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The Irving-William series and the 2-His-1-carboxylate facial triad: A thermodynamic study of Mn^{2+} , Fe^{2+} , and Co^{2+} binding to taurine/ α -ketoglutarate dioxygenase (TauD)

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Abstract

Taurine/ α -ketoglutarate (α KG) dioxygenase (TauD) is a nonheme Fe^{2+} and α KG dependent metalloenzyme, which catalyzes the hydroxylation of taurine eventually leading to sulfite production in *Escherichia coli*. The metal-dependent active site in TauD is formed by two histidine and an aspartate side-chains coordinating to one face of the octahedral coordination geometry, known as the 2-His-1-carboxylate facial triad. This motif is found in many nonheme Fe^{2+} proteins, yet limited information is known regarding the thermodynamic parameters that govern transition metal ions binding to this site. Here we report data associated with three cytosolic metal ions competing for this site. Isothermal titration calorimetry and related biophysical techniques were used to generate complete thermodynamic profiles of Mn^{2+} and Co^{2+} binding to TauD, and these values are compared to the Fe^{2+} data reported earlier [Henderson *et al.*, *Inorg. Chem.* **2015** 54, 2278–2283]. The pH and buffer independent binding constant (K) were measured to be 1.1×10^6 , 2.4×10^7 , and 2.9×10^9 , for Mn^{2+} , Fe^{2+} , and Co^{2+} , respectively. The corresponding G values were calculated to be -8.1 , -10.1 , and -12.9 kcal/mol, respectively. The measured enthalpy changes for these binding events (ΔH) are $-12.2 (\pm 0.1)$, $-12.8 (\pm 0.1)$, and $-17.8 (\pm 0.6)$ kcal/mol, respectively. These data are fully consistent with the Irving-Williams series, which shows an increasing affinity for transition metal ions from left to right across the periodic table. It appears that the periodic increase in affinity, however, is a result of increasing enthalpic favorability (ΔH) and variation in the penalty associated with entropy (ΔS) associated with these processes.

Keywords

Metal-binding; Manganese(II); Cobalt(II); Irving-William series; Isothermal titration calorimetry; Thermodynamics

Introduction

In Nature, matching a specific transition metal ion to its correct protein involves complex processes that include metal ion uptake, regulation, export, sequestration, and compartmentalization [1–15]. This complexity is needed to ensure the appropriate metal ion binds to its target protein [16]. Biologically available metal ions exhibit varied affinities for particular metal binding sites [17,18], but it was Irving and Williams who made the initial observation that metal ion affinity generally increases across the row of transition metals from Mn^{2+} to Cu^{2+} then slightly lessens when considering the main group metal ion Zn^{2+} [19]. This observation, known as the Irving-Williams series, has been the focus of a variety of chemical and biochemical studies where the underlying physical interactions that impact the thermodynamics of metal ion coordination have been well-described for simple small molecule systems [20]. In proteins and other biomacromolecules, metal ion binding is more complicated. Metal ions or Lewis acids bind to biomolecules, where they can impact both local and globular structure, and these dynamic changes temper these primarily thermodynamically controlled events [21–23]. Herein, we report our current efforts to study the thermodynamics of binding by biologically available transition metal ions to the *Escherichia coli* protein TauD, a taurine/ α -ketoglutarate (α KG) dioxygenase that catalyzes the O_2 -dependent hydroxylation of taurine that subsequently decomposes to sulfite and aminoacetaldehyde. Specifically our study focuses on the coordination of Mn^{2+} , Fe^{2+} , and Co^{2+} , which are metal ions commonly found in reasonable concentrations in the cytoplasm of *E. coli* [4]. Other common biological metal ions including Cu^{2+} and Zn^{2+} are known to have negligible concentrations in the cytoplasm of *E. coli*, where TauD is known to fold and bind metal ions [24]. The thermodynamic data reported herein is illustrative of the periodic affinity of these metal ions to the 2-His-1-carboxylate facial triad (Figure 1) found in the active site of TauD [22, 25–29], but also begins to unravel the underlying enthalpy and entropy driving forces behind these affinities.

