Supplemental Information

"Thermodynamics of Pb(II) and Zn(II) binding to MT-3, a neurologically important metallothionein"

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Description of the determination of the formation enthalpy (ΔH) and stability constant (K) for the formation of metal-EDTA complexes at a specific pH.

To provide more detail about how data from the NIST database was used in the thermodynamic analyses, the treatment of Zn^{2+}EDTA formation at pH 6.0 in Bis-Tris buffer is outlined in detail. An analogous approach was used for Pb^{2+}EDTA formation at pH 6.0 in MES buffer.

$$Zn^{2+} + H^{+}_{n}EDTA + n\ Buffer \rightleftarrows Zn^{2+}EDTA + n\ BufferH^{+}$$

Enthalpy of Formation: ΔH

$\Delta H_{Zn\text{-}EDTA}(pH\ 6.0) = -n\Delta H_{EDTA\text{-}H} + \Delta H_{Zn\text{-}EDTA} + n\Delta H_{Buffer\text{-}H}$

Here, n is the number of protons that are displaced from the EDTA by the metal ion and subsequently protonate the buffer.

To determine n, the protonation state of the EDTA at the desired pH is found with the Henderson-Hasselbalch relationship.

$pH = pK_a + \log([A^-]/[HA])$

Since two of the pK_a values of EDTA are well below pH 6.0, they are not included in the analysis. The percentage of protonation associated with pK_a 6.13 is determined by the following analysis.

EDTA (NIST database)

	pKa	ΔH
4H+	2	0.3
3H+	2.69	1.5
2H+	6.13	-4.2
1H+	9.52	-5.6

$6.0 = 6.13 + \log([A^-]/[HA])$
$-0.13 = \log([A^-]/[HA])$
$10^{-0.13} = [A^-]/[HA]$
$0.741 = [A^-]/[HA] = X$

% protonated = $[A^-] + [HA] = [HA]X + [HA] = [HA](X + 1)$

$$\% \text{ protonated} = \frac{[HA]}{(X + 1)}$$

$$\% \text{ protonated} = \frac{100\%}{(0.741 + 1)} = 57.4\ \%$$

Repeating this calculation for pK_a 9.52 results in 99.7% protonation. This gives an overall EDTA protonation of 0.574 + 0.997 = 1.57 protons at pH 6.0.

Now, using values for $\Delta H_{Buffer\text{-}H}$ and $\Delta H_{Zn\text{-}EDTA}$ from NIST, the above equation is solved.

$\Delta H_{Zn\text{-}EDTA}$(pH 6.0)

$= -n\Delta H_{EDTA\text{-}H} + \Delta H_{Zn\text{-}EDTA} + n\Delta H_{Buffer\text{-}H}$

$= -0.574(-4.2\ kcal/mol) - 0.997(-5.6\ kcal/mol) + (-4.7\ kcal/mol) + 1.57(-6.86\ kcal/mol)$

$= -7.5\ kcal/mol$

Stability Constant: K

The NIST value for the Zn^{2+}EDTA stability constant is independent of pH, so this value needs to be adjusted to the experimental pH to account for proton competition with Zn^{2+} for EDTA.

$$K_{Zn\text{-}EDTA}(\text{pH } 6.0) = \frac{K_{Zn\text{-}EDTA}}{[1 + K_{EDTA\text{-}H1}[H^+] + K_{EDTA\text{-}H1}K_{EDTA\text{-}H2}[H^+]^2]}$$

$$= \frac{3.16 \times 10^{16}}{[1 + (10^{9.52})(10^{-6.0}) + (10^{9.52})(10^{6.13})(10^{-6.0})^2]}$$

$$= \frac{3.16 \times 10^{16}}{[1 + (3.3 \times 10^{9})(1 \times 10^{-6}) + (3.3 \times 10^{9})(1.3 \times 10^{6})(1 \times 10^{-12})]}$$

$$= \frac{3.16 \times 10^{16}}{1 + 3300 + 4290}$$

$$= 4.1 \times 10^{12}$$