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A Calorimetric Assessment of Fe²⁺ Binding to α -Ketoglutarate/Taurine Dioxygenase: Ironing Out the Energetics of Metal Coordination by the 2-His-1-Carboxylate Facial Triad

Kate L. Henderson[†], Tina A. Müller[‡], Robert P. Hausinger[‡], and Joseph P. Emerson^{*,†}

[†]Department of Chemistry, Mississippi State University, Mississippi State, MS 39762

[‡]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824-4320

Abstract

The thermodynamic properties of Fe²⁺ binding to the 2-His-1-carboxylate facial triad in α -ketoglutarate/taurine dioxygenase (TauD) were explored using isothermal titration calorimetry. Direct titrations of Fe²⁺ into TauD and chelation experiments involving the titration of ethylenediaminetetraacetic acid into Fe²⁺-TauD were performed under an anaerobic environment to yield a binding equilibrium of $2.4 (\pm 0.1) \times 10^7$ ($K_d = 43$ nM), and a ΔG° of $-10.1 (\pm 0.03)$ kcal/mol. Further analysis of the enthalpy/entropy contributions indicate a highly enthalpic binding event, where $\Delta H = -11.64 (\pm 0.25)$ kcal/mol. Investigations into the unfavorable entropy term led to the observation of approximately 6.5 water molecules becoming organized within the Fe²⁺-TauD structure.

TOC image

Thermodynamic profile associated with Fe²⁺ binding to the 2-His-1-carboxylate facial triad in TauD.

*Corresponding Author: Phone: 662-325-9500. Fax: 662-325-1618. jemerson@chemistry.msstate.edu.

Keywords

isothermal titration calorimetry; thermodynamics; non-heme iron; TauD

Mononuclear Fe^{2+} sites can be found throughout nature at the catalytic centers of many important enzymes.^{1–3} Many complexes of Fe^{2+} spontaneously react with dioxygen to generate high-valent intermediates which are fundamental for the biological and environmental processes they catalyze. A common motif found in a range of non-heme Fe^{2+} oxygenases is the 2-His-1-carboxylate facial triad.^{4,5} This metal binding configuration involves the side-chains of two histidine and one aspartate/glutamate amino acid residues to occupy one face of the Fe^{2+} octahedral coordination sphere, leaving the adjacent face coordinated to solvent ligands which are easily exchanged for binding substrates (Figure 1). In the dioxygenase family of enzymes, the 2-His-1-carboxylate facial triad affords proximity of the substrate(s) and dioxygen to ensure efficient catalysis of reactions such as the repair of alkylated DNA/RNA, the biosynthesis of penicillin, and the degradation of aromatic compounds for carbon sequestration.^{6–9}

A subfamily of oxygenases containing the 2-His-1-carboxylate motif are the α -ketoglutarate (α KG)-dependent enzymes.¹⁰ The best studied member of this subfamily is α KG/taurine dioxygenase or taurine hydroxylase (TauD), an enzyme that couples the oxidative decarboxylation of α KG and oxidation of taurine to liberate sulfite (Scheme 1).¹¹ In TauD, the mononuclear Fe^{2+} center is coordinated facially to the side-chains of residues His255, His99, and Asp101 (Figure 2). This affords the bidentate binding of α KG on the adjacent face, with the carbonyl oxygen binding *trans* to the Asp ligand, and the C1 carboxylate oxygen binding across from His99.^{12,13} The final coordination site is open for dioxygen binding and activation. Previous studies have indicated that several divalent metals can bind to the active site of TauD; however, Fe^{2+} is the only metal that is capable of supporting its catalytic activity.^{14,15} On the basis of perturbations to the protein UV absorbance spectrum, the K_d of Fe^{2+} binding to TauD apoprotein was reported to be 90 ± 50 nM. Here, we explore the thermodynamic properties of Fe^{2+} binding to TauD and discuss the underlying driving forces behind Fe^{2+} coordination by the 2-His-1-carboxylate facial triad.