Materials and methods

Reagents

Metal salts, ethylenediaminetetraacetic acid disodium salt (EDTA), and buffers were purchased at the highest grade available and were used as received. All solutions were made using 18 M Ω water purified by a Millipore Ultrapurification system.

Overexpression and purification of TauD

The taurine/ α KG dioxygenase TauD was produced in *E. coli* BL21(DE3) [pME4141] cells that were induced with 0.1 mM isopropyl- β -D-1-thiogalactose and grown terrific broth containing kanamycin (50 $\mu\text{g ml}^{-1}$) at 30 °C [24]. Cells were collected by centrifugation, cell-free extracts were prepared, and TauD was purified by sequential DEAE-Sepharose and

phenyl Sepharose chromatographies as previously described [29]. Purified apoprotein was stored in 25 mM tris(hydroxymethyl)aminomethane (Tris), pH 8.0, buffer containing 1 mM EDTA at -80°C .

Sample preparation

Purified TauD stock was prepared by dialyzing against 3 L of 25 mM buffers at pH 7.4 for 18 h. TauD concentration was determined by UV spectroscopy, using the calculated $\epsilon_{280} = 46,410 \text{ M}^{-1} \text{ cm}^{-1}$ based on the protein sequence. Metal solutions were made by dilution of metal ion salts with the dialyzing buffer. All samples were made anaerobic by purging with argon gas for 15 min.

Isothermal Titration Calorimetry (ITC)

The MicroCal VP-ITC instrument was sealed in an anaerobic chamber with a constant nitrogen flow. Experiments were run at 25°C . The metal solutions were titrated into samples of TauD apoprotein at a stirring rate of 307 rpm over 6 s, with 300 s between each injection. Chelation titrations were performed by titrating EDTA solutions into metal-TauD samples. Titrations of metal(II)-buffer samples into EDTA solutions were performed in 2-(carbamoylmethylamino)ethanesulfonic acid (ACES), 2-(N-morpholino)ethanesulfonic acid (MOPS), Tris, piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) and N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES) buffers.

Differential Scanning Calorimetry (DSC)

Samples of TauD were diluted to a final concentration of $20 \mu\text{M}$ in the dialysate buffer. The sample and reference cells of the differential scanning calorimeter (N-DSC II, Calorimetry Sciences Corp) were made anaerobic using a glove bag purged for 30 min with Ar, which actively displaces the aerobic atmosphere. Metal-enzyme samples were thermally denatured by increasing the temperature from 15 to 85°C at a scan rate of 2°C per min, then cooling to 15°C at the same rate. The data were analyzed using CPCalc software (Calorimetry Sciences Corp.) A final temperature scan was used as the baseline for each experiment, and this baseline was subtracted from the raw data. All data were fit to single, two-state scaled models. Experiments were replicated 2–3 times and averaged. Error bars are shown for one standard deviation from the mean.

Circular dichroism (CD) spectroscopy

TauD apoprotein and metal-bound TauD species were prepared as described above, diluted to a final concentration of $5 \mu\text{M}$ in the dialysate buffer, and CD data were measured between 200–250 nm using a quartz cell (1-cm pathlength) and an OLIS DSM-20 spectropolarimeter (Bogart, GA).

