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INTERACTION OF HEAVY METALS WITH 7S SOYBEAN GLOBULIN: MOLECULAR DYNAMICS STUDY

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Molecular dynamics (MD) simulations were performed to examine the structural and dynamic effects of Cd^{2+} and Co^{3+} binding on 7S soybean globulin. Using a 200 ns simulation at 300 K with GROMACS and the CHARMM General Force Field, key structural parameters—including root-mean-square deviation (RMSD), radius of gyration (Rg), solvent-accessible surface area (SASA), and root-mean-square fluctuations (RMSF)—were analyzed to assess protein stability, flexibility, and compactness under varying metal ion concentrations. The results of the MD simulation indicate: i) at low metal concentrations, the protein maintained structural stability with minimal deviations; ii) increasing metal ion concentrations induced distinct structural changes in the protein structure depending on the ion type; iii) lower metal concentrations primarily affected specific regions of the α-subunit, whereas higher concentrations influenced both the α- and β-subunits; iv) fluctuations in secondary structure elements—α-helices, 3_{10}-helices, and β-strands—suggested potential destabilization, particularly in systems with high metal concentrations; v) α-helical content remained stable throughout the simulation, a slight decrease in β-sheet content was observed at higher metal concentrations. This suggests that heavy metal binding may have a destabilizing effect on β-sheet structures, altering the overall conformation of 7S globulin. These insights are valuable for the development of protein-based nanomaterials for heavy metal detection and sorption.
Keywords: *Protein-metal interaction; Heavy metals; Molecular dynamics*
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Over the past few decades, soybean proteins have gained significant attention not only for their nutritional benefits in the food industry but also for their role in developing protein-based nanomaterials for industrial and biomedical applications [1-12]. For instance, soybean proteins have been effectively utilized in the fabrication of biodegradable nanocomposite films for food packaging [1,2], thermally conductive composites [3,4], and soy protein-based coatings [5], among others. Additionally, soybean proteins have been employed in the synthesis of various nanoparticles for nutraceutical and drug encapsulation and delivery [6-8]. Teng et al. [6] developed curcumin-loaded soy protein nanoparticles with high encapsulation efficiency and a controlled biphasic release profile. Furthermore, Zare-Zardini et al. [7] proposed slow-release curcumin-containing soy protein nanoparticles as potential anticancer agents for osteosarcoma.

Recent studies indicate that soybean proteins are specifically promising for the development of nanosystems for metal detection and sorption owing to their diverse amino acid functional groups, which exhibit strong metal-binding properties [9-12]. Ju et al. [9] introduced a soybean protein fiber (SPF)-derived skeleton to regulate Li deposition and enhance Li metal battery stability. Their findings demonstrate that, during battery cycling, SPF facilitates the formation of a lithium fluoride (LiF) nanocrystal-enriched interface, which reduces interfacial impedance and enhances charge transfer kinetics. In the realm of heavy metal removal, a soy protein-based polyethylenimine hydrogel exhibited high efficiency in adsorbing Cu(II) ions from aqueous solutions, even in the presence of competing heavy metal ions such as Zn(II), Cd(II), and Pb(II) [10]. Similarly, soy protein microsponges have shown exceptional Pb(II) adsorption capabilities [11]. Yamada et al. [12] explored an inorganic composite material composed of soy protein and 3-glycidoxypropyltrimethoxysilane for heavy metal sorption from wastewater. Their study revealed that the composite material exhibits selectivity for divalent light metal ions, based on a comparative analysis of accumulation rates for different heavy metals [12].

Although numerous studies highlight the potential of soybean proteins for the development of protein-based nanocomposites for heavy metal removal and accumulation, the underlying factors influencing nanosystem metal selectivity remain insufficiently understood and require further investigation. Specifically, detailed insights into the specific binding sites and intermolecular interactions between heavy metal ions and soybean proteins are still lacking. In our previous research, we identified the binding sites for a range of heavy metal ions (Cu^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Ni^{2+}, Hg^{2+}, Co^{2+}, Cu^{+}, Au^{+}, Ba^{2+}, Pb^{2+}, Pt^{2+}, Sm^{3+}, and Sr^{2+}) on two main soybean protein (7 S globulin and glycinin) and explored the nature of their interactions [13]. The present study employs molecular dynamics simulations to investigate the effects of selected heavy metal ions (Cd^{2+} and Co^{3+}) on the structure and dynamics of soybean 7S globulin.