MATERIALS AND METHODS

Reagents and General Procedures

All reagents and buffers were of the highest grade available and used as received. All solutions and media were prepared in 18 M Ω water purified through a Millipore system. Glass dialysis containers and vials for apoprotein storage were rinsed with ethylenediaminetetraacetic acid (EDTA) and thoroughly rinsed with 18 M Ω water before use.

Overexpression and Purification of α -Ketoglutarate/Taurine Dioxygenase

TauD was produced in *Escherichia coli* BL21(DE3) cells harboring plasmid pME4141.¹⁵ Cultures were grown at 30 °C and induced by addition of 0.1 mM isopropyl- β -D-1-thiogalactose. Cells were harvested by centrifugation and resuspended in TE (25 mM Tris

buffer, pH 8.0, and 1 mM EDTA) containing 1 mM phenylmethylsulfonyl fluoride. Cell-free extracts were prepared by sonication followed by centrifugation at $100,000 \times g$ for 1 h. The lysate (approx. 50 ml) was applied to a DEAE-Sepharose column (2.5×19 cm, Sigma) equilibrated with TE and protein was eluted using a three step protocol: a linear gradient of 0 to 0.25 M NaCl in TE for 2 column volumes (CV), isocratic flow of 0.25 M NaCl in TE (1 CV), followed by a linear gradient from 0.25 M to 1 M NaCl in TE in 1 CV. Fractions were analyzed by sodium dodecylsulfate polyacrylamide gel electrophoresis and those containing TauD were pooled, concentrated using a centrifugal filter (Millipore), and dialyzed overnight into TE containing 0.5 M $(\text{NH}_4)_2\text{SO}_4$. The sample was loaded onto a phenyl Sepharose column (2.6×20 cm, GE Healthcare) equilibrated with TE buffer containing 0.5 M $(\text{NH}_4)_2\text{SO}_4$. TauD was eluted using a gradient from 0.5 to 0.05 M $(\text{NH}_4)_2\text{SO}_4$ in TE (1 CV) followed by decreasing $(\text{NH}_4)_2\text{SO}_4$ concentration to 0 M in TE in 0.75 CV. Fractions were analyzed and concentrated as before and dialyzed into TE. When appropriate, TauD was further purified by gel filtration chromatography using Superdex-200 with TE buffer containing 150 mM NaCl. Eluted TauD was again concentrated and dialyzed into TE. Purified TauD was stored in aliquots at -80°C .

Enzyme activity assays were carried out by incubating TauD (3–5 $\mu\text{g}/\text{ml}$) in assay buffer containing 25 mM Tris, pH 8, 1 mM taurine, 1 mM αKG , 50 μM Fe^{2+} , and 100 μM ascorbate for 1–5 min at 37°C . The assays were stopped by addition of EDTA (5 mM) and sulfite production was determined spectrophotometrically at 415 nm after addition of Ellman's reagent (0.1 mg/ml final concentration). One unit of enzyme activity is defined as the amount of enzyme that releases 1 μmol of sulfite per min at 37°C .

Isothermal Titration Calorimetry (ITC)

Purified TauD apoprotein stock was diluted to 100 μM and dialyzed against one of three different 25 mM buffers (as specified) at pH 7.4 for 18 h. TauD and dialysate were made anaerobic by passing Ar over the solutions. A 3.3 mM Fe^{2+} acetate solution was made by using the degassed dialysate. The MicroCal VP-ITC instrumentation was sealed in an anaerobic chamber (Plaslabs) with constant N_2 flow during the course of the experiment. Data were collected at 25°C , unless specified otherwise, for 3 μL injections of the Fe^{2+} acetate solution into a 1.5 mL cell containing the TauD solution to generate Fe^{2+} -TauD. Injections were carried out at a stir rate of 307 rpm over 6 s periods with 300 s spacings between injections. Chelation titration experiments were performed with 50 μM anaerobic Fe^{2+} -TauD, and 800 μM EDTA in 25 mM HEPES buffer at pH 7.4. Data were collected at 25°C for 6 μL injections of EDTA over 12 s periods with 300 s spacings between injections, while stirring at a rate of 307 rpm. For all experimental sets, control experiments were performed and raw heats were subtracted as necessary. The integrated heats obtained by titrating buffer into buffer or Fe^{2+} into buffer (Figure S1 in supplementary information) were negligible. Data were analyzed using a one-site model in the MicroCal data analysis software package in Origin (OriginLabs) and using CHASM software, developed by the laboratory of Edwin Lewis (Mississippi State University), which uses a nonlinear least squares fitting algorithm to fit the change in heat per injection to equilibrium binding model equations.¹⁶ Isotherms for Fe^{2+} binding to TauD in various buffers were replicated 2–4 times.

