A Model for Local Melting of Metalloprotein Structure

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Received: October 3, 1996[⊗]

Metalloprotein complexes that are able to explore a range of structures will isomerize as the temperature is raised when the isomeric state is coupled to a soft mode in the vibrational spectrum. A model based on a local mode description of the metal center provides insight into the driving forces for the isomerization reaction, which is the local analog of global melting transitions well-known for cooperative systems (proteins, nucleic acids, and crystals). Thermal isomerization of the active site in manganese superoxide dismutase appears to be an example of this type of transition, the azide complex converting between six- and five-coordinate forms at a temperature $T_m = 220$ K. In superoxide dismutase, mode melting defines the pathway for ligand binding at ambient temperatures, stabilizing the functional form of the active site. Thermal isomerization may be a very general characteristic of complex molecules in solution or in the gas phase, reflecting a transition in molecular dynamics.

Introduction

Metal ions in proteins add new biological functions, serving as active sites for catalysis and as redox centers in electron transfer reactions.[1,2] In metalloproteins, the massive metal ions bound in a light-element background are associated with relatively low-frequency vibrations, and unusual thermal behavior can be expected for these sites. Excitation of vibronically active modes in high-symmetry metal complexes having (nearly) degenerate electronic ground states gives rise to characteristic temperature-dependent changes in structure and spectra.[3−5] More generally, a thermally excited complex may isomerize, with distinct structures being stabilized over different temperature ranges. This complete conversion to a distinct form resembles the melting of a molecular crystal and is very different from the linear response to a simple constrained harmonic structure. In biology, local melting of protein structure and the stabilization of active site isomers are likely to be an important mechanism for controlling reaction surfaces in catalysis. An oscillator model for thermal isomerization illustrates these points.

Background

Molecules oscillate about their equilibrium geometry in a locally harmonic potential $V(\mathbf{x})$ with normal modes $\mathbf{Q}_k$ and frequencies ω_k satisfying the dynamical equation[6,7]

$$\mathbf{M}^{-1/2}\mathbf{F}\mathbf{M}^{-1/2}\mathbf{Q}_k = \omega_k{}^2\mathbf{Q}_k \tag{1}$$

where the matrix $\mathbf{M}$ contains the atomic masses and $\mathbf{F}$ is the Hessian of the molecular potential evaluated at the equilibrium point $\mathbf{x}_0$, associated with eigenvalues $\epsilon_{j,k} = (j + {}^1/_2)\hbar\omega_k$ for the energy of the jth excitation of the kth vibrational mode.[6,7] At $T = 0$ K, all vibrations are frozen, and the stable structure is the most tightly bound form, corresponding to the equilibrium geometry $\mathbf{x}_0$ at the global minimum of the potential energy surface (Figure 1, A). The vibrational ladder is populated on warming, the energy partitioning over all $3N - 6$ modes with the lower frequencies predominating:[8,9]

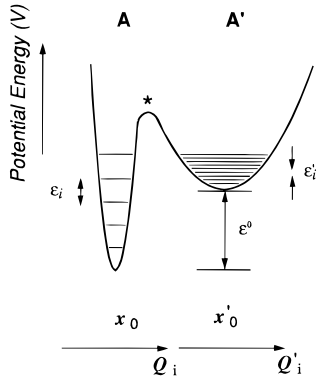

Figure 1. Potential energy diagram for isomeric states. The more tightly bound isomer (A) having equilibrium geometry $\mathbf{x}_0$ will in general have a relatively steep potential surface associated with relatively high-frequency modes (Q_i). A more weakly bound isomer (A′) trapped in a potential minimum at a displaced coordinated ($\mathbf{x}_0'$) will be associated with a distinct set of $3N - 6$ vibrational modes (Q_i') with a larger set of lower frequencies if the potential is flatter in the higher energy form. Trapping of A′ implies a saddle point (*) in the trajectory linking the two structures.

