Application of the Thermorheologically Complex Nonlinear Adam-Gibbs Model for the Glass Transition to Molecular Motion in Hydrated Proteins

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ABSTRACT The nonlinear thermorheologically complex Adam Gibbs (extended "Scherer-Hodge") model for the glass transition is applied to enthalpy relaxation data reported by Sartor, Mayer, and Johari for hydrated methemoglobin. A sensible range in values for the average localized activation energy is obtained (100–200 kJ mol^{-1}). The standard deviation in the inferred Gaussian distribution of activation energies, computed from the reported KWW β-parameter, is ~30% of the average, consistent with the suggestion that some relaxation processes in hydrated proteins have exceptionally low activation energies.

INTRODUCTION

Annealing of hydrated proteins (1,2) and B-DNA (3) has been reported to produce endothermic heat capacity peaks in DSC scanned samples, that occur ~20 K above the annealing temperatures when scanned at 10–30 K min^{-1}. Sartor et al. (1) also reported that the Tool-Narayanaswamy-Moynihan (TNM) formalism (4,5) for describing enthalpy relaxation within and below the glass transition temperature range reproduced these endothermic heat capacity peaks as a function of annealing temperature with good accuracy, although the computed peaks were narrower than observed. It is firmly established that the TNM formalism accounts for such annealing-induced endotherms very well (5,6), which strongly suggests that the phenomenological similarities between the glass transition and protein dynamics should be taken seriously. The two phenomena are known to have many similarities of course (7). Green et al. (2) (GFA hereafter) noted that protein dynamics are characterized by ''great departures from thermorheological simplicity'' and by fast dynamic processes that were ascribed to a merging of polymerlike side chain dynamics (β-relaxations) and the glass transition (α-relaxation). We offer an interpretation of the extreme breadth of the transition in terms of a Gaussian distribution of activation energies that has a physically sensible standard deviation, and of course immediately generates thermorheological complexity. This interpretation has elements in common with the GFA suggestion of merging α-and β-relaxations (the latter are known to be thermorheologically complex, for example), but has the advantage of being quantifiable and of allowing the standard deviation in a Gaussian distribution of activation energies to be determined explicitly from the value of β. This standard deviation is found to be 30% of the average, suggesting that the fast processes discussed in GFA may simply be the tail of an extravagantly broad, but single, distribution of relaxation times (note that the short time components of the distribution are responsible for annealing endotherms). As noted in GFA, these very low activation energy fast processes may also be Nature's way of making biological processes robust with respect to temperature variability.

Our analysis draws on a recently described extension (8) of the nonlinear Adam-Gibbs model (9,10), increasingly referred to as ''Scherer-Hodge'' (SH). For convenience, this extension is referred to here as ESH (extended SH).

PHENOMENOLOGY OF THE GLASS TRANSITION

This subject has been reviewed (6), but is briefly summarized here for convenience.

1. The structural state of an amorphous substance, within and below the glass transition temperature range, is parameterized by the enthalpic fictive temperature $T_{f,H}$, defined as the temperature at which the observed enthalpy (in excess of the crystalline value) would be the equilibrium value. A change in $T_{f,H}$ of magnitude $\Delta T_{f,H}$ is approximately related to the corresponding change in enthalpy ΔH by

$$\Delta H \approx \Delta C_p \Delta T_{f,H}, \quad (1)$$

where ΔC_p is the change in heat capacity across the glass transition temperature range.

2. The TNM expression for the relaxation time τ for $T_{f,H}$ is the generalized Arrhenius equation

$$\tau_{T_{f,H}}(t) = A \exp[x\, h/RT(t) + (1-x)\cdot h/RT_{f,H}(t)], \quad (2)$$

where A, x, and h are adjustable parameters. The appearance of $T_{f,H}$ in the argument of the exponential reflects the nonlinearity of the kinetics, and for this reason the parameter x is often referred to as the nonlinearity parameter.

3. The kinetics are linearized using the reduced time formalism (11,12), in which the relaxation variable t/τ is replaced by the reduced time ξ defined by

$$\xi(t) = \int_0^t dt'/\tau(t'), \quad (3)$$

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where $\tau(t')$ is given by Eq. 2 for $T(t)$ and $T_{f,H}(t)$ during DSC scans and isothermal annealing.