RESULTS

Binding of metal ions by macromolecules can be monitored by ITC through the direct measurement of the heat changes associated with the binding reaction.^{17,18} In a single experiment, the binding equilibrium (K) and observed enthalpy (H_{obs}) can be directly obtained, permitting the calculation of Gibbs free energy (G°) and entropy (S) terms using equations 1 and 2.

$$\Delta G^\circ = -RT \ln K \quad (1)$$

$$\Delta G^\circ = \Delta H_{\text{obs}} - T \Delta S \quad (2)$$

All entropy terms in this study are reported in terms of $-T \Delta S$ in order to provide a straightforward comparison to the G and H terms.

The observed enthalpy terms obtained from metal binding ITC experiments are more appropriately described as a complex series of competitive binding events that involve the Fe^{2+} ion. Moreover, these competitive binding events often result in the release or uptake of protons, resulting in the ionization of the buffer ($H_{\text{ionization}}$). A plot of the H_{obs} versus the $H_{\text{ionization}}$ yields a linear relationship where the slope is equal to the number of protons (n_p) released/consumed during the binding event as shown in equation 3.

$$\Delta H_{\text{obs}} + \Delta H_{\text{Fe-buffer}} = (\Delta H_{\text{Fe-TauD}} - n_p \Delta H_{\text{H-TauD}}) + n_p (\Delta H_{\text{ionization}}) \quad (3)$$

Control experiments involving the titration of the Fe^{2+} -buffer complexes into EDTA were performed to elucidate the $H_{\text{Fe-buffer}}$ for our thermodynamic analyses (see Figures S3–S5 and the corresponding thermodynamic cycles in Tables S2–S4 supplementary information). These experiments were performed in 2-(carbamoylmethylamino)ethanesulfonic acid (ACES), 2-(*N*-morpholino)ethanesulfonic acid (MOPS), and 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) buffers, which were chosen for their pH range and minimal heats of interaction with the metal ion or protein. For the direct titration of Fe^{2+} -acetate into a solution of TauD apoprotein, the raw data were baseline corrected and the isotherms were fit for binding at a single site, yielding H_{obs} values in ACES, MOPS, and HEPES buffers (Figure 3). A plot of the $H_{\text{obs}} + H_{\text{Fe-buffer}}$ against the $H_{\text{ionization}}$ indicates 0.9 protons are released during the binding event, and the y -intercept of -1.40 kcal/mol is the heat associated with Fe^{2+} binding to TauD minus the protonation enthalpy of TauD ($H_{\text{Fe-TauD-H}}$), as illustrated in Figure 3C. A complete list of the observed thermodynamic parameters for the direct titration of Fe^{2+} into TauD in each buffer can be found in Table 1. Error bars on the data points obtained in ACES buffer are larger in magnitude than those observed for other buffers due to the averaging of titrations between different preparations of TauD. However, due to the goodness of fit in our slope ($R^2 = 1.00$), we believe the data to be accurate and represent the average thermodynamic values for Fe^{2+} binding to TauD.

Using the observed enthalpy values from the titrations of Fe^{2+} into TauD, thermodynamic cycles can be generated that incorporate all known equilibria taking place in solution for the Fe^{2+} titration in each buffering system (Table 2 for MOPS buffer, and Tables S5 and S6 for ACES and HEPES buffers, respectively). Cycles similar to these have been used in the analysis of metal binding to small peptides and protein systems.^{19–21} The overall reaction contains the dissociation of the Fe^{2+} ion from the Fe^{2+} -buffer complex, Fe^{2+} binding to the 2-His-1-carboxylate facial triad, and protonation of the buffer which stems from a loss of 0.9 protons, presumably from the Fe^{2+} bound waters due to a shift in $\text{p}K_a$ when bound to the metal. Deconvolution of the observed enthalpy data in MOPS results in the estimation of the $H_{\text{Fe-TauD}}$ to be $-11.7 (\pm 0.2)$ kcal/mol. A complete list of the changes in enthalpy for Fe^{2+} binding to TauD from the thermodynamic cycles in each buffer can be found in Table 3.