MOLECULAR DYNAMICS SIMULATIOS

The three-dimensional X-ray crystal structure of basic 7S globulin from soybean was retrieved from the Protein Data Bank (https://www.rcsb.org/) using PDB ID 3AUP. Molecular dynamics (MD) simulations and trajectory analyses

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were performed using GROMACS (version 5.1) with the CHARMM36m force field. For the simulations, chain A of the 7S globulin structure was selected. The input files for MD simulations were generated using the Solution Builder module within the web-based CHARMM-GUI interface [14]. The system was solvated using a TIP3P water rectangular solvation box, with a variable side length adjusted according to the protein size and heavy metal ion concentration, ensuring a minimum distance of 10 Å between the protein and the box edges. Cadmium and cobalt ions were introduced to the system at five concentrations (5, 50, 100, 500 and 550 ions, respectively). The control systems included the protein along with the necessary Na^+ ions to neutralize its net charge were also simulated. Heavy metal ions were introduced and randomly distributed within the system. Molecular dynamics (MD) simulations and trajectory analyses were conducted using GROMACS (version 2023.3) with the CHARMM36m force field in the NPT ensemble and a 2-fs time step. Simulations were performed at 300 K, with system minimization and equilibration carried out over 50,000 and 250,000 steps, respectively. During equilibration, the Berendsen algorithm was used for temperature and pressure control, while the LINCS algorithm was applied to constrain hydrogen-containing bond lengths.The total simulation time was 200 ns. The GROMACS commands gmx rms, gmx gyrate, gmx rmsf were used to calculate the protein backbone root-mean-square deviation (RMSD), protein radius of gyration (R_g), root-mean-square fluctuations of the C-alpha atoms (RMSF). Visualization of the snapshots of the MD runs and analysis of the protein secondary structures and protein solvent-accessible surface area (SASA) were performed in VMD. The evolution of the secondary structure was followed using the VMD Timeline tool [15] and Tcl scripts.

RESULTS AND DISCUSSION

Representative snapshots from a 200 ns simulation of cadmium heavy metal binding to 7S globulin are presented in Figure 1.