Results

Calorimetry is a powerful technique for studying the thermodynamics of metal ion binding to biological ligands. Through a single ITC experiment, the enthalpy (H_{obs}) of the reaction and the equilibrium constant (K_{obs}) are directly measured. These data can be used with two common thermodynamic equations ($G_{\text{obs}} = -RT\ln K_{\text{obs}}$ and $G_{\text{obs}} = H_{\text{obs}} - T S_{\text{obs}}$) to

calculate the Gibbs free energy (G_{obs}) and an entropy term ($-T \Delta S_{\text{obs}}$). A representative isotherm for the binding of Co^{2+} to TauD in Tris buffer is shown in Figure 2. For the direct binding reaction of metal(II) to TauD, the raw heat data were baseline corrected and the isotherms were fit to one-site binding equilibria to yield the observed equilibrium constants (K_{obs}) and enthalpy values (H_{obs}) (Table 1). Parallel experiments were done in other buffer systems, where the enthalpy of ionization of the buffer can give insight into changes in acidity induced by metal ion binding. Similar analyses were carried out for both Mn^{2+} and Co^{2+} . These observed thermodynamic terms for metal binding to TauD are best considered to be a complex series of metal-chelate and related interactions, which are fit to a model that includes buffer-metal ion interactions, metal ion-protein interactions, H^+ release, and buffer ionization resulting from release or uptake of protons during the competitive binding event [29–31].

To elucidate the thermodynamic properties of metal(II)-buffer interactions, metal(II)-buffers complexes (using ACES, MOPS, Tris, PIPES, and TES buffers) were titrated into EDTA solutions to measure $H_{\text{metal-buffer}}$. Then a plot of $H_{\text{obs}} + H_{\text{metal-buffer}}$ against the buffer $H_{\text{ionization}}$ was generated for each data set, with the linear relationships yielding slopes that are equal to the number of protons (n_p) released/consumed. Analysis of the ITC data for Mn^{2+} and Co^{2+} binding to TauD suggests that 1.5 and 2.0 protons are released during these processes, respectively (Figure 3). For comparison, the parallel Fe^{2+} binding data reported earlier are also shown [29], where Fe^{2+} binding to TauD releases 1.1 proton (Figure 3, black line).

The heats measured in the ITC experiments are the summation of all heats produced from the metal ion coordination. We and others have used a relatively simple thermodynamic analysis of such data to estimate the change in enthalpy (ΔH) associated with metal ion binding [24, 29, 30]. By applying this analysis to metal ion binding with TauD, the pH and buffer independent thermodynamic parameters can be determined for metal(II) ion binding to TauD. The thermodynamic reactions modeled for Mn^{2+} and Co^{2+} binding to TauD in Tris buffer are illustrated in Tables 2 and 3. The buffer-independent Mn^{2+} and Co^{2+} binding data obtained through this analysis were averaged with the buffer-independent enthalpies derived from the other metal-buffer binding experiments to yield ΔH for metal ion binding to TauD of $-12.2 (\pm 0.1)$ and $-17.8 (\pm 0.6)$ kcal/mol for Mn^{2+} and Co^{2+} , respectively (Table 4). Notably, these data correlate well with the previously reported Fe^{2+} binding event to TauD, which has an enthalpy of $-12.8 (\pm 0.1)$ kcal/mol [29].

The K_{obs} value for the direct titration of metal into TauD was also affected by additional equilibria in solution, which includes an association constant K_a of M^{2+} -TauD and a dissociation constant (K_d) for the M^{2+} -buffer interaction. To obtain K_a of M^{2+} -TauD, EDTA was titrated into the M^{2+} -TauD complex. Using the equation: $K_a = K_{\text{M}^{2+}\text{-EDTA}}/K_{\text{obs}}$, where $K_a = 1/K_d$, K_a values were obtained for Mn-TauD (1.1×10^6) and Co-TauD (2.9×10^9). The corresponding ΔG values are -8.1 kcal/mol and -12.9 kcal/mol for Mn^{2+} and Co^{2+} binding to TauD, respectively. When compared to the ΔG value of -10.1 kcal/mol measured for Fe^{2+} binding to TauD [29], we note a regular increase in the affinity of metal(II) ions for TauD, when going from Mn^{2+} to Fe^{2+} to Co^{2+} (Table 5 and Figure 4). This trend is fully consistent with the Irving-Williams series. Using the ΔH and ΔG terms, we calculated the entropy