The additional equilibria in solution also have an effect on the observed K value in the direct titration of Fe^{2+} into TauD. Because there are interactions between metal ions and buffers, K for the direct titration is the product of the individual equilibrium constants occurring in solution. This includes the association constant (K_a) of the Fe^{2+} -TauD, and the dissociation constant ($K_{\text{d Fe-buffer}}$) of the Fe^{2+} -buffer. To obtain the K_a term of Fe^{2+} -TauD, a second set of experiments was performed involving a chelation titration where the Fe^{2+} -EDTA binding equilibrium (K_{EDTA}) is known, allowing for the calculation of the Fe^{2+} -TauD K_a using the following equation:²⁰

$$K_a = K_{\text{EDTA}} / K \quad (\text{where } K_a = 1 / K_{\text{d Fe-TauD}}) \quad (4)$$

The integrated isotherm from the ITC chelation titration of EDTA into Fe^{2+} -TauD in 25 mM HEPES buffer at pH 7.4 is shown in Figure 4 (shown in blue). The integrated isotherm of the observed chelation includes one-site binding with a significant endothermic feature associated with the dilution of EDTA into buffer solution. These dilutions appear to be associated with a second binding process, seen in both direct titration and chelation experiments for TauD and other enzyme systems. This second process could be attributed to a weak, adventitious binding site,¹³ or structural rearrangements of the new Fe^{2+} -EDTA complex. A non-protein Fe^{2+} ITC control experiment was performed and the data (Figure 4, shown in red) are subtracted to yield a more symmetric one-site binding event (Figure 4, gray).²⁰ The K for the chelation titration is $8.4 (\pm 0.4) \times 10^6$. Deconvolution of the series of equilibria taking place in the chelation experiment yields a K_a for Fe^{2+} -TauD of $2.4 (\pm 0.1) \times 10^7$ (or a K_d of $42 (\pm 2)$ nM, in excellent agreement with the previously published value.¹⁴ This K_a value then allows for the back calculation of the $K_{\text{a Fe-buffer}}$ from the set of direct titration experiments, which is found in Table 1. Using the $K_{\text{a Fe-TauD}}$ and the $H_{\text{Fe-TauD}}$, we obtain a ΔG° of $-10.1 (\pm 0.03)$ kcal/mol and a $-\Delta S^\circ$ contribution of $-12.1 (\pm 0.03)$ kcal/mol, indicating an enthalpy-driven binding event. A complete thermodynamic profile is illustrated in Figure 5.

A heat capacity study was performed to help determine the nature of the unfavorable entropy term during the Fe^{2+} coordination event. By performing the Fe^{2+} binding titration at different temperatures and plotting the observed enthalpy against temperature, the change in heat capacity (ΔC_p) was determined by the slope of the linear correlation between

points.^{22–25} The direct titration of Fe^{2+} into TauD in MOPS buffer was performed at 5, 15, and 25 °C. The plot of H_{obs} vs. temperature yields a C_p of 0.0389 (± 0.0138) kcal/mol·K (Figure 6). Using the statistical thermodynamics equation of $C_p = N3R$, where R is the gas constant and N is the number of solvent molecules gained/released, the Fe^{2+} binding to TauD event has a net gain of approximately 6.5 (± 2.3) water molecules.

DISCUSSION

Nature has adapted to provide a few common metal-binding sites to catalyze oxidation reactions in biology. The 2-His-1-carboxylate facial triad motif is often detected in non-heme oxygenase enzymes, where the representative carboxylate ligand is either a glutamate or aspartate residue.¹ This residue supplies a negative charge in the binding motif, dramatically stabilizing the divalent metal ion and lowering the overall charge on the Fe^{2+} complex. In this study, we measured Fe^{2+} binding to TauD as a means to directly determine the thermodynamic driving forces behind the 2-His-1-carboxylate facial triad coordination of the metal ion in a representative of this family of non-heme Fe^{2+} oxygenases.