$$Z = \prod_{k=1}^{3N-6} Z_k = \prod_{k=1}^{3N-6} \sum_j \mathrm{e}^{-\beta\epsilon_{j,k}} \tag{2}$$

where Z is the molecular partition function and $\beta = (k_B T)^{-1}$ is the reciprocal thermal energy. The equilibrium between two isomers differing only in internal energy (vibrational and electronic) is given by the ratio of their vibrational partition functions (Z'/Z) weighted by the Boltzmann factor for the electronic energy difference ($e^{-\beta\epsilon_0}$):

$$K_{eq} = \prod_{k'=1}^{3N-6} Z_{k'}{}' \, \mathrm{e}^{-\beta\epsilon_0} / \prod_{k=1}^{3N-6} Z_k \tag{3}$$

with the index k running over all molecular vibrations. Many vibrations are either unchanged by the isomerization or remain frozen at ambient temperatures ($\epsilon_k >> k_B T = 200$ cm^{-1} at 300 K) and cancel in this expression, leaving a small number of active modes whose contribution to the molecular partition function changes in the transition. These active modes are

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⊗ Abstract published in *Advance ACS Abstracts,* January 1, 1997.

S1089-5647(96)03049-0 CCC: $14.00

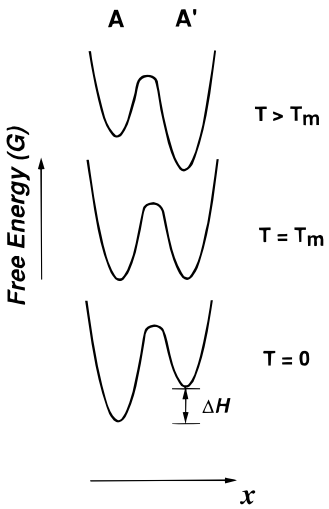

Figure 2. Temperature dependence of the isomeric states. The relative thermodynamic stability of isomers A and A′ defined in terms of the free energy ($G = H - TS$) varies with the degree of vibrational excitation. Progressively raising the temperature stabilizes A′ as a result of entropic contributions to the free energy and at the transition temperature (T_m) A is converted into A′. Above the transition, the A′ structure is stable.

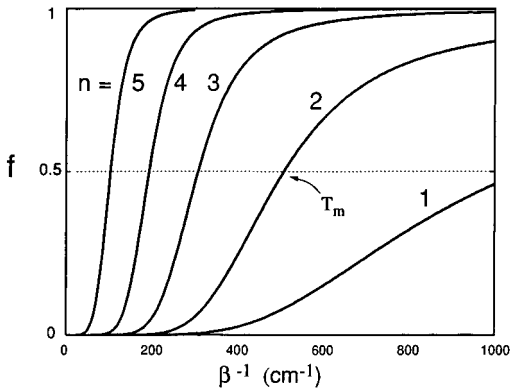

Figure 3. Two-state statistical model for thermal conversion. Fractional conversion (f) as a function of energy (β^{-1}) for five-mode model for $\epsilon_0 = 5000$ cm^{-1}, $\epsilon = 1000$ cm^{-1}, and $\epsilon' = \alpha\epsilon$ ($\alpha = 0.1$), with 1−5 active modes (n).

associated with a softening of the potential in the region of the displaced equilibrium geometry ($\mathbf{x}_0'$) for the A′ isomer, resulting in compression of the eigenvalues and a larger set of low-frequency vibrations, adding to the configurational entropy of A′ and progressively stabilizing that form as the temperature is raised (Figure 2). At the transition temperature (T_m) both A and A′ coexist in equilibrium, the relative stabilities of the two forms reversing at higher temperature as a result of mode melting.[10]