4. The KWW response function is used with the reduced time argument:

$$\phi(t) = \exp\{-[\xi(t)]^{\beta}\}, \quad (4)$$

where β is another adjustable parameter. This linearized function is then incorporated into a Boltzmann superposition integral for computing the response to rate cooling, annealing, and rate heating.

5. The SH model replaces Eq. 2 with an expression derived from a nonlinear form (9,10) of the familiar Adam-Gibbs model (13):

$$\tau = A' \exp[B/T(1 - T_2/T_{f,H})], \quad (5)$$

where A', B, and T_2 are adjustable constants. The parameter T_2 can be identified with the Kauzmann temperature, but is not discussed here. Despite appearances, Eqs. 2 and 5 produce very similar results (6,10). Their parameters are related by

$$x \approx 1 - T_2/T'_{f,H}, \quad (6)$$

where T_f' is the glassy state value of T_f (and is the definition of choice for T_g), and

$$B \approx x^2 \cdot h/R. \quad (7)$$

The Adam-Gibbs model is based on the concept of cooperatively rearranging regions whose size increases with decreasing temperature, for which direct experimental evidence has recently been reported (14).

6. The localized activation energy $\Delta\mu$ in the SH model is related to the parameters h and B of Eqs. 2 and 5 by

$$\Delta\mu = B \cdot R \cdot \Delta C_p/s_c^* \quad (8)$$

$$\approx x^2 \cdot h \cdot \Delta C_p/s_c^*, \quad (9)$$

where s_c^* is the entropy associated with the minimum number W of configurations required for relaxation:

$$s_c^* = R \cdot \ln(W). \quad (10)$$

Traditionally, W has been set equal to 2 (the configurations before and after rearrangement), although it has been suggested (10) that for chain polymers, where a crankshaft motion is the only viable candidate for a localized relaxation event that does not require concomitant rearrangement of atoms far removed from the relaxation site, W is better approximated as 2^3.

Equation 9 indicates that $\Delta\mu$ is very different from h. In essence, h is not a true activation energy because it corresponds to the rearrangement of many moieties and cannot be identified with individual transitions. On the other hand, $\Delta\mu$ is by definition an activation energy per moiety (13), and a distribution in $\Delta\mu$ therefore corresponds directly to a distribution in individual relaxation times.

7. The KWW exponent β is constant in both the TNM and SH models, but the distributions in B and $\Delta\mu$ introduced in ESH easily removes this restriction (8). The implications of this modification are discussed below.

ADAM-GIBBS PARAMETERS

The TNM parameters reported by SMJ are $\ln(A/s) = -135.2$, $h = 300$ kJ mol^{-1}, $x = 0.25$, and $\beta = 0.07$. The uncertainties in these are presumably large, since the predicted endotherms are sharper than the observed ones, and the sensitivity of the computed curves to changes in parameters may be unusually small because of the unprecedented extremely small value of β. The derived SH parameter B is

$$B \approx x^2 h/R = (0.0625)(300\times10^3)/8.31 = 2.26\,\text{kK}, \quad (11)$$

and the putative Kauzmann temperature is

$$T_2 \approx T_g(1 - x) = (180)(0.75) = 135\,\text{K}, \quad (12)$$

where 180 K is the observed ''onset'' value of T_g for the TNM calculated C_p reported in SMJ (the onset value corresponds closely to T_f' of Eq. 6 for canonical glasses).

Estimation of the localized SH activation energy $\Delta\mu$ from Eq. 9 requires a value for ΔC_p that cannot be obtained from the SMJ data because sample weights were not reported. However, Table 1 of GFA reports $\Delta C_p = 0.6$ J g^{-1} K^{-1} for poly-L-asparagine with the same water content, and we adopt this value with the recognition that other proteins such as hydrated methemoglobin may have different values. If water is assumed to participate in the relaxation event, as discussed in GFA, then the relaxing moiety would have a molar mass of ~20–40 g mol^{-1} (1–2 water molecules and 1–2 C/N/O atoms), and the molar value of ΔC_p is estimated to be ~30–60 J K^{-1} mol^{-1}. If $s_c^* = R \cdot \ln(2) = 6\,\text{J K}^{-1}$ is assumed, then Eq. 9 yields

$$\Delta\mu \approx (2260)(8.31)(30-60)/6 \approx 100-200\,\text{kJ mol}^{-1}. \quad (13)$$

This is a reasonable range for hydrogen bond strengths in hydrated proteins.