terms ($-T \Delta S$) for metal(II) binding to TauD (Table 5). For both Mn^{2+} and Co^{2+} binding to TauD the $-T \Delta S$ terms were unfavorable. These data are summarized graphically in Figure 4, where it is clear there is an increasing affinity for the metal ions as one shifts from Mn^{2+} to Fe^{2+} to Co^{2+} . By contrast, the ΔH and $-T \Delta S$ values do not show a regular or periodic trend. In fact, Mn^{2+} and Fe^{2+} have similar enthalpy terms, whereas Co^{2+} binding was associated with significantly more heat. The regular increase in affinity as measured by an increasingly negative ΔG values going from Mn^{2+} to Co^{2+} results from a compensating relationship between ΔH and $-T \Delta S$ terms [32].

A series of differential scanning calorimetry (DSC) experiments were also performed to explore the global stability of various metal bound forms of TauD. Although the thermal denaturation of TauD and its metal-substituted complexes is not reversible, an approximate change in enthalpy for these systems can be derived [23, 33–34]. The TauD apoprotein unfolding event (Figure 5, dashed curve) was fit to a single two-state model, giving T_{mTauD} at $59.2 (\pm 0.1) ^\circ\text{C}$ and ΔH_{TauD} of $132.3 (\pm 1.8) \text{ kcal/mol}$ (Table 6). For Mn-TauD (Figure 5, red curve), a similar model indicated unfolding at T_{mTauD} of $59.7 (\pm 0.2) ^\circ\text{C}$ with ΔH_{TauD} of $143.6 (\pm 0.2) \text{ kcal/mol}$ (Table 6). The difference in the unfolding enthalpies of the two forms of the protein yielded the enthalpy of binding of Mn^{2+} to the enzyme: ($\Delta H_{\text{binding}} = \Delta H_{\text{TauD}} - \Delta H_{\text{MnTauD}}$). This $\Delta H_{\text{binding}}$ of -11.3 kcal/mol is in good agreement with that derived from our ITC data of -12.2 kcal/mol . The Fe-TauD sample was previously reported to unfold (Figure 5, black curve) according to a two-state model, providing the thermodynamic parameters shown in Table 6. For Co-TauD (Figure 5, blue curve), the unfolding event was fit to a single two-state model to obtain T_{mTauD} of $65.2 (\pm 0.2) ^\circ\text{C}$ and ΔH_{TauD} of $148.2 (\pm 0.4) \text{ kcal/mol}$ (Table 6). The $\Delta H_{\text{binding}}$ for Co-TauD was calculated to be -15.9 kcal/mol , again similar to the value derived from the ITC data presented here. The Co-TauD denaturation curve also fit well to two, two-state binding models with similar goodness of fit as the single two-state model (Table 6). The first two-state denaturation event was assigned as a small amount of TauD apoprotein in the sample, due to the similarities of the sample T_{m} and ΔH , which compared well to the DSC data collected for TauD. The second unfolding event is assigned to the denaturation profile of Co-TauD with a T_{m} at $65.4 (\pm 0.1) ^\circ\text{C}$ and ΔH_{TauD} of $148.6 (\pm 0.1)$. If we consider the difference in the enthalpy terms, $\Delta H_{\text{binding}}$ yielded a value of -16.0 kcal/mol , which is the same as the value obtained from the single two-state model. In summary, the enthalpy of metal binding obtained from both ITC and DSC experiments show the largest magnitude for Co-TauD within ITC-obtained enthalpy and DSC-obtained enthalpy, -17.8 kcal/mol and -16.0 kcal/mol respectively, where the magnitude can be ordered as $\text{Co-TauD} > \text{Fe-TauD} > \text{Mn-TauD}$. There are subtle differences (1–2 kcal/mol) between DSC and ITC values measured here, but that difference is likely due to some alternative processes that arise from the irreversibility of the DSC experiments.

