

Analysis of BSA adsorption by using ITC, DSC, and equilibrium isotherm

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In order to elucidate the adsorption behavior of BSA onto aluminium hydroxide gel (alum gel) in terms of biothermodynamics, isothermal titration calorimetry (ITC) experiments were performed. Effects of 'ligand' (BSA) concentration and temperature on the adsorption to 'macromolecules' (alum gel) were investigated by using two different BSA concentrations (17 and 26 mg/mL) at two different temperatures (25 and 35°C). Overall, the adsorption to alum gel was an endothermic reaction, but the dilution (of BSA by the buffer) itself was exothermic. Association constant (K_a) to form the BSA-alum gel complexes ranged $10^6 \sim 10^7 \text{ M}^{-1}$. This value was ca. 3 orders-of-magnitude lower than those of antigen-antibody coupling reactions. ΔH at 35°C and 25°C for 26 mg/mL were around 175 and 75 kJ/mol. However, ΔG for the entire experiments was ca. -40.0 kJ/mol. ΔS at 35°C and 25°C for 26 mg/mL were ca. 0.70 and 0.38 kJ/(mol•K), respectively. In conclusion, the adsorption of BSA onto aluminium hydroxide gel adjuvant was entropically driven reaction. It means the water molecules shielding both the gel and the protein were expelled during the adsorption. Currently equilibrium isotherm and differential scanning calorimetry (DSC) experiments are being performed.