A two-state model with a small number of active oscillator modes can reproduce the thermal interconversion of isomers, providing a picture of the microscopic origins of this behavior. The parameters in this model are the energy difference (ϵ_0), the frequency shift for active modes in the higher energy isomeric state ($\epsilon' = \alpha\epsilon$), and the number of active modes (n). For simplicity, a fixed frequency shift (α) and number of energy levels (j) are set for each oscillator mode within each of the isomeric states. For a five-mode model with $\epsilon_0 = 5000$ cm^{-1} (typical of values for metal−ligand bond enthalpies), $\alpha = 0.1$, and $j_{max} = 250$, the population of the upper state grows at the expense of the lower until the latter is completely depleted as β^{-1} ranges from 0 to 1000 cm^{-1}, as shown in Figure 3. The number of microstates increases exponentially with the number of active modes, and increasing the contributions from multiple vibrational modes results in a narrower transition range, the transition temperature (T_m) being determined by ϵ_0, n, and α. At the midpoint of the transition, $\ln K = 0 = \ln Z' - \beta\epsilon_0 - \ln Z$, so

$$T_m = \frac{\epsilon_0}{k_B \ln Z' - k_B \ln Z} = \frac{\epsilon_0}{nk_B \ln (Z'_{k,active}/Z_{k,active})} \quad (4)$$

making T_m directly proportional to ϵ_0 and α and inversely proportional to the number of active modes, n. The denominator in this expression represents the configurational entropy for the transition. The molecular partition function that determines melting behavior in proteins can in principle be evaluated by substituting local mode frequencies for isomeric forms into eq 3.

Generalization

The requirements for a temperature-dependent structure (a softening of molecular potentials with decreased bonding and sufficiently deep trapping of the higher energy isomer to support a distinct vibrational manifold) are quite unrestrictive and can be expected to be met by a wide range of molecules. Trapping of the higher energy state is essential for melting to occur: without trapping, the structure is expected to smoothly transform with a continuous change in the properties rather than undergoing a transition between discrete populations. Molecular transitions tend to be relatively broad compared to the abrupt phase transitions associated with cooperative changes in extended systems and may occur over a temperature range of 100 K or more, the width being determined by the enthalpy and entropy changes in the reaction. The transition temperature, T_m, is likewise determined by the compensation point between the enthalpy and entropy ($T_m = \Delta H/\Delta S$). Entropy-compensated transitions of this sort have been extensively studied in the context of protein folding and its converse, the melting of higher-order structure as a macromolecule reversibly unwinds,[11−13] where the importance of these compensation terms in defining the statistical energy landscape of a folding protein has been discussed in detail.[13] Local melting of protein structure produces a cascade of discrete folded structures stabilized over different temperature ranges, introducing additional complexity into the phase diagram for protein folding.

Melting may in fact be expected in a wide class of molecules where loss of bonding relaxes dynamical constraints. This type of behavior has been observed for vibrationally excited argon and water clusters in the gas phase where solidlike and liquidlike isomeric states have been characterized.[14−17] In solution, individual molecules can rearrange weakly bound substructures, interconverting between isomeric states that can serve as intermediates in chemical reactions. Since intermediates are reached by activated processes (they are trapped), the contribution of these states will clearly be temperature dependent, stabilized by mode-melting mechanisms. The temperature dependence of local structure for complex biomolecules having a relatively rich range of isomeric structures can be expected to have important implications for biological function. Several examples illustrate these points.

The inversion of cyclohexane is a classic in conformational analysis.[18] At ambient temperatures, the chair conformer is stable, with the unstable twist form at significantly higher energy ($\Delta H = 3.5$ kcal/mol) serving as an intermediate in the interconversion of degenerate chair isomers. Chair−twist isomerization involves an increase in entropy ($\Delta S = 4.5$ cal/(mol deg)), and above the transition temperature $\Delta H/\Delta S = T_m \approx 1600$ K the twist form will become the stable structure. At

