Equilibrium Thermodynamic Analysis of Protein Adsorption Mechanism to Mixed-Mode Chromatography Resin

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Mixed-mode resins containing mercapto-ethyl-pyridine (MEP) as a binding motif, for example, is developed for antibody capturing as an alternative to Protein A resin. It is known that the mechanisms of protein interaction with a mixed-mode resin are 'mixed', i.e., adsorption primarily by hydrophobic interactions and desorption mainly by electrostatic interactions. However, the mechanisms are not yet fully elucidated. In this study, we performed equilibrium adsorption experiments with BSA (bovine serum albumin) and MEP resin (purchased from Pall Life Sciences, Inc., USA) and applied the classical van't Hoff analysis to examine the thermodynamic properties. The parameters were: NaCl concentration (0, 50, 100 mM), pH (4, 5, 6, 7, 8, 9), and temperature (25, 35, 45℃). The results showed that Ka and qmax were affected mostly by temperature, i.e., higher temperature increased the binding capacity by 2-5 times. Also, qmax was increased by higher salt concentration. But, there was no clear effect of pH. Considering the pI of BSA is 4.8, the surface charge variation did not influence the binding. We could infer the binding mechanism was entropy-driven, i.e., hydrophobic interactions.