Fe^{2+} binding to the 2-His-1-carboxylate facial triad in TauD is a highly favorable reaction in which one metal ion binds per monomer with a ΔG° of $-10.1 (\pm 0.03)$ kcal/mol. The substoichiometric number of metal ions (n) bound to TauD (Table 1) may be due to residual Fe^{2+} , Fe^{3+} , or other contaminating metal ion in the solution of the apoprotein. A control experiment was performed where EDTA was titrated into apoprotein (see Fig. S2 in supplemental information), resulting in approximately 0.2 molar equivalents of Fe^{2+} (or other cation) binding to EDTA. This result justifies the substoichiometric metal content for the direct titration experiments.

The favorable ΔG° of Fe^{2+} binding to TauD is driven by the enthalpic contribution of the coordination process. Included in the observed enthalpy term (approximately -2.8 kcal/mol) is the Fe^{2+} coordination to His255, His99, and Asp101, as well as a proton release event. We hypothesize this proton release event is associated with the ionization of water coordinated to the Fe^{2+} ion. The Lewis acidity of the metal ion results in an average reduction in the $\text{p}K_a$ of the coordinated waters to approximately 7.8, however each coordinated water molecule most likely has a unique $\text{p}K_a$ value which is slightly shifted based on its exact location in the coordination sphere and its H-bonding network. The net release of 0.9 protons as a result of this event provides an additional 12.0 kcal/mol in enthalpic instability for Fe^{2+} binding. When accounting for the proton release and buffer ionization, the change in enthalpy (ΔH) for Fe^{2+} binding is a highly favorable $-11.6 (\pm 0.3)$ kcal/mol. Alternatively, some proton density could be released from His255 and His99 when Fe^{2+} binds. At pH 7.4, approximately 96 % of free histidine in solution is in neutral form. If the histidines within the active site pocket are in this protonation state, then the Lewis acidity of the Fe^{2+} when bound to the ϵ nitrogen will lower the $\text{p}K_a$ of the proton residing on the δ nitrogen, which could result in additional proton loss. The ionization enthalpy of the δ nitrogen for a free histidine residue has been reported to be 10.5 kcal/mol,^{26,27} which would also have a destabilizing effect on the enthalpy of Fe^{2+} binding to TauD, much like water as proposed above and within approximately 3 kcal/mol of the enthalpy value. However, the ionization

enthalpy values of histidine bound to Fe^{2+} are not known, hindering the accurate thermodynamic analysis for this possibility.

There are few comparable Fe^{2+} binding studies in the literature. One recent report measured Fe^{2+} binding to the histidine rich peptide sequence of the iron-regulated transporter IRT1, yielding a ΔH of -6.5 kcal/mol, however it is unclear whether or not this value includes the ionization of water.¹⁹ The difference in enthalpy between this system and TauD could lie in the contribution of the aspartate side chain residue, where the negatively charged residue provides charge stabilization with the positively charged Fe^{2+} which in turn would have direct effects on the enthalpy and entropy terms for the system. In another example, Fe^{2+} binding to an α -helical-rich keratin complex suggests Fe^{2+} coordination to two glutamate residues within the α -helix which supplies an observed enthalpy of -0.86 kcal/mol.²⁸ This enthalpic value is largely due to complete charge stabilization between the two negatively charged glutamate side chain residues and the divalent metal ion (accounting for a large binding equilibrium of 2.8×10^5), whereas a negligible enthalpy indicates a highly entropically driven binding event, as expected by charge-charge interactions.²⁹ Our results indicate a large binding equilibrium and distinctly favorable change in enthalpy for Fe^{2+} binding to TauD. This result is consistent with charge stabilization contributions from the aspartate residue in addition to the favorable contacts with the histidine side chain residues to provide the favorable thermodynamic terms. However, solvation and conformational changes also play a role in the free energy of metal ion coordination, limiting a detailed comparison of TauD with IRT1 and keratin.