The CD spectra (Figure 6) of three metal TauD complexes varied subtly, with Fe-TauD showing a maximum negative ellipticity band at 225 nm whereas the Mn-TauD and Co-TauD samples exhibited their maximal CD features at 226 and 221 nm, respectively. Overall, these CD data support the notion that the metallated forms of TauD have similar secondary structural content, with the CD signal blue shifting with Co^{2+} binding and red shifting with

Mn²⁺ which provides evidence that the energetics of metal ion binding is transduced into the secondary structural features of these metal substituted TauD samples.

Discussion

In this study, the buffer and pH independent thermodynamic driving forces are measured for Mn²⁺ and Co²⁺ metal ions binding to the 2-His-1-carboxylate facial triad of TauD. These data are compared to the published Fe²⁺ thermodynamic data to give insight into how the thermodynamics observed for possible cytosolic transition metal ions compares to the affinity predicted by the Irving-Williams series [29].

The thermodynamic parameters derived from thermal denaturation curves measured for Mn-TauD and Co-TauD using DSC match well with the data from the parallel ITC experiments for Mn²⁺ and Co²⁺ binding to TauD. There is a 1–2 kcal/mol difference in the H values when comparing the DSC and ITC data; although this difference is unlikely to be significant, it raises questions regarding the precision of the simplistic thermodynamic model used to assess the ITC data (Tables 2 and 3) and the role aggregation may play in the DSC derived data.

The binding of Mn²⁺ and Co²⁺ to TauD are highly favorable with G values of –8.1 kcal/mol and –12.8 kcal/mol, respectively. Binding experiments of Mn²⁺, Fe²⁺, and Co²⁺ to TauD show incremental gains in the magnitude of the free energy, typically following the Irving-Williams series of stability [19]. Like Fe²⁺, Mn²⁺ and Co²⁺ binding equilibria are both enthalpically driven. This notion is consistent with previous thermodynamic studies of metal-ligand interactions, where oxygen or nitrogen donor atoms are present [29]. Mn²⁺ and Fe²⁺ have similar enthalpy values for metal ion coordination to TauD, whereas the binding enthalpy for Co²⁺ is significantly more stabilizing (–17.8 kcal/mol). This trend is also evident from the DSC data, where both T_m and H increase from TauD < Mn-TauD < Fe-TauD < Co-TauD. Some of this stability is translated into the dominant secondary structure of TauD, where the CD signal of the β barrel blue shifts with more stabilizing metal ions. Although metal ion binding to TauD is driven primarily by enthalpy changes, the entropic contributions of these metal binding events play a key role in the periodic increasing stability associated with changes in the free energy.

Mn²⁺ and Co²⁺ have similar entropic penalties for metal ion coordination, which are somewhat larger than the entropy penalty reported for Fe²⁺ binding to TauD [29]. It is not initially clear what gives rise to the differences between solutions of Mn²⁺, Fe²⁺, and Co²⁺, and how these differences can induce dramatic changes to their entropy penalties [29]. All three of these metal ions have similar ionic radii [35], solvation radii [36], coordination numbers in aqueous media [37], and water induced pK_a changes [38], which begs the question “where do these dramatic entropic changes arise for Mn²⁺, Fe²⁺, and Co²⁺?” One possibility is that the Lewis acidity of the metal ions are playing a more dramatic role in these metal binding equilibria than previously thought. The Lewis acidity of the metal ions can be ranked simply by considering the number of protons released when binding to TauD. Cobalt(II) binding releases 2.0 H⁺, Mn²⁺ binding releases 1.5 H⁺, whereas Fe²⁺ releases only 1.1 H⁺, which lets us rank their Lewis acidity as follows: Co²⁺ > Mn²⁺ > Fe²⁺.