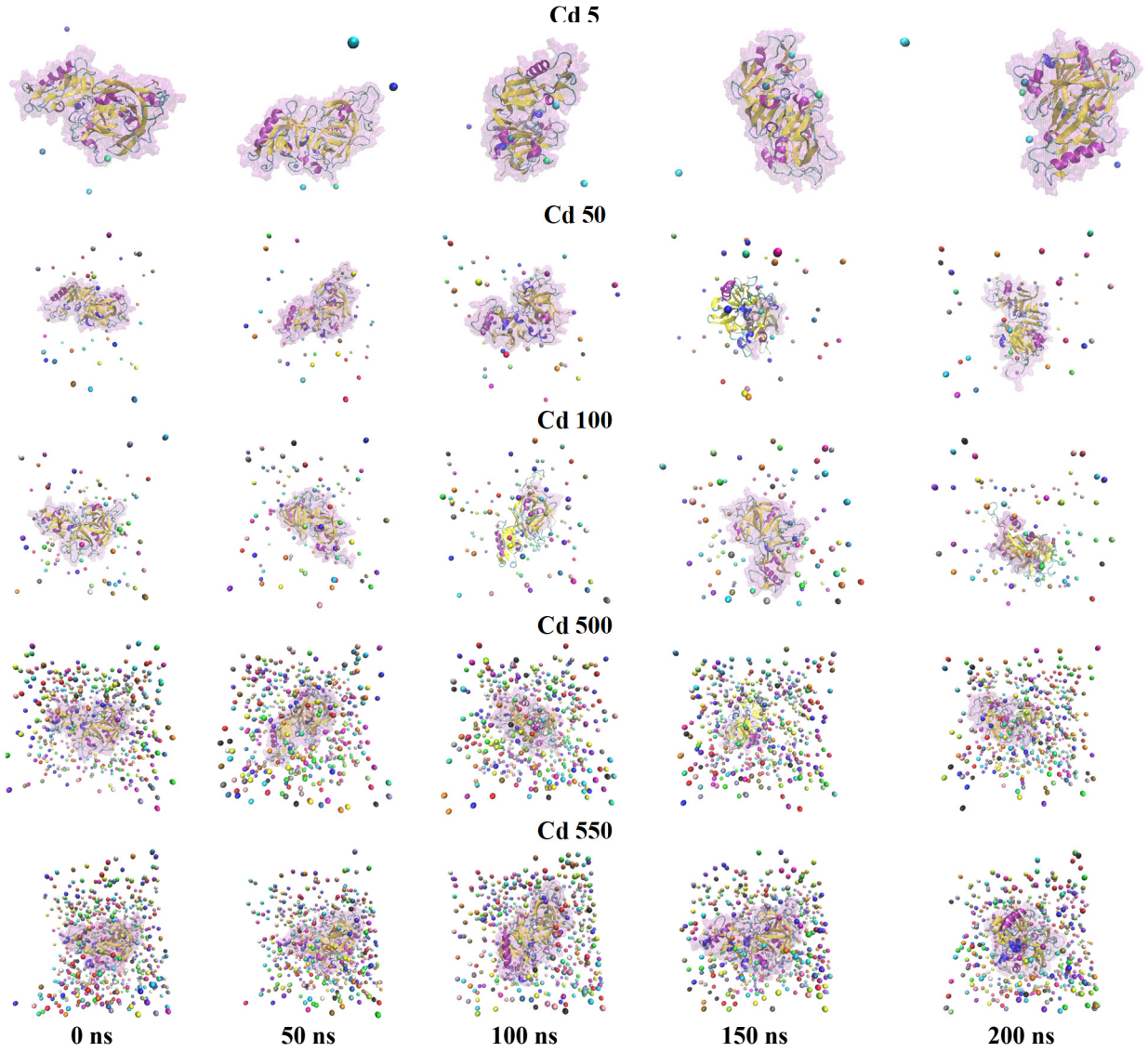

Figure 1. The snapshots of the Cd-protein complexes

In the initial phase of our study, we assessed the overall stability of the protein during the simulation by analyzing the time evolution of key structural parameters, including the backbone root-mean-square deviation (RMSD), radius of gyration (Rg), root-mean-square fluctuations (RMSF) of C-alpha atoms, and solvent-accessible surface area (SASA). Figure 2 depicts the temporal variations in the backbone RMSD, offering insights into the dynamic stability of the protein throughout the simulation period.

