consumption of inorganic phosphates can be traced back to the widespread availability of processed foods and ready-to-eat fast foods. The rate of inorganic phosphate consumption is unprecedented. In the past 3 decades, inorganic phosphate consumption increased over 2-fold.[33−35] This represents a sizable increase in the total amount. Patients with chronic kidney disease and young, healthy humans have a strong correlation between elevated serum phosphate levels and the risk of mortality; therefore, the public and policy-makers should be aware of the potential harm caused by the addition of inorganic phosphates to processed foods. This is because there is a strong correlation between high serum phosphate levels and an increased risk of mortality in people who already have chronic kidney disease. There is a 22% increase in mortality risk for every 1 mg/dL elevation in serum phosphate levels, according to observational studies.[16]

This work is significant biologically because it addresses the complex link among phosphates, food additives, and human health. At physiological pHs, some enzymes found in the gastrointestinal system exist in the cationic state. For example, lysozyme in the saliva pH of 6.7, pepsin at stomach pH of 2.0, and trypsin at the duodenum pH of 6.0. Proteins and enzymes in the gastrointestinal system that exist in cationic states are highly susceptible to denaturation and aggregation in the presence of strong anionic additives, such as hexametaphosphate. Concerns arise from the extensive use of polyphosphate, such as sodium hexametaphosphate, in processed foods. These additives can improve food quality but also have harmful health effects by interfering with food digestion. The study highlights the importance of considering the impact of food additives on digestive processes, potentially influencing health outcomes, especially in populations exposed to high phosphate diets.

5. CONCLUSIONS

Based on the results of this study, it appears that the solubility of the food protein HEWL changed in a hexametaphosphate concentration-dependent manner. The conformational changes were a bimodal concentration-dependent effect. The loss of structure and solubility was detected in micromolar concentrations of hexametaphosphate. Interestingly, HEWL gained a native-like structure and solubility at the millimolar concentrations of hexametaphosphate. More study is needed to elucidate the mechanism of the bimodal effect of hexametaphosphate. Potentially, hexametaphosphate can bind and change the solubility of processed food proteins present in the cationic state. Hexametaphosphate also has the potential for electrostatic interactions with a wide variety of proteins and substances found in the gut. There is a lack of data on the effects of these types of interactions. Consistent usage of hexametaphosphate may worsen pre-existing problems and impair digestion.

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Notes

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