Interestingly, there is a linear correlation between protons released from Mn^{2+} , Fe^{2+} , and Co^{2+} binding to TauD versus the entropy penalty associated with these metal ions binding to TauD (Figure 7). Based on these data points, there appears to be an entropic penalty of approximately +2.6 kcal/mol per proton during the metal binding event. This penalty is similar to those measured for metal ion coordination to other proteins where proton release is observed [39]. Normally, metal ion binding is modeled from a series of linked equilibria, where metal ions are passed from a buffer chelate to the biomacromolecule, as illustrated in equation 1, where a metal-Tris complex is added into the TauD apoprotein in Tris buffer to generate the holoprotein (Equation 1). From this simplistic equation, it is not evident where the proton-dependent entropic penalty arises. Perhaps a more plausible mechanism for explaining the thermodynamic data presented here is that the metal binds rapidly, creating a highly charged species (Equation 2, bold), which we expect to be entropically unfavorable due to a localization of positive charge. The metal ion/Lewis acid then modulates local pK_a values within the protein allowing for protons to be released, alleviating the strain in this high-energy species.

Generally it is assumed that at pH 7.4, Mn^{2+} , Fe^{2+} , and Co^{2+} form metal ion-buffer complex ions in aqueous solution with similar ionic charge across this portion of the periodic table. Additionally, in our analysis we assume the ionization of metal bound water/hydroxide is the source of H^+ release in these systems [29]. The dramatically different number of protons released for Mn^{2+} , Fe^{2+} , and Co^{2+} suggest that (1) one of the assumptions used to model this data is problematic, or (2) that the metal ion/Lewis acid binding event impacts the stability of local ionizable groups in close proximity to the metal binding site. In either case, further studies defining metal ion speciation in aqueous solution and assessing the overall impact of metal binding on local ionizable residues are needed to continue to unravel the driving forces behind the Irving-Williams series and how these periodic trends can impact biological processes.

In summary, the thermodynamic profiles associated with transition elements 25–27 (Figure 4) show that the free energy of metal ion binding to TauD follows the Irving-Williams series for these metal ions. It is unclear how the changes in enthalpy and entropy facilitate increasing the magnitude of the free energy term and thereby increasing the affinity of Fe^{2+} over Mn^{2+} , and Co^{2+} over Fe^{2+} . In small molecule coordination chemistry, these interactions can be linked to stabilization of bonding orbitals, where notions like absolute hardness, and ligand field stabilization energies can lead to strong enthalpically favorable interactions [20]. However, the change in enthalpy terms for these binding events with biological and complex ligands are complicated. The blue shift in the CD spectra of Mn-, Fe-, and Co-TauD indicates that the metal binding event impacts the relative stability of the intramolecular interactions which stabilize the cupin fold in TauD. This observation may indicate that some

of the stabilizing energy from metal ion coordination is converted into secondary structure stabilization in TauD. This observation suggests the complex ion equilibria associated with metal ion coordination can be modelled with a simple set of equilibria, but on an atomic level the metal ion coordination to biomolecules is a vastly more complicated process than just ligand coordination alone.

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Figure 1.

(A) The monomer unit of Fe-TauD and (B) the Fe^{2+} -binding active site in TauD. The metal is shown as an orange sphere, taurine is highlighted with yellow carbon atoms, αKG is shown with violet carbons, and the 2-His-1-carboxylate facial triad is shown with blue carbon atoms. The α -helices are shown in cyan and β -sheets are shown in red. Coordinates for this structure are taken from the protein data bank (access code 1OS7).

Figure 2.

A representative set of raw data and the integrated isotherm for Co^{2+} binding to TauD. This analysis was performed in 25 mM Tris buffer, pH 7.4, to obtain the binding equilibrium and enthalpy for the reaction.

Figure 3.

Observed enthalpies corrected for metal(II) buffer interactions plotted against buffer ionization enthalpies. These plots yield the number of protons released (slope). The reactions include Co²⁺ added to TauD (blue, $y = 2.0x + 1.4$, $r^2 = 0.983$), Mn²⁺ added to TauD (red, $y = 1.5x + 7.9$, $r^2 = 0.999$), and Fe²⁺ added to TauD (black, $y = 1.1x + 1.4$, $r^2 = 0.999$) [29].