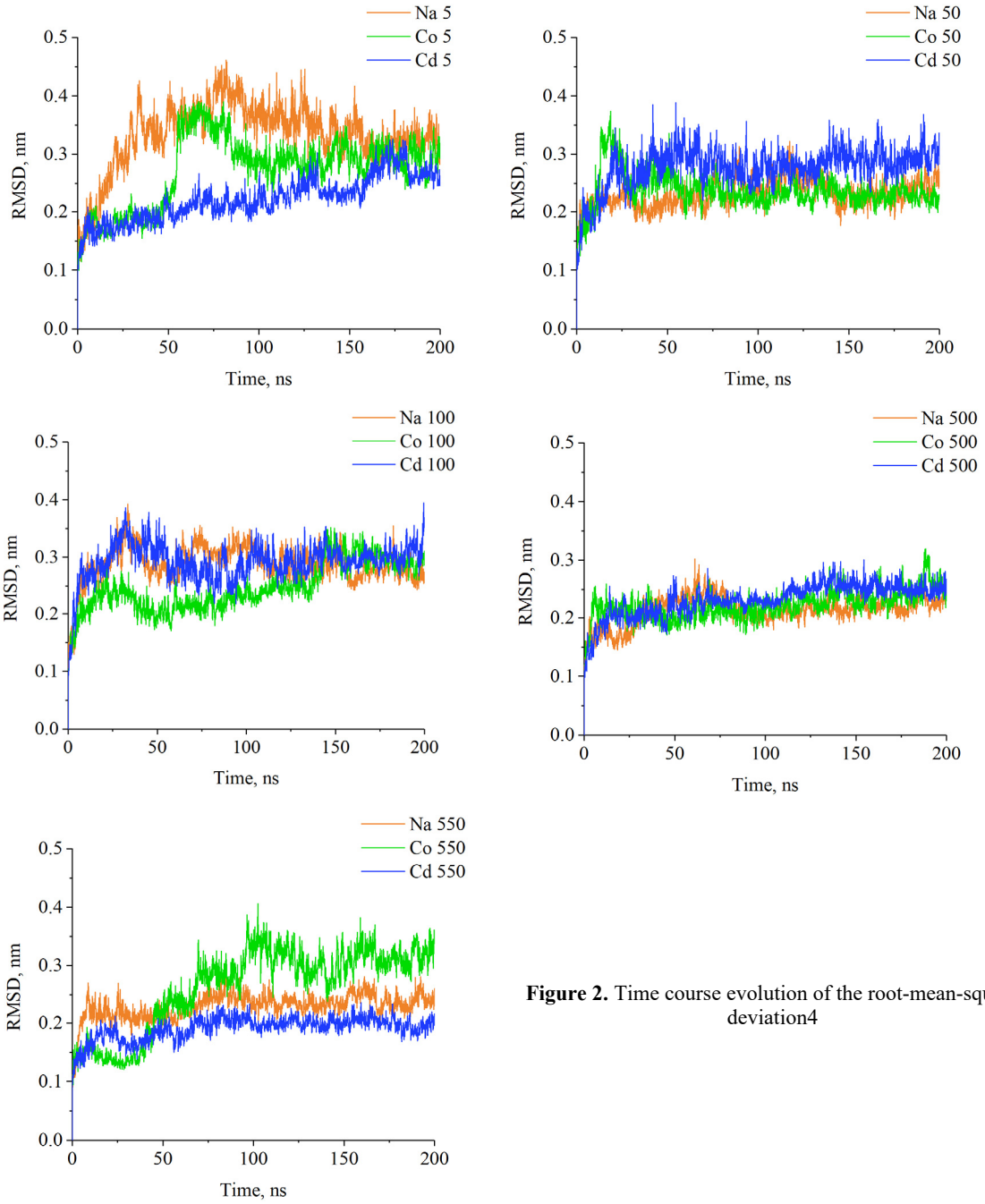

Figure 2. Time course evolution of the root-mean-square deviation4

As shown in Figure 2, the calculated RMSD values for the $Cd^{2+}$and Co^{3+}-containing systems do not exceed 0.4 nm remaining below 0.25 nm for the majority protein-metal complexes, except for brief fluctuations observed in the Cd 100/7S globulin system during the first 50 ns. The RMSD time profile for the Co5/protein system exhibits two clear stages: i) the first phase shows small fluctuations around 0.15 nm during the initial 50 ns of the simulation, indicating minimal structural deviation; ii) in the second phase, there is a sharp rise in RMSD to 0.4 nm at around 52 ns, after which the value decreases to roughly 0.2 nm. From this point, the protein's structure stabilizes and fluctuates around this value for the rest of the simulation, suggesting a stable conformation. For the Co550/protein system, a notable increase in RMSD values was observed starting from 46 ns, after which the RMSD values stabilized and fluctuated around an average of 0.33 nm for the rest of the simulation, indicating equilibration and structural stability. Therefore, it can be concluded that the molecular dynamics simulations achieved satisfactory convergence.

To determine how different concentrations of heavy metal ions affect the dynamic behavior of amino acid residues of 7S globulin, the RMSF values of the C-alpha atom were calculated (Fig. 3). The RMSF analysis revealed detailed information on the fluctuations of amino acid residues in all the heavy-metal/protein systems examined. It identified specific regions of the protein that experienced heightened spatial fluctuations, which were attributed to the binding of metal ions. The 7S globulin from soybean, also known as β-conglycinin, is a major storage protein composed of multiple subunits that adopt a compact, globular structure. Its secondary structure is predominantly composed of β-sheets, with a smaller proportion of α-helices and random coil regions [16]. A defining feature of this protein is the presence of 12 cysteine residues [36], forming five intra-chain and one inter-chain disulfide bridges [16].

