Determination of experimental Sb binding constant

Determination of Sb binding constant was performed through titration of preformed ZF with the trivalent antimony compound potassium Sb^{III} tartrate and taking advantages of the known equilibrium constants for the formation of potassium Sb^{III} tartrate[17] and ZnF peptide.[16b] Experiment was performed at 298 K in 20 mM potassium phosphate, KCl 0.075 M, pH 6 (I = 0.1 M). Initial peptide concentration was 3.3 μM and Sb concentration varied from 3.3 to 95 μM (8 data points).

The following reactions and equilibrium constants were considered:

$SbO^+ + H_2T^{2-} \leftrightarrow SbT^- + H_2O$ $\quad K_{SbT} = [SbT^-]/[SbO^+][H_2T^{2-}]$ (1)

$SbO^+ + H_3F \leftrightarrow SbF + H^+ + H_2O$ $\quad K_{SbF} = [SbF][H^+]/[SbO^+][SbF]$ (2)

$Zn^{II} + H_3F \leftrightarrow ZnF^- + 3H^+$ $\quad K_{ZnF} = [ZnF^-][H^+]^3/[Zn^{II}][H_3F]$ (3)

Where SbO^+, H_2T^{2-} and H_3F, Zn^{II}, SbT, SbF and ZnF^- represent the reactive antimony species in solution, the fully deprotonated form of tartrate, the apopeptide, the free Zn^{II} cation, the 1:1 Sb^{III}-tartrate complex, the 1:1 Sb^{III}-peptide complex and the 1:1 Zn^{II}-peptide complex, respectively.

From reactions (1), (2) and (3), the following reaction and equilibrium constant were deduced:

$SbT^- + ZnF^- + 2H^+ \leftrightarrow SbF + H_2T^{2-} + Zn^{II}$ $\quad K = [SbF][H_2T^{2-}][Zn^{II}]/[SbT^-][ZnF^-][H^+]^2$ (4)

Since $[SbF] = [H_2T^{2-}]$, $[Zn^{II}] = [Zn]_{tot} - [ZnF^-]$ and $[SbT^-] = [Sb]_{tot} - [SbF]$,

where $[Zn]_{tot}$ and $[Sb]_{tot}$ represent the concentrations of total (free and bound) Zn and Sb, respectively, the equilibrium constant K could be expressed as follows:

$$K = [SbF]^2 ([Zn]_{tot} - [ZnF^-])/[ZnF^-]([Sb]_{tot} - [SbF])[H^+]^2 \quad (5)$$

From the fluorimetric titration data, [SbF] and $[ZnF^-]$ were calculated as follows:

$[SbF] = [F]_{tot}(I - I_{Zn})/(I_{Sb} - I_{Zn})$

$[ZnF^-] = [F]_{tot}(I - I_{Sb})/(I_{Zn} - I_{Sb})$

Where $[F]_{tot}$ represent the concentrations of total (bound to Zn and Sb) peptide,

Where I is the fluorescence measured from any added potassium Sb^{III} tartrate concentration and I_{Zn} and I_{Sb} correspond to the fluorescence intensities of the fully Zn^{II}- and Sb^{III}-saturated peptide, respectively.

The plot of $[SbF]^2 ([Zn]_{tot} - [ZnF^-])/[ZnF^-]$ as a function of $([Sb]_{tot} - [SbF])[H^+]^2$ followed by linear regression, according to eq 5, allowed the determination of K:

$K = 22820 \pm 1890$

Since K is related to the equilibrium constants of reactions (1), (2) and (3), according to the equation $K = K_{SbF}/K_{ZnF}K_{SbT}$, $K_{SbT} = 1.638\text{x}10^8$ is given in the literature[17] and Zn binding constant to the peptide at pH 6 and 298 K is: $K'_{ZnF} = K_{ZnF}/[H^+]^3 = 10^{7.5}$ M^{-1} according to literature,[16b] K_{SbF} was calculated as: $K_{SbF} = 118$.

A binding constant $K'_{SbF} = K_{SbF}/[H^+] = 1.18\text{x}10^8$ M^{-1} was determined at pH 6 and 298 K.