412d Understanding Lactalbumin Structural Change in Hydrophobic Interaction and Reverse Phase Chromatography

Abigail H. Laurent, Jie Chen, Jace L. Fogle, Yunzhi Xiao, Steven M. Cramer, John P. O'Connell, Erik Fernandez, and Todd M. Przybycien

Separation in reversed-phase chromatography (RPC) and hydrophobic interaction chromatography (HIC) is based on hydrophobic interactions of the target molecule with the chromatography media. RPC and HIC media differ in the coverage of nonpolar ligands on the support, with HIC media being more sparsely covered than RPC. RPC has not found routine industrial use for proteins because it is denaturing. This is likely the very reason why RPC is so highly resolving, as denaturation provides the media with access to more hydrophobic interior residues [1, 2]. HIC is currently used in protein bioprocessing as it is not as denaturing as RPC. However the relationship between separation in HIC and protein structural change is not yet known.

In this work, we studied the calcium-binding protein bovine α-lactalbumin which has been shown to exhibit structural change upon interaction with both HIC [3] and RPC media [4]. Alpha-lactalbumin is a useful model protein in that it has two well-characterized forms with differing stabilities – the holo form which is attained on binding calcium ions and the less stable apo form which is attained in the absence of calcium ions. Raman and circular dichroism (CD) spectroscopy results for holo/apo form mixtures indicate that α-lactalbumin structural change on HIC media differs in type from what is seen on RPC media. The protein loses helical content and gains sheet content on exposure to HIC media and gains helical content on exposure to RPC media. Our subsequent spectroscopic studies explore how α-lactalbumin's structural response on adsorption may compare across different types of hydrophobic chromatography media, both HIC and RPC, for similar mobile phase conditions and at different points on the apparent adsorption isotherms for both the holo and apo forms. Batch adsorption experiments will be performed on two types of HIC media, a butyl- and a phenyl-derivatized resin, and two types of RPC media, a C4 and a C18 resin. Measurements of the free energy of unfolding of α-lactalbumin under the solution conditions of interest will be used to inform the interpretation of the structural response in terms of a previously proposed 4-state thermodynamic model. This will allow effects of salt and organic solvent mobile phase modifier to be separated from the effects of the media surfaces.

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