139c Deactivation of Enzymes in Concentrated Salt Solutions: an Investigation of Hofmeister Effects

James M. Broering and Andreas S Bommarius

Enzymes and non-catalytic proteins are rarely found alone in solution. Instead, they are surrounded by a wide array of cosolutes which can have beneficial or detrimental effects on their stability in solution. Understanding the effects of these cosolutes on dissolved proteins can lead to better formulation of bioprocessing media, improved shelf life studies of protein-based pharmaceuticals, and more robust biocatalyst design. Most published work has focused on the effects of strongly deactivating cosolutes such as guanidinium salts or urea, or strongly stabilizing cosolutes such as trehalose or trimethylamine oxide (TMAO). The focus on studying these extreme cases has left a noticeable gap in what is understood about the influences of cosolutes with intermediate stabilizing or destabilizing effects. Despite the fact that proteins are more likely to encounter salts like sodium chloride than guanidinium or TMAO in solution, especially in vivo, common salts fall into this intermediate category of poorly understood cosolutes.

Since Hofmeister's original work which ranked salts based on their effectiveness at salting-in or salting-out proteins, the same ranking has been found to predict the relative effects of salts on a number of other protein properties. However, descriptions or predictions of different salts' effects on protein properties have rarely moved beyond the qualitative ranking of the Hofmeister series. Ion hydration, as implied by the magnitude and sign of the Jones-Dole B-viscosity coefficient has been suggested as an indicator of an ion's effect on a protein. Though B-viscosity coefficients have been used as relative indicators of Hofmeister series position, a quantitative link between B-viscosity coefficients and salt effects on protein stability has not been established. Our work seeks to evaluate the usefulness of B-viscosity coefficients as predictors of an ion's effect on protein deactivation kinetics and develop a quantitative relationship between B-viscosity coefficients and observed kinetic deactivation constants of proteins.

We have measured the kinetics of horse liver alcohol dehydrogenase (HL-ADH) and □-chymotrypsin deactivation and the fluorescence loss of a novel monomeric red fluorescent protein in sodium salt solutions of constant water activity and the calculated the observed kinetic deactivation constants. These deactivation constants were compared to the Jones-Dole B-viscosity coefficients of the anions in solutions, and though the three proteins are not structurally similar, common trends was observed for all three. We use differential scanning calorimetry to measure the unfolding constants of our proteins and, in combination with our observed deactivation rates, show that the deactivation is unfolding limited. We will also investigate the preferential interactions of the salts with the proteins to determine their role in modifying protein kinetic stability and any correlation with ion hydration.