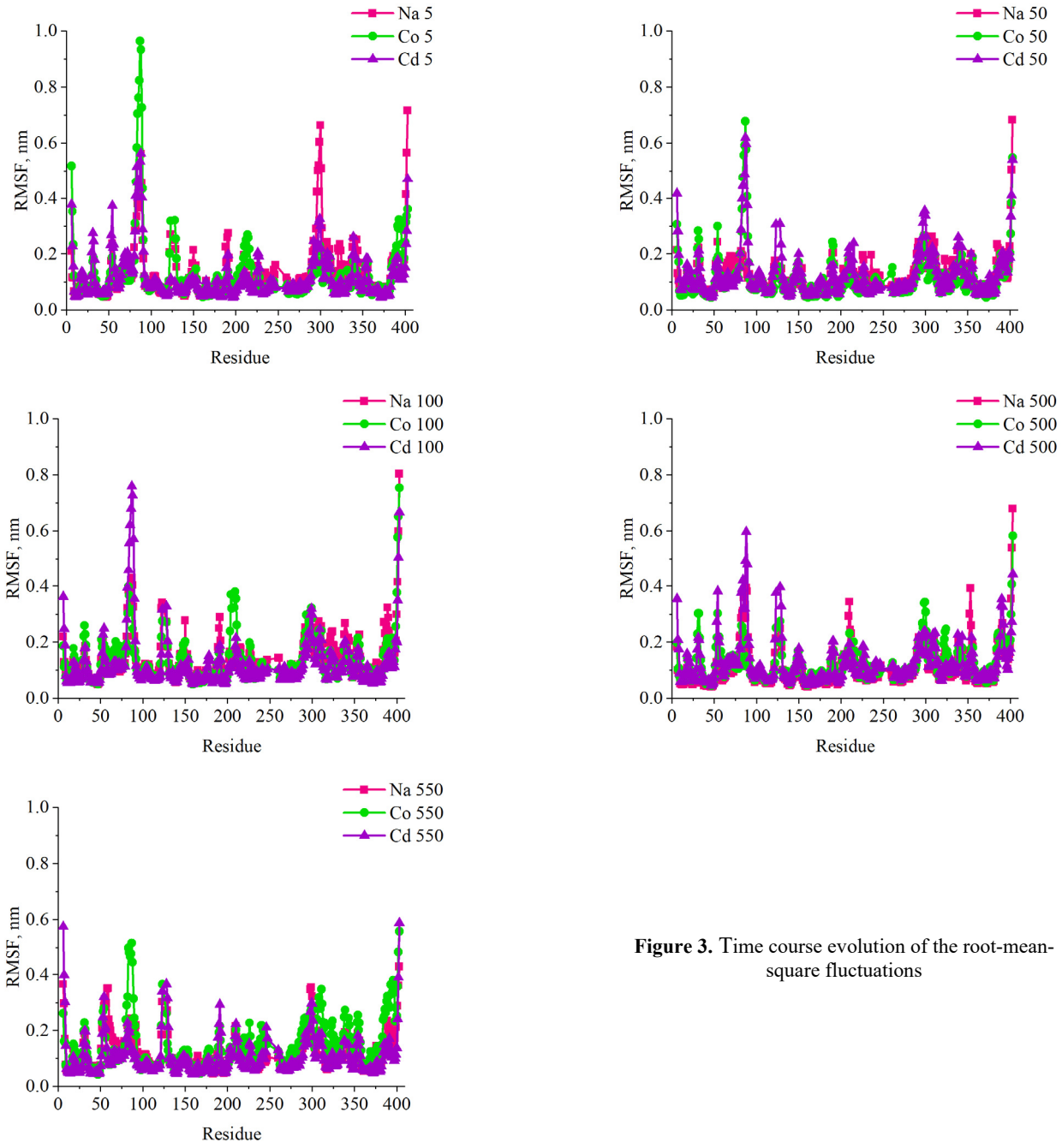

Figure 3. Time course evolution of the root-mean-square fluctuations

As shown in Figure 3, the binding of Cd^{2+} and Co^{3+} at low ion concentrations (5 and 50 ions) was associated with noticeable fluctuations in specific protein regions, particularly residues 81-92, 120-130, and 290-305. A further increase in heavy metal ion concentration led to a rise in RMSF values in the 81-92 residue region, with this effect being more pronounced in the presence of 100 and 500 cadmium ions. Moreover, in systems containing 550 heavy metal ions, the most significant RMSF increase in the 81-92 residue region was observed in the presence of cobalt ions, suggesting a stronger destabilizing effect of Co^{3+} at high concentrations. Notably, the high heavy metal ion concentrations (500 and 550 ions) lead to the fluctuation increase of all residues starting from V271 to K310. This region of 7S globulin contains the α-helix, 3_{10}-helix and β-strands, indicating the possible destabilization of the protein structure. Notably, as illustrated

in Figure 3, heavy metal binding appears to primarily affect the α-subunit (residues 25–275) in systems containing 5, 50, and 100 ions. However, at higher metal concentrations, RMSF changes were observed in both the α-subunit and the low-molecular-weight β-subunit (residues 276–427), indicating a broader impact on protein flexibility and structural dynamics.

Another parameter, reflecting the protein's structural compactness is the radius of gyration (Rg). with lower Rg values signifying a more tightly folded conformation and higher Rg values reflecting a more expanded or less compact structure. To assess how heavy metal binding influences protein structure, the Rg values for the metal/protein complexes were computed and monitored throughout the simulation. Figure 4 presents the time evolution of Rg, providing insights into changes in protein compactness throughout the simulation.