Figure 4.

Complete thermodynamic profiles for Mn²⁺, Fe²⁺, and Co²⁺ binding to TauD. The Gibbs free energy terms (ΔG) are shown as black columns, the change in enthalpy (ΔH) are shown in red, and the temperature dependent change in entropy terms ($-T\Delta S$) are shown in green. Data for Fe²⁺ are from Henderson *et al.* [29].

Figure 5.
Heat capacity curves for the thermal denaturation of TauD species: TauD apoprotein (dashed), Fe-TauD (black), Mn-TauD (red), and Co-TauD (blue).

Figure 6.
CD spectra of metal-TauD species: Fe-TauD (black), Mn-TauD (red), and Co-TauD (blue).
CD intensities were normalized at 245 nm.

Figure 7. Proton release plotted versus entropy penalties for Mn^{2+} , Fe^{2+} , and Co^{2+} . These data correlate well ($r^2 = 0.98$) with a linear relationship to the entropic penalty and proton release.

Table 1.

Observed thermodynamic parameters of manganese(II) acetate tetrahydrate and cobalt(II) nitrate binding to TauD^a

Metal ion	25 mM buffer (pH 7.4)	$H_{\text{ionization}}^b$ (kcal/mol)	K_{obs}	H_{obs} (kcal/mol)	G_{obs} (kcal/mol)
Mn ²⁺	ACES	−7.27	$3.2 (\pm 0.2) \times 10^4$	−4.7 (± 0.1)	−6.2 (± 0.1)
	TES	−7.70	$6.0 (\pm 0.6) \times 10^5$	−4.3 (± 0.2)	−7.9 (± 0.1)
	Tris	−11.36	$3.7 (\pm 0.5) \times 10^5$	−9.5 (± 0.1)	−7.4 (± 0.1)
Co ²⁺	MOPS	−5.04	$3.4 (\pm 0.8) \times 10^6$	−2.3 (± 0.2)	−9.0 (± 0.1)
	TES	−7.7	$1.1 (\pm 0.4) \times 10^5$	−6.5 (± 0.8)	−6.9 (± 0.4)
	Tris	−11.36	$6.4 (\pm 0.1) \times 10^6$	−13.3 (± 0.8)	−9.3 (± 0.1)

^aError associated with the data presented represents one-standard deviation from the mean of 2–4 independent experiments.

^bBuffer ionization enthalpy values from reference [40].

Table 2.Thermodynamic cycle for Mn^{2+} binding to TauD in Tris buffer.

Reaction	Coefficient	<i>H</i> (kcal/mol)
$\text{Mn-Tris} \rightleftharpoons \text{Mn}^{2+} + \text{Tris}$	1	-0.16^a
$\text{TauD-H}^+ \rightleftharpoons \text{TauD} + \text{H}^+$	1.5	13.34^b
$\text{Tris} + \text{H}^+ \rightleftharpoons \text{H}^+\text{-Tris}$	1.5	-11.36^c
$\text{Mn}^{2+} + \text{TauD} \rightleftharpoons \text{Mn-TauD}$	1	-12.34
$0.5 \text{ Tris} + \text{Mn-Tris} + \text{TauD} - 1.5 \text{ H}^+ \rightleftharpoons \text{Mn-TauD} + 1.5 \text{ H}^+\text{-Tris}$		-9.53^d

^aCalculated from the control experiments of EDTA chelating Mn^{2+} in a Tris buffer.^bRepresents the ionization of water within the $\text{H}_2\text{O-Mn}^{2+}\text{-TauD}$ complex involving 1deprotonation to the $\text{HO-Mn}^{2+}\text{-TauD}$ species. Value is taken from ref [41].^cValue taken from ref [40].^dData from Table 1.