The entropy term for Fe^{2+} binding to TauD is slightly unfavorable, indicated by a $-T \Delta S$ term of $+1.6 (\pm 0.4)$ kcal/mol. The unfavorable entropy compensation for enthalpically-driven reactions in macromolecular systems usually stems from structural reorganization upon the binding event. However, previous studies on Fe^{2+} binding to TauD using UV analysis indicate that there is no substantial structural reorganization when the enzyme coordinates Fe^{2+} .¹⁴ Likely candidates accounting for the small, unfavorable $-T \Delta S$ include: an altered H-bonding network between amino acid residues once Fe^{2+} is bound; an overall gentle relaxation of the TauD structure providing more flexibility to the enzyme; water reorganization within or at the surface of the enzyme; proton release, as indicated by our ITC titration studies; and buffer release (approximately 1–2 molecules per metal ion, dependent on the buffer used) to bulk solvent from the Fe^{2+} -buffer complex. The orchestration of all of these terms during the dynamic Fe^{2+} binding reaction, in addition to charge stabilization at the Fe^{2+} center, may result in a negligible net change during metal coordination; this would explain why the entropy term is so small. To gain insight into the role of solvent in the metal coordination process, we have also performed the metal titrations at a series of temperatures, which gives rise to the heat capacity of Fe^{2+} binding to TauD. Although there are no major structural changes associated with the metal ion coordination to TauD, this study indicates a small, positive change in the heat capacity ($+38.9$ cal/mol·K) over a 20 K temperature range. As temperature increases, our observed data reflect a decrease in ΔH , while a slight increase in $-T \Delta S$ occurs. Higher temperatures can cause more favorable ΔS terms, where overall increases in vibrational and rotational energies are more pronounced, which could contribute to the more favorable $-T \Delta S$ term. This process creates

balance between the enthalpy and entropy terms, resulting in no net change in G_{obs} over the 20 °C temperature range.

When we further analyze the C_p term using statistical thermodynamics, the positive C_p value corresponds to the ordering of approximately 6.5 water molecules within Fe^{2+} -TauD upon metal coordination to the 2-His-1-carboxylate ligands. This result can be rationalized as a shift in the H-bonding network surrounding the metal binding site, where local water molecules help stabilize the organized structure once Fe^{2+} is bound. This notion also helps to explain the source of unfavorable entropy measured from this equilibrium.

CONCLUSION

The data presented herein combine the direct titration of non-heme Fe^{2+} into TauD with the chelation titration of EDTA into Fe^{2+} -TauD to obtain all of the thermodynamic properties for non-heme Fe^{2+} coordination to the 2-His-1-carboxylate facial triad of TauD (Scheme 2). The complete binding process yields a highly favorable G° of $-10.1 (\pm 0.03)$ kcal/mol, and is clearly an enthalpy driven process which releases 0.9 protons from bound waters coordinated to the metal center. Upon Fe^{2+} binding, water is reorganized within the Fe^{2+} -TauD structure suggesting a change in the H-bonding network within the enzyme. This provides clarity to the driving forces behind Fe^{2+} binding in TauD. However it remains to be seen if this trend is generalized across the 2-His-1-carboxylate facial triad enzymes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

αKG	alpha-ketoglutarate
TauD	alpha-ketoglutarate/taurine dioxygenase
EDTA	ethylenediaminetetraacetic acid
ITC	isothermal titration calorimetry
ACES	2-(carbamoylmethylamino)ethanesulfonic acid
MOPS	2-(<i>N</i> -morpholino)ethanesulfonic acid
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid

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Figure 1.

Representation of the 2-His-1-carboxylate facial triad, where the histidine and carboxylate side chains are bound to one face of the iron. The adjacent sites of the coordination sphere are solvated until cofactor and substrates bind.

Figure 2.

The structure of the Fe^{2+} -TauD active site pocket, with His255, His99, and Asp101 making up the 2-His-1-carboxylate facial triad (green). α -Ketoglutarate (red) chelates the metal (orange sphere) and taurine (yellow) is bound nearby, but does not coordinate the Fe^{2+} . Coordinates were taken from PDB:1OS7 and the image was generated using PyMOL.¹²

Figure 3.