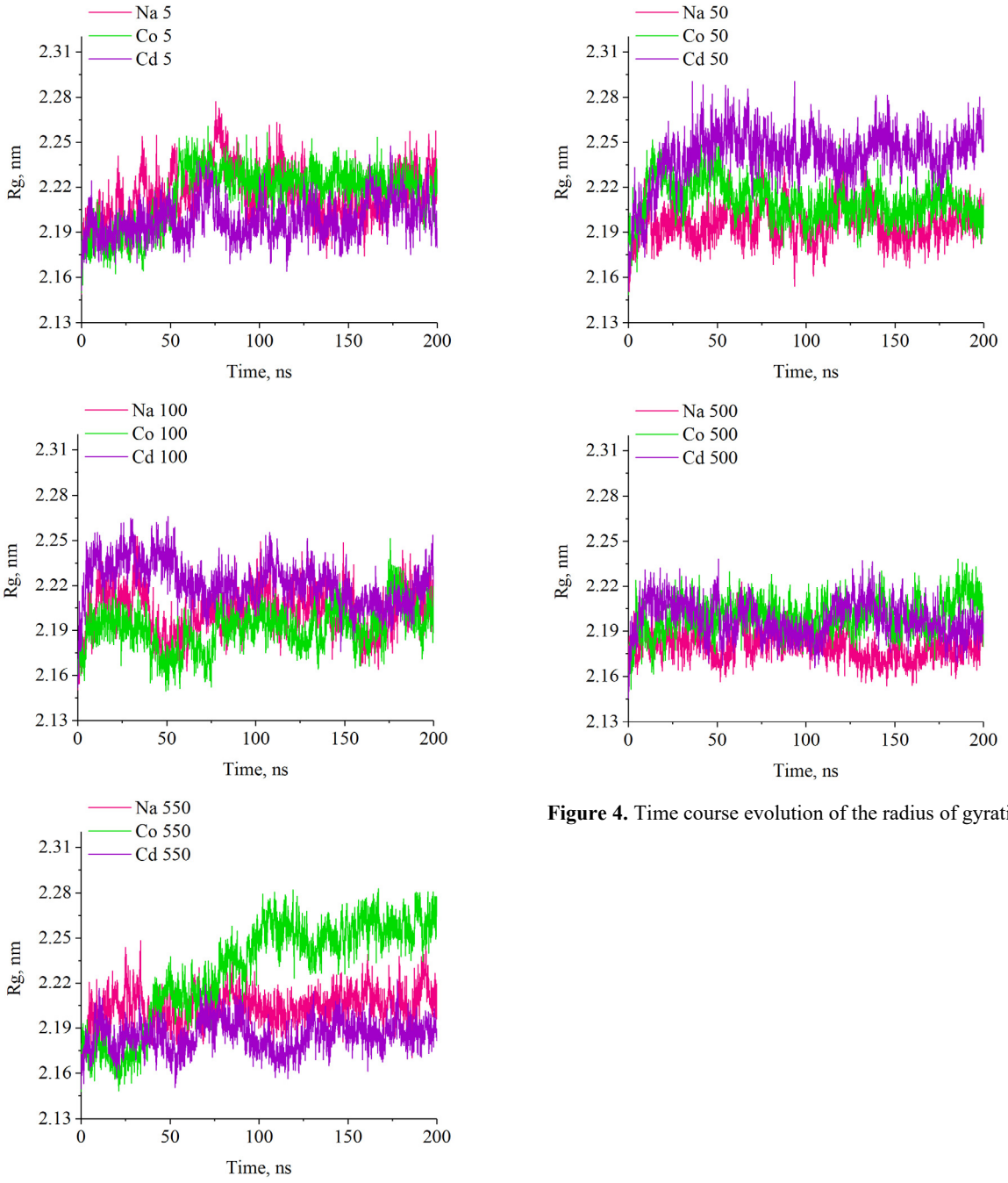

Figure 4. Time course evolution of the radius of gyration

The findings presented in Figure 4 indicate that at a low heavy metal ion concentration (5 ions), the 7S globulin structure remained highly stable throughout the simulation, with an average radius of gyration (Rg) of 2.20 nm, suggesting minimal structural perturbation. However, as cobalt concentration increased, the protein structure expanded, with the most pronounced effect observed in the Co550 protein/metal system. A linear approximation of the Rg time evolution revealed a clear increasing trend, with Rg values rising from approximately 2.16 nm at the start of the simulation to 2.27 nm at 200 ns. This suggests a spatial rearrangement of amino acid residues, potentially influencing secondary structural

elements. In contrast, for systems containing cadmium ions, the radius of gyration decreased at high metal concentrations, with the most significant reduction observed in the Cd550 system. This decrease suggests that the protein structure becomes more compact under these conditions.

To evaluate changes in the hydrophobic residue environment throughout the simulation, we analyzed the relative variations in solvent-accessible surface area (SASA) per residue (Figure 5).

