Studies of the transition metal complex with Human Serum Albumin

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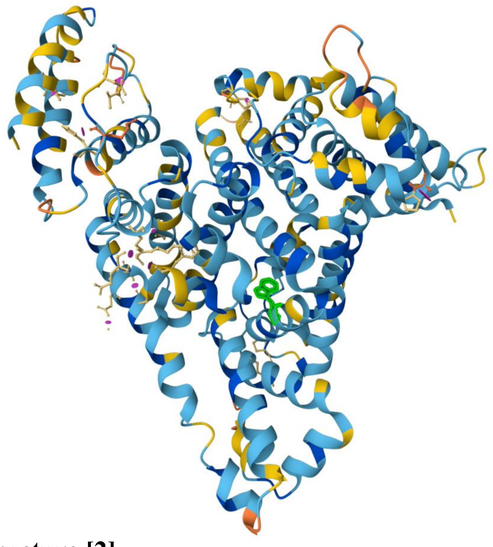
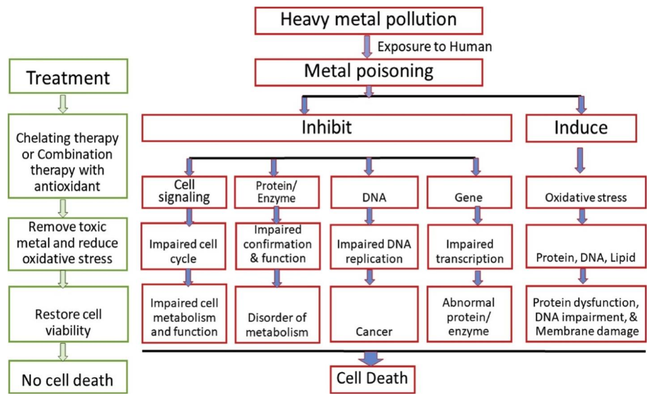
Background

Human Serum Albumin (HSA) is the most abundant protein present in the plasma. It plays vital roles in maintaining osmotic pressure and transporting various substances, including drugs. HSA has multiple metal-binding sites that facilitate the interaction and binding of metal ions without significantly compromising the structure of the protein [1]. Albumin contains four metal-binding sites that are partially selective, with distinct preferences for certain metals. It is an important regulator of physiological metal ions, such as Cu(II) and Fe(III) [1]. This unique ability, attributed to HSA's structure and multiple binding sites, enables it to interact with a wide range of ligands, serving key roles in transport and antioxidant defense.



HSA structure [2]

Motivation and Significance



Cu(II) [1]

- HSA contains approximately 15% of physiological Cu(II) always bound as Cu(II) because of the oxidative conditions in the blood.

Fe(II) [1]

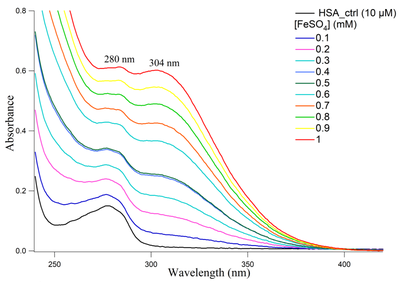
- While present in the body, iron is a cofactor of many enzymes.
- HSA can bind with Fe^{2+} when physiological concentrations are in iron overload.

Tabulation of Binding Site

Metal Ions	Binding Site
Fe^{2+}	Gln29, Tyr30, Gln32
Cu^{2+}	Gln29, Tyr30, Gln32

Optical Spectroscopy Results

With $FeSO_4$ (Figure 1)



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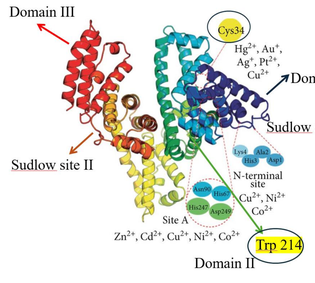
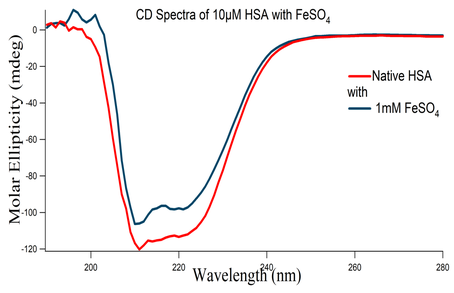
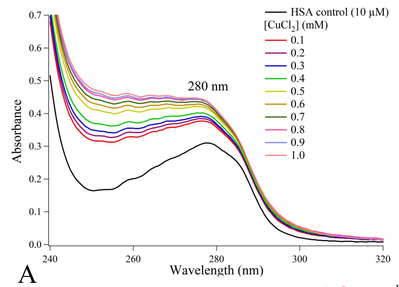


- Figure 1 A. Absorption Spectra of HSA with Fe^{2+}.
- Figure 1 B. CD spectra of HSA with Fe^{2+}.



B

With $CuCl_2$ (Figure 2)



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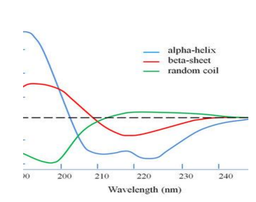
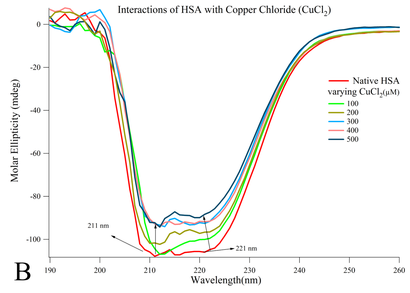


- Figure 2 A. Absorption Spectra of HSA with Cu^{2+}.
- Figure 2 B. CD spectra of HSA with Cu^{2+}.



B

Objectives and Approaches

Hypothesis: Specific transition metals bind to HSA without causing significant structural changes and enabling the protein to adapt its function.

Main objective: Explore the effects of transition metal complexes on the structural integrity of human serum albumin.

The approaches taken: Optical Spectroscopic techniques and titration methods.

Observations

- With gradual addition of both Cu^{2+} and Fe^{2+} there is an increase in the abs at 280 nm, indicating interaction of the ions with HSA.
- With Fe^{2+}, there is a growth of a new peak at 304 nm, suggesting the formation of a new chromophore.
- The addition of $CuCl_2$ to HSA appreciably change the secondary structure at concentrations > 0.2 mM.
- The addition of $FeSO_4$ did not significantly alter the structure of the protein at concentrations under 1 mM.

Future Studies

- Explore the effect of other transition metals, such as silver and manganese, on HSA's structural conformation.
- Use fluorescence spectroscopy to examine these interactions further.
- Explore the new chromophore at 304 nm with $FeSO_4$.
- Use oxidized protein and see the effect of metals.
- Perform SDS-PAGE and HPLC.

References:
1. Wojciech B.; Magdalena S.; Ewa K.; Peter F. Binding of transition metal ions to albumin: Sites, affinities and rates. *Biochimica et Biophysica Acta (BBA) - General Subjects*. **2013**, *1830* (12), 5444-5455. https://doi.org/10.1016/j.bbagen.2013.06.018. (Accessed 2025-2-23 from Science Direct).
2. Ischemia-Modified Albumin: Origins and Clinical Implications - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Structure-of-human-serum-albumin-a-The-molecule-consists-of-a-single-polypeptide_fig2_353667584 [accessed 23 Feb 2025]

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