The reported value of 0.07 for the KWW β-parameter is by far the smallest ever reported—the smallest published value for canonical glasses is 0.25 for PVC (5), and most materials are characterized by values well above this, in the range 0.4–0.7 (6). Since all the hydrated proteins studied by Sartor et al. (1) behaved very similarly to one another, and to the data of GFA, this low value for β is probably a characteristic of hydrated proteins and reinforces the idea that they are characterized by extravagantly broad distributions of relaxation times (2). We believe that this value of β should be taken seriously, and to this end we now compute the width of the distribution of activation energies in terms of β. It is known that in the limit of small β the KWW distribution is a log Gaussian (15), corresponding to a Gaussian

distribution in activation energies (8). In this limit the standard deviation in the (natural) log Gaussian distribution of relaxation times, $\sigma_{\ln\tau}$, is related to β by (15)

$$\sigma_{\ln\tau} = 1/\beta, \tag{14}$$

so that $\beta = 0.07$ gives $\sigma_{\ln\tau} = 14.3$, corresponding to $14.3/2.3 \approx 6$ decades for the standard deviation in the distribution of $\log_{10}(\tau)$. For consistency, the TNM ''onset'' value of $T_g \approx 180\,\mathrm{K}$, as estimated from the SMJ-computed $C_p(T)$, is used here to compute the standard deviation in B of Eq. 14, σ_B. This yields (8)

$$\begin{aligned}\sigma_B &\approx (T_g - T_2)\sigma_{\ln\tau} \approx xT_g\sigma_{\ln\tau} \\ &\approx (0.25)(180)(14.3) \approx 650\,\mathrm{K},\end{aligned} \tag{15}$$

which is ~30% of the average value of B. Thus ~0.05% (half of the 3.3σ-confidence level) of the barriers $\Delta\mu$ are zero in these hydrated proteins (assuming that the distribution in B is entirely due to that in $\Delta\mu$, i.e., that there is no distribution in s_c^*). This is obviously a rough estimate, of course, because it assumes that the low energy tail of the true distribution remains Gaussian, but it is nonetheless considerably larger than those for canonical glasses, that are typically characterized by distributions that have (nonlog Gaussian) half-widths of ~1.5–2 decades (as estimated from the approximation (8) that KWW distribution half-widths are $1.5(1/\beta - 1)$ decades). These widths are ≈5–10% of the average $\Delta\mu$ for canonical glasses, and imply negligibly small probabilities of zero activation energies ($<10^{-14\%}$ for a log Gaussian). We suggest that it is the significant probability of near-zero energy barriers in hydrated proteins that quantitatively distinguishes them from canonical glasses.

We turn now to the relevance of the SMJ best fit TNM-computed C_p data to the experimental data for hydrated proteins. It is apparent from SMJ and GFA that the only quantitatively significant difference between the various hydrated proteins is ΔC_p and the shift in T_g. The latter can be accommodated by simply changing the preexponential factors A and A' in Eqs. 2 and 5, while keeping the other TNM parameters the same. This needs to be tested, however. The value of ΔC_p relates $\Delta\mu$ to h, and needs to be evaluated for many more systems as well.

In view of the significance of thermorheologically complexity, the recently described ESH model (8) seems well-suited to describe these systems more accurately. This approach may provide a superior description of the broad annealing endotherms observed in hydrated proteins, for example, and might also yield tighter estimates of enthalpy relaxation parameters for hydrated proteins.

CONCLUSIONS

The ESH model, when applied to published data for scanned DSC heat capacities of hydrated methemoglobin, yields a sensible range of localized activation energies and a standard deviation equal to 30% of the average that implies ~5 in 10^4 processes with zero activation energies. This lends quantitative support to the suggestion by GFA that temperature invariant relaxation processes in proteins may be Nature's solution to robust performance in variable temperature environments. Since the analysis given here does not draw on any specific characteristics of methemoglobin, and since the experimental annealing results for several different hydrated proteins are very similar, these results may be applicable to many, perhaps even most, hydrated proteins. To determine if this is quantitatively true, however, TNM and/or ESH parameters need to be determined for many more hydrated proteins.

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