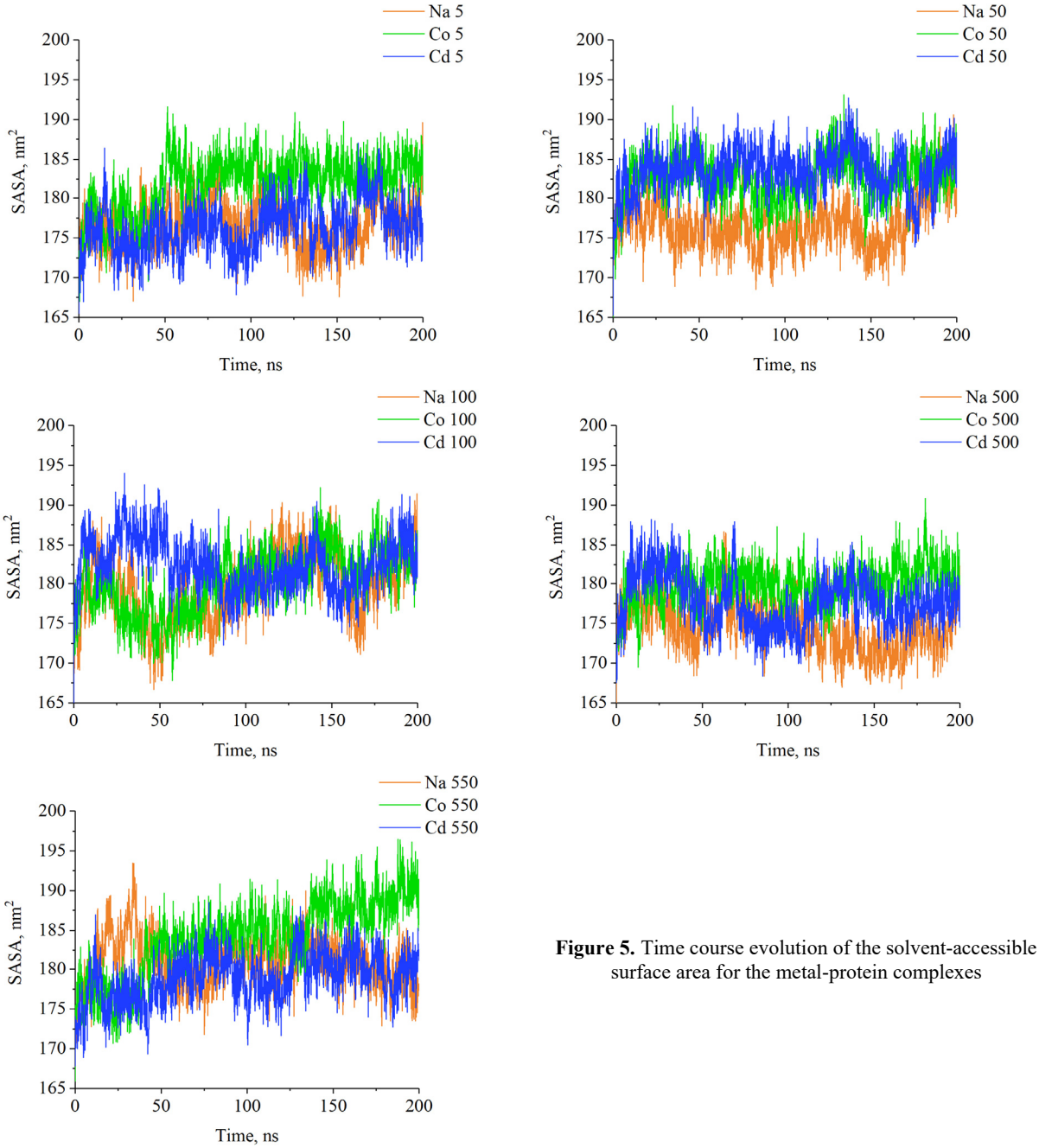

Figure 5. Time course evolution of the solvent-accessible surface area for the metal-protein complexes

The analysis showed no significant variations in SASA values across most protein-metal complexes at metal ion concentrations below 500. In the majority of studied systems, SASA remained within the range of 175–185. However, a slight increase in SASA was observed in systems containing 550 metal ions, suggesting a minor expansion of the protein's solvent-exposed surface at higher ion concentrations.

To characterize the changes in the secondary structure of 7S globulin in the presence of heavy metals during the simulation, we analyzed the time evolution of α-helices and β-sheet content (Fig. 6).

The binding of Cd^{2+} and Co^{3+} does not significantly alter the α-helical content of the protein. This finding suggests that while an increase in RMSF values was observed in the α-helical regions, it primarily reflects residue-level fluctuations rather than disruptions in the secondary structure, which remained stable throughout the simulation. Conversely, heavy metal-protein complexation resulted in a slight reduction in β-sheet content, as the proportion of residues adopting β-sheet conformations gradually decreased throughout the simulation. This effect became more pronounced at higher heavy metal concentrations, suggesting a potential destabilization of β-sheet structures in response to metal binding.

