# ABSTRACT

Alpha-2-macroglobulin ($\alpha_2$M) belongs to the alpha-macroglobulin family of secreted proteins and is constitutively abundant in human biological fluids. The functions of $\alpha_2$M are dependent on its conformation, which can be native, transformed, or dimeric. Each conformation is functionally distinct, all playing a crucial role in the regulation of inflammatory and immune responses. It has been demonstrated that dissociation of the native $\alpha_2$M tetramer into dimers is induced by hypochlorite, an inflammatory oxidant, that significantly enhances the holdase-type chaperone activity of $\alpha_2$M. Preliminary investigations by the Wyatt laboratory identified that phenolic compounds such as rosmarinic acid (RA), caffeic acid (CA) and salvianolic acid β (Saβ), induce $\alpha_2$M to dissociate into dimer-like molecules. The purpose of this study was to demonstrate the effect of a drug or drug-like phenolic compounds on the conformation of $\alpha_2$M and compare this to the effect of hypochlorite.

Considering the superior chaperone activity of dimeric $\alpha_2$M compared to native and transformed $\alpha_2$M tetramers, this study undertook a series of experiments to characterise the structure and function of phenolic compound-treated $\alpha_2$M in order to generate proof-of-principle data, to demonstrate whether phenolic compounds could be used to target the chaperone activity of $\alpha_2$M. Specifically this study aimed to characterise in the holdase-type chaperone activity and cell surface receptor binding of phenolic compound-modified $\alpha_2$M. The evaluation of holdase-type chaperone activity involved conducting a Thioflavin T (ThT) assay with the Alzheimer's disease-related amyloid beta peptide, $A\beta_{1-42}$. Assessment of cell surface binding of biotinylated phenolic compound-modified $\alpha_2$M was accomplished through flow cytometry, utilizing SH SY5Y neuroblastoma cells. The structural characterization of

phenolic compound-modified $\alpha_2$M included native gel electrophoresis and a bis-ANS assay to analyse migration patterns and surface exposed hydrophobicity, respectively. The proof-of-principle data generated in this study support the conclusion that although phenolic compound-modified $\alpha_2$M enhanced the ability of $\alpha_2$M to stabilise A$\beta_{1\text{-}42}$, this effect does not occur via a canonical holdase chaperone action. The results also suggest that phenolic compounds may induce a slightly transformed conformation in $\alpha_2$M, which is electrophoretically fast but shields the hydrophobic regions that are normally exposed on $\alpha_2$M dimers. The findings support the idea that treatment of $\alpha_2$M with phenolic compounds may expose the internal thiol ester that forms a covalent bond with A$\beta_{1\text{-}42}$ and exposes the LRP-1 binding domain. However additional studies are required to confirm this model.

A greater understanding to the functions of phenolic compound-modified $\alpha_2$M has the potential to lay the groundwork for developing treatments for conditions characterized by protein misfolding and inflammation.