Thermodynamic analysis of Human Serum Albumin interaction with uremic toxins.

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Uremic toxins accumulate in large numbers in the blood of patients suffering from chronic kidney disease (CKD) and interact detrimentally with plasma proteins [1-3]. Thereby, several protein-bound toxins mainly bind to the transport protein Human Serum Albumin [1]. A well-studied toxin is Indoxylsulfate (IS), where binding to HSA is known to inhibit the drug binding ability of the protein in addition to structural modifications []. Another representative member of small hydrophobic uremic toxins, which has not been well studied yet is Phenylacetic acid (PhAA) [4]. Jankowski et al. have achieved a more effective clearance of PhAA and IDS under high ionic strength, where the fraction of uremic toxins was significantly decreased during hypertonic predilution hemodiafiltration [5]. However the mechanisms underlying the interaction have not been fully explored. Furthermore, albumin is modified in pathophysiological condition as in chronic renal failure: urea-induced carbamylation on multiple lysine and arginine chains occur [6].
To elucidate thermodynamic information, isothermal titration calorimetry (ITC) is the best method of choice to measure enthalpy, entropy and binding affinity directly in one experiment [7]. We therefore use ITC to investigate the binding of IS and PhAA to HSA under the influence of varying temperature and ionic strength to access full thermodynamic information. Additionally, the effect of in vitro urea modification of HSA upon its binding affinity towards the uremic toxins is studied.
It is found that two PhAA molecules bind to HSA in a sequential binding process with a binding constant k_b in the order of $\approx 10^4$ and $\approx 10^3$. In contrast, IDS binds much stronger to HSA with a total of 3 molecules to a high and low affinity binding site in the order of $\approx 10^5$ and $\approx 10^3$. All binding isotherm show negative binding affinity dependency with increasing temperature and higher ionic strength. Representative binding isotherms are shown in Fig. 1 with the binding of PhAA to native HSA at (a) different temperature and constant ionic strength I=20mM and (b) constant temperature 37°C and varying ionic strengths.

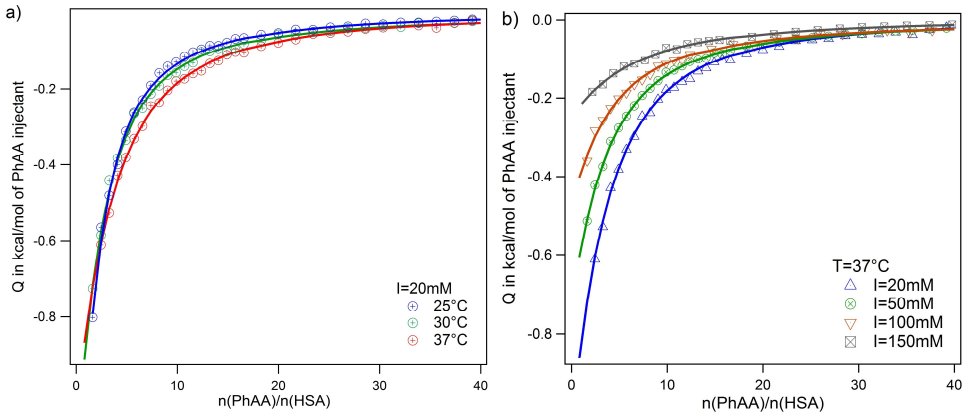

Fig. 1: Binding isotherms for the binding of PhAA to native HSA. (a) Influence of temperature varying from room to body temperature at ionic strength I=20mM. (b) influence of ionic strength at body temperature 37°C.

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