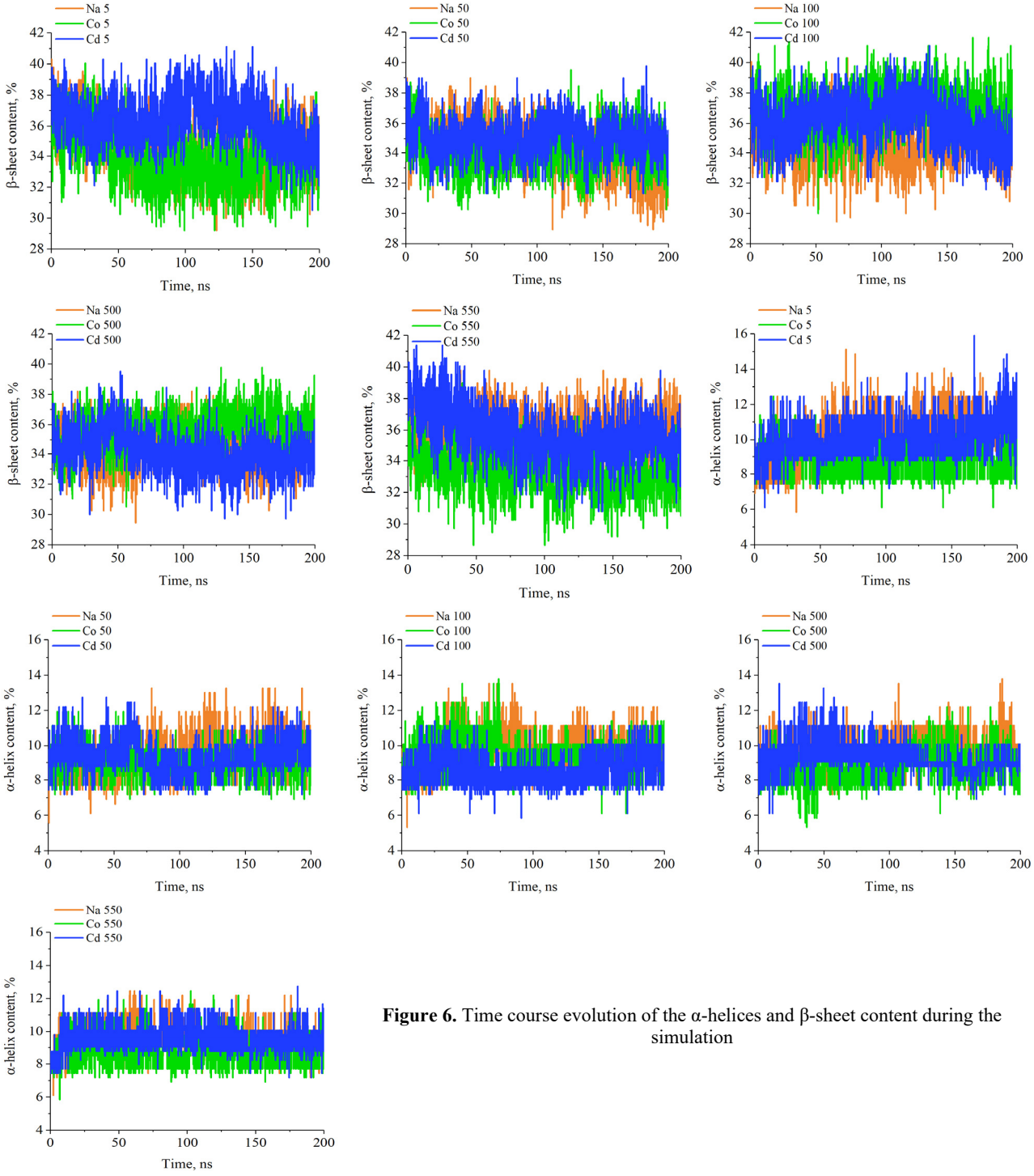

Figure 6. Time course evolution of the α-helices and β-sheet content during the simulation

CONCLUSIONS

The present study employed molecular dynamics (MD) simulations to investigate the effects of Cd^{2+} and Co^{3+} binding on the structure and dynamics of 7S globulin. Key structural parameters—including RMSD, Rg, RMSF, and SASA—were analyzed over a 200 ns MD simulation at 300 K using GROMACS and the CHARMM General Force Field to assess protein stability, flexibility, and compactness at varying metal ion concentrations.Our MD results indicate that at low metal concentrations (5 ions), the protein remained highly stable with minimal structural deviations. However, as metal concentrations increased, distinct effects were observed depending on the ion type. RMSF analysis revealed that heavy metal binding primarily affected specific regions of the α-subunit at lower concentrations, while both the α- and β-subunits were impacted at higher concentrations. Increased fluctuations in regions containing secondary structural elements (α-helices, 3_{10}-helices, and β-strands) suggest potential destabilization, particularly in systems with high metal ion concentrations (500 and 550 ions). Secondary structure analysis demonstrated that α-helical content remained stable, indicating that observed fluctuations were primarily residue-level rather than structural disruptions. In contrast, a gradual

reduction in β-sheet content was observed, particularly at high metal concentrations, suggesting a destabilizing effect of heavy metal binding on β-sheet structures.

Overall, our findings highlight the distinct structural responses of 7S globulin to cadmium and cobalt binding and contribute to a deeper understanding of protein-metal interactions. These insights may have implications for the development of protein-based nanomaterials for heavy metal detection and sorption.

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ВЗАЄМОДІЯ ВАЖКИХ МЕТАЛІВ З 7S ГЛОБУЛІНОМ З СОЇ: ДОСЛІДЖЕННЯ МЕТОДОМ МОЛЕКУЛЯРНОЇ ДИНАМІКИ

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У цьому дослідженні були використано метод молекулярної динаміки (MD) для дослідження структурних і динамічних ефектів зв'язування Cd^{2+} і Co^{3+} з 7S соєвим глобуліном. Використовуючи 200-нс симуляцію при 300 K з GROMACS і CHARMM General Force Field, були проаналізовані ключові структурні параметри, зокрема корінь середньоквадратичного відхилення остову ланцюга (RMSD), радіус інерції (Rg), площа поверхні, доступна для розчинника (SASA), та середньоквадратичні флуктуації (RMSF), для оцінки стабільності, гнучкості та компактності білка при різних концентраціях іонів металів. Результати MD дослідження показали: i) при низьких концентраціях металів білок зберігав структурну стабільність з мінімальними відхиленнями; ii) зростання концентрацій металевих іонів викликало більш суттєві структурні зміни в структурі білку залежно від типу іону; iii) на низьких концентраціях металів зміни переважно стосувалися конкретних регіонів α-субодиниці глобуліну, в той час як на вищих концентраціях впливали як на α-, так і на β-субодиниці; iv) коливання в елементах вторинної структури — α-спіралях, 3_{10}-спіралях та β-ланцюгах — вказували на потенційну дестабілізацію, особливо в системах з високими концентраціями металів; v) вміст α-спіралей залишався стабільним протягом симуляції, однак спостерігалося незначне зменшення вмісту β-ланцюгів при високих концентраціях металів. Це свідчить, що зв'язування важких металів може мати дестабілізуючий ефект на β-ланцюгові структури та змінювати загальну конформацію 7S глобуліну. Ці результати є корисними для розробки наноматеріалів на основі білків для виявлення та сорбції важких металів.

Ключові слова: *взаємодія білок-метал; важкі метали; молекулярна динаміка*