Modeling and Characterization of Thymosin β4 as a Multi-Metal System: Iron, Zinc and Calcium Binding, Peptide Aggregation and Ferroptosis

Joanna Izabela Lachowicz[1]

[1] Department of Environmental Health, Occupational Medicine and Epidemiology, Wroclaw Medical University, Mikulicza-Radeckiego 7, 50-368 Wroclaw, PL, Poland, joanna.lachowicz@umw.edu.pl
[2] Department of Medical Science and Public Health, University of Cagliari, Cittadella Universitaria, 09042 Monserrato, CA, Italy;

Thymosin β4 (Tβ4) is a highly conserved, intrinsically disordered peptide traditionally associated with actin sequestration and tissue repair. However, emerging evidence suggests a broader functional role involving metal ion coordination and regulation of redox-dependent cellular processes. In this work, we present an integrated view of Tβ4 as a multi-metal system, combining experimental characterization and conceptual modeling to describe its behavior as a complex biochemical network.

Using a multi-technique approach, including spectroscopic, structural, computational, and cellular analyses, we demonstrate that Tβ4 is capable of coordinating multiple biologically relevant metal ions, including iron (Fe^{2+}/Fe^{3+}), zinc (Zn^{2+}), and calcium (Ca^{2+}), through distinct and partially overlapping binding regions. Iron binding plays a central role in modulating intracellular redox balance and contributes to the regulation of ferroptosis, an iron-dependent form of regulated cell death. In this context, Tβ4 acts as an endogenous metal chelator capable of limiting labile iron pools and influencing oxidative stress-related pathways.

Beyond iron coordination, we show that zinc binding induces peptide aggregation, revealing an additional layer of complexity in Tβ4 behavior. This aggregation process suggests the emergence of higher-order structures driven by metal-mediated interactions, potentially affecting peptide bioavailability and function. In parallel, calcium coordination highlights the involvement of Tβ4 in ion-dependent signaling environments, suggesting a role in coupling structural dynamics with cellular signaling processes.

Importantly, the combination of biophysical characterization (e.g., NMR, spectroscopy), molecular modeling, and cellular functional studies enables a translational interpretation of Tβ4 activity, bridging molecular-scale mechanisms with biological outcomes. This integrative strategy is essential to capture the multifactorial nature of Tβ4, where metal binding, conformational plasticity, aggregation phenomena, and redox regulation are tightly interconnected.

By integrating these findings, we propose that Tβ4 can be described as a multi-parametric, metal-dependent system, in which binding affinity, metal competition, and peptide conformational flexibility collectively determine functional outputs. From a complex systems perspective, ferroptosis regulation, peptide aggregation, and metal homeostasis emerge as interconnected phenomena, rather than isolated processes.

This framework positions Tβ4 as a metal-driven molecular hub, where coordination chemistry, structural plasticity, and cellular responses converge. The adoption of a multi-technique, translational approach not only advances the fundamental understanding of Tβ4 biology but also opens new perspectives for biomedical applications, including the design of novel therapeutic strategies targeting metal dysregulation, oxidative stress, and ferroptosis-related pathologies.

REFERENCES

1. Lachowicz, J.I.; Pichiri, G.; Piludu, M.; Fais, S.; Orrù, G.; Congiu, T.; Piras, M.; Faa, G.; Fanni, D.; Dalla Torre, G.; et al. Thymosin β4 Is an Endogenous Iron Chelator and Molecular Switcher of Ferroptosis. Int. J. Mol. Sci. 2022, 23, 551.
2. Lachowicz, J.I.; Jaremko, M.; Jaremko, Ł.; Pichiri, G.; Coni, P.; Piludu, M. Metal Coordination of Thymosin β4: Chemistry and Possible Implications. Coord. Chem. Rev. 2019, 396, 117–123.
3. Piludu, M.; Pichiri, G.; Coni, P.; Piras, M.; Congiu, T.; Faa, G.; Lachowicz, J.I. Cell Starvation Increases Uptake of Extracellular Thymosin β4 and Its Complexes with Calcium. Int. Immunopharmacol. 2023, 116, 109743.