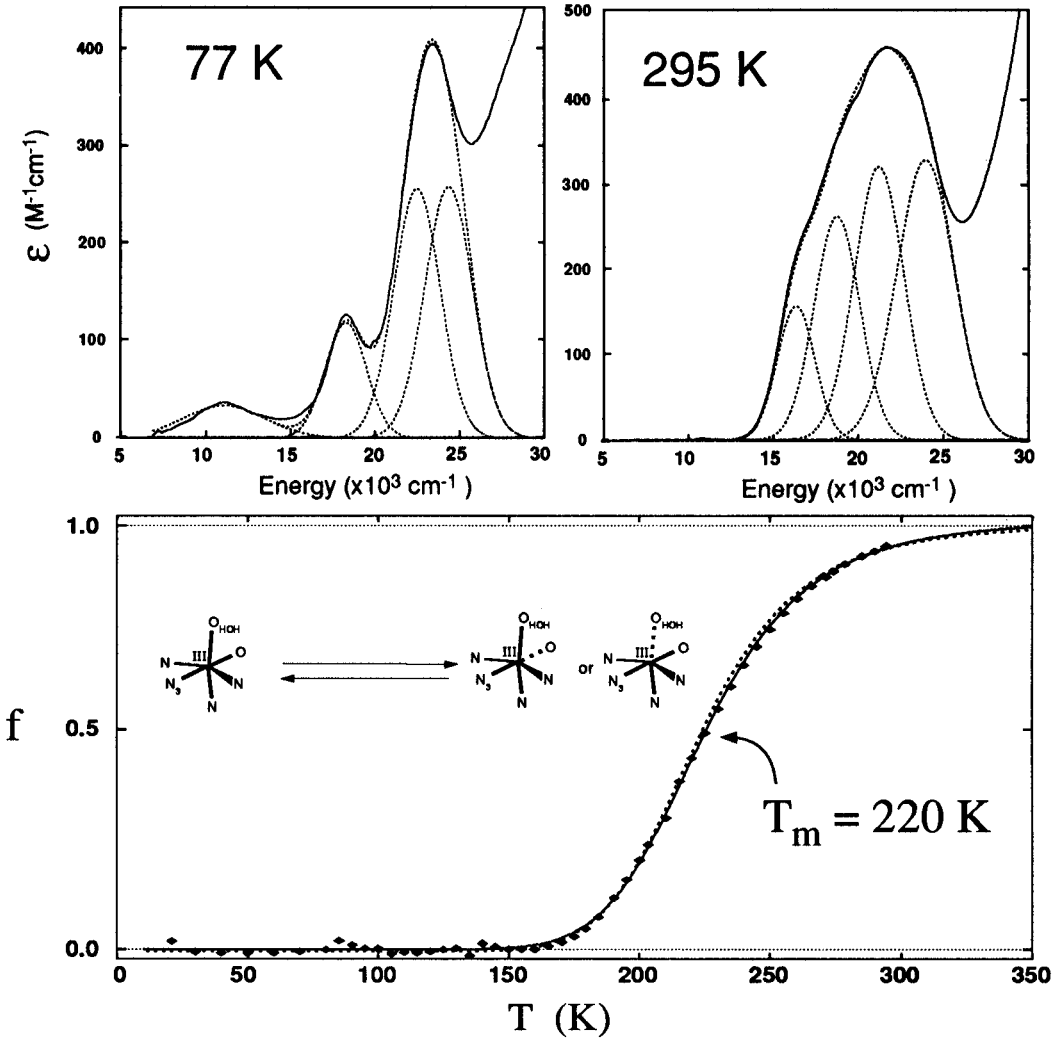

Figure 4. Thermochromism for azide complex of manganese superoxide dismutase. (top) Optical absorption spectra for 5.5 mM Mn(III) SD in 50% (v/v) glycer(ol-d_3):D_2O glassing solvent containing 25 mM KPO_4 buffer, pH 7, with 80 mM KN_3 at 77 and 295 K, resolved into Gaussian components. The appearance of a near-IR absorption band is characteristic of a six-coordinate Mn(III) complex. (bottom) Conversion curve for fractional conversion (f) as a function of temperature (T) obtained by analysis of absorption changes at 500 nm following subtraction of a linear temperature-dependent term from the entire data set. (◆) experiment; (—) theoretical curve based on $\Delta H_{vH} = 5$ kcal/mol, $\Delta S_{vH} = 22$ cal/(mol K); (···) oscillator model $K = e^{-\beta\epsilon_0}\prod_{k=1}^{15}(\sum_{j=0}^{250}e^{-\beta(j+1/2)h\nu_k})/\prod_{k=1}^{15}(\sum_{j=0}^{250}e^{-\beta(j+1/2)h\nu_k})$ with $\epsilon_0 = 2500$ cm^{-1} and M−L vibrational frequencies calculated *ab initio* for $[MnF_6]^{3-}$ (six-coordinate octahedral) and $[MnF_5]^{2-}$ (five-coordinate quare pyramidal) complexes using density functional methods (DMol, Biosym Technologies) and a softened potential for the displaced ligand ($h\nu_1 = H\nu_2 = h\mu\nu_3 = 5$ cm^{-1}).