Table 3.Thermodynamic cycle for Co^{2+} binding to TauD in Tris buffer.

Reaction	Coefficient	<i>H</i> (kcal/mol)
$\text{Co-Tris} \rightleftharpoons \text{Co}^{2+} + \text{Tris}$	1	0.9 ^a
$\text{TauD-H} \rightleftharpoons \text{TauD} + \text{H}$	2	13.34 ^b
$\text{Tris} + \text{H}^+ \rightleftharpoons \text{H}^+-\text{Tris}$	2	-11.36 ^c
$\text{Co}^{2+} + \text{TauD} \rightleftharpoons \text{Co-TauD}$	1	-18.12
$\text{Tris} + \text{Co}^{2+}-\text{Tris} + \text{TauD}-2\text{H}^+ \rightleftharpoons \text{Co}^{2+}-\text{TauD} + 2\text{H}^+-\text{Tris}$		-13.26 ^d

^aCalculated from the control experiments of EDTA chelating Co^{2+} in a Tris buffer.^bRepresents the ionization of water within the $\text{H}_2\text{O}-\text{Co}^{2+}-\text{TauD}$ complex involving deprotonation to the $\text{HO}-\text{Co}^{2+}-\text{TauD}$ species. Value is taken from ref [41].^cValue taken from ref [40].^dData from Table 1.

Table 4.

pH and buffer independent enthalpy values associated with Mn^{2+} , Fe^{2+} , Co^{2+} binding to TauD.

25 mM buffer	H_{MnTauD}^a (kcal/mol)	H_{FeTauD}^b (kcal/mol)	H_{CoTauD}^a (kcal/mol)
ACES	-12.4 ($\pm$ 0.1)	-12.6 ($\pm$ 0.8)	--
HEPES	--	-12.9 ($\pm$ 0.5)	--
MOPS	--	-12.8 ($\pm$ 0.1)	-18.3 ($\pm$ 0.2)
TES	-12.0 ($\pm$ 0.2)	--	-17.0 ($\pm$ 0.7)
Tris	-12.3 ($\pm$ 0.1)	--	-18.1 ($\pm$ 0.8)
Average	-12.2 ($\pm$ 0.1)	-12.8 ($\pm$ 0.1)	-17.8 ($\pm$ 0.6)

^a pH and buffer independent enthalpies associated with metal binding. Data derived from this study.

^b pH and buffer independent enthalpies associated with metal binding. Data derived from Henderson *et al.*[29]

Table 5.

A complete set of thermodynamic data from ITC experiments associated with metal ion binding to TauD.^a

	<i>G</i> (kcal/mol)	<i>H</i> (kcal/mol)	- <i>T S</i> (kcal/mol)	ref
Mn ²⁺	-8.2 (±0.2)	-12.2 (±0.1)	4.0 (±0.2)	This work
Fe ²⁺	-10.1 (±0.1)	-12.8 (±0.1)	2.7 (±0.1)	Ref 29
Co ²⁺	-12.8 (±0.3)	-17.8 (±0.6)	5.0 (±0.7)	This work

^aError range is calculated as one standard deviation from the mean for a minimum of 3 trials

Table 6.

Thermodynamic data for the thermal denaturation of metal-TauD species as measured by DSC

	Fitting model	T _m (°C)	H (kcal/mol)	Species observed in experiment
TauD	2-state	59.2 (± 0.1)	132.3 (± 1.8)	TauD
Mn-TauD	2-state	59.7 (±0.2)	143.6 (± 0.2)	Mn-TauD
Fe-TauD ^a	2-state	61.4 (± 0.9)	145.1 (± 1.8)	Fe-TauD
Co-TauD	2-state	65.2 (± 0.2)	148.2 (± 0.4)	Co-TauD
	two 2-state	59.9 (± 0.1)	135.6 (± 0.1)	TauD
		65.4 (± 0.1)	148.6 (± 0.1)	Co-TauD

^aData from ref [29].