A representative raw heat (A) and integrated isotherm (B) for Fe²⁺ binding to TauD in MOPS buffer. Observed enthalpies versus the ionization enthalpies of the ACES, MOPS, and HEPES buffers (C). The slope of the line yields the number of protons released from the system. Linear fit is $y = 0.91x - 1.40$. $R^2 = 1.00$.

Figure 4. Titration of EDTA into Fe^{2+} -TauD. Integrated data (blue), non-protein Fe^{2+} control (red), and the baseline subtracted, adjusted isotherm (gray).

Figure 5.

The complete thermodynamic profile for Fe²⁺ binding to TauD. Fe²⁺-buffer interactions and buffer ionization have been accounted for to give a more accurate representation of the thermodynamic properties for the Fe²⁺ binding event.

Figure 6.

Effect of temperature on ΔH of Fe^{2+} binding to TauD. The calculated ΔH associated with Fe^{2+} titration into TauD at various temperatures in MOPS buffer at pH 7.4 yields a slope equal to the C_p for the binding reaction. Values are averaged and the error is one standard deviation from the mean. The data (Table S1) are found in the supporting information.

Scheme 1.
The reaction catalyzed by TauD.

Scheme 2.

The thermodynamic cycle for Fe²⁺ binding to TauD.

Table 1Observed thermodynamic values from the ITC titration of Fe²⁺ acetate into TauD

25 mM Buffer (pH 7.4)	<i>n</i>	<i>K</i>	<i>H</i> _{obs} (kcal/mol)	<i>H</i> _{ionization} (kcal/mol)	<i>G</i> (kcal/mol)	− <i>T S</i> _{obs} (kcal/mol)	<i>K</i> _{Fe-buffer}
MOPS	0.78 (± 0.13)	5.4 (± 0.7) × 10 ⁵	−2.2 (± 0.3)	−5.04	−7.8 (± 0.1)	−5.7 (± 0.4)	42.0 (± 5)
HEPES	0.85 (± 0.13)	3.3 (± 0.6) × 10 ⁵	−1.9 (± 0.5)	−4.86	−7.5 (± 0.1)	−5.6 (± 0.4)	72.5 (± 14)
ACES	0.94 (± 0.07)	1.0 (± 0.7) × 10 ⁷	−3.9 (± 0.8)	−7.27	−9.2 (± 1.0)	−5.3 (± 1.5)	19.7 (± 13)

Values are the average of 2–4 replicates and the errors associated with the values are one standard deviation from the mean.

Table 2The thermodynamic cycle for Fe²⁺ binding to TauD in MOPS buffer

Reaction	Coefficient	<i>H</i> (kcal/mol)
Fe ²⁺ -MOPS + TauD-H _{0.9} ⁺ →		-2.17 ^a
Fe ²⁺ -TauD + MOPS-H _{0.9} ⁺ + 0.1 MOPS		
Fe ²⁺ -MOPS →	1.0	1.83 ^b
Fe ²⁺ + MOPS		
TauD-H ⁺ →	0.91	13.34 ^c
TauD + H ⁺		
MOPS + H ⁺ →	0.91	-5.04 ^d
MOPS-H ⁺		
Fe ²⁺ + TauD →	1.0	-11.55
Fe ²⁺ -TauD		

^aFrom Table 1.^bCalculated from control experiments of EDTA chelation of Fe²⁺ in MOPS buffer.^cRepresents the ionization of water within the Fe²⁺-TauD complex. Value is taken from reference.²⁶^dValue taken from reference.³⁰

Table 3

The average enthalpy values in each buffer for Fe²⁺ binding to TauD

25 mM Buffer, pH 7.4	$H_{\text{Fe-TauD}}$ (kcal/mol)
MOPS	-11.46 ($\pm$ 0.01)
HEPES	-11.53 ($\pm$ 0.49)
ACES	-11.94 ($\pm$ 0.83)
Average	-11.64 ($\pm$ 0.25)

Values were calculated based on the thermodynamic cycle for the binding reaction in the respective buffering system. Values were averaged and the error represents one standard deviation from the mean.