higher temperatures, the relative stability of the two forms reverses, and the chair form becomes an intermediate in the interconversion of degenerate twist structures. Although complete conversion has not been observed experimentally in this case, a significant fraction of twist structures (25%) are found at high temperature (1070 K).[19]

Many inorganic complexes lose weakly coordinated solvent under much milder conditions near ambient temperatures, converting to a form with decreased metal−ligand bonding at higher energy. In many cases this transition is associated with thermochromism.[20−22] Low-frequency deformations of the metal complex have been identified as the active modes for isomerization in certain cases.[23] Based on the very broad requirements for molecular isomerization, examples of temperature-dependent structures in biology can be expected to be even more abundant. The relatively low vibrational frequencies associated with metal centers in metalloproteins make local melting of structure around these site likely. While the model developed here emphasizes localized excitations of a metal complex as the basis for temperature-dependent structures, isomerization of the metal center may more generally involve protein deformations that are coupled to active site geometry.[24−28] Structural transitions would then occur as the enzyme explores its multiple conformational substates. Thermal isomerization of metalloprotein complexes can lead to subtle changes in ligation affecting spin state and redox potential, directly relating to the catalytic chemistry of the active sites. In some cases, local changes in protein structure is revealed in thermochromism of the active site metal complex. Thermochromism of hemoproteins[29−31] has been difficult to interpret because of the complexity of the spectra. Thermochromism has also been reported for nonheme metalloenzymes. For manganese superoxide dismutase, spectra of anion complexes that are expected to mimic the interactions of the active site with superoxide or peroxide exhibit a dramatic temperature dependence that can be interpreted in terms of a change in ligation of the metal center.[32] At ambient temperature approximating biological conditions, the spectra of the Mn center are characteristic of a five-coordinate Mn(III) ion, changing as the metal picks up an additional ligand to become six-coordinate at low temperature ($T_m = 220$ K) (Figure 4). Based on model calculations that reproduce the transition, the softer interligand potentials associated with five-coordination and the freedom of the dissociated ligand appear to be responsible for the stabilization of the

lower-coordinate complex at elevated temperature. The six-coordinate complex that becomes unstable under physiological conditions may resemble an intermediate in an associative displacement turnover reaction for MnSD,[32] and destabilization of this structure will contribute to efficient binding of substrate molecules. Other metalloenzyme complexes also exhibit thermal transitions. Thermochromism in the copper metalloenzyme galactose oxidase and glyoxal oxidase is related to interconversion of isomeric structures differing in protonation state of the metal ligands. In this case, the isomeric states map out a proton transfer coordinate important for redox catalysis, and the relative stability of these structures is expected to be important for active site chemistry.[33] Thermochromism of cobalt complexes of carbonic anhydrase has also been reported, reflecting changes in stability of coordination geometries related to catalytic structures in the functional enzyme.[34]

Conclusions

Temperature-dependent structures that result from softened potentials in a trapped isomeric state may be expected to be the rule rather than the exception in chemistry and more particularly biology, where complex molecules stabilized by relatively weak intramolecular interactions representing a large number of isomeric structures are especially common. Structural, spectroscopic, and computational approaches can be combined to gain deeper insight into these transformations of molecular structure.

Acknowledgment. Support for this project from the National Institutes of Health (GM 42680) is gratefully acknowledged.

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