Entropy Is the Major Driving Force for Fragmentation of Proteins and Protein−Ligand Complexes in the Gas Phase

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Received: February 27, 2003

This paper presents a critical analysis of Arrhenius parameters for gas-phase fragmentation of proteins and protein−ligand complexes reported in the literature. We demonstrate that there is a surprisingly strong correlation between the Arrhenius activation energy (E_a) and the preexponential factor (A). This correlation becomes extremely important for reactions characterized by very high or very low values of A. This correlation is a direct consequence of the relative change in the spacing between vibrational levels of the reactant and the transition state for reaction. Converting the Arrhenius activation energy into the threshold energy for the reaction using Tolman's theorem reveals the true magnitude of the correlation between molecular complexity and stability. Tolman's correction factor (ΔE_{corr}) increases linearly with log(A) from 3 kcal/mol for log(A) = 16.2 to 36.4 kcal/mol for log(A) = 39.2. Threshold energies extracted from the Arrhenius activation parameters for 56 different reactions are the same within the experimental error bars, while the preexponential factors differ by many orders of magnitude. This indicates that activation entropy is the major driving force for dissociation of proteins and protein−ligand complexes in the gas phase.

Introduction

Gas-phase binding energies of protein complexes provide important information on the intrinsic interactions between proteins and ligands in the absence of solvent. In some cases, when solution-phase interactions are preserved in the gas phase, the gas-phase and solution-phase stabilities of protein−ligand complexes are strongly correlated. Mass spectrometric methods for quantifying noncovalent binding interactions in biomolecular structures have been recently reviewed.[1] Blackbody infrared radiative dissociation (BIRD),[2−5] a valuable method for studying thermal kinetics in the gas phase, has been successfully applied to relatively small biomolecules[4,5] and more recently to oligonucleotide complexes[6−8] and protein−ligand complexes.[9−12] This method utilizes the photon flux generated by the vacuum chamber walls and the long time scale of a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) to heat the ions radiatively and to follow their fragmentation as a function of wall temperature. Arrhenius activation energies and preexponential factors for reactions of interest are determined in BIRD experiments. A strong correlation of the strength of protein−ligand interactions with relative changes in Arrhenius activation energies has been presented recently.[9−12]

Arrhenius activation energy (E_a) represents an average over the entire ensemble of activated species. It can be converted into the dissociation threshold (E_0) using Tolman's theorem[13−15]

$$E_a = E_0 + \langle E' \rangle (T) - \langle E \rangle (T) + k_B T \qquad (1)$$

where k_B is Boltzmann's constant; $\langle E' \rangle (T)$ and $\langle E \rangle (T)$ are the average energy of the transition state (TS) and the average energy of all molecules, respectively. We denote $\Delta E_{corr} = \langle E' \rangle - \langle E \rangle$ as Tolman's correction factor. We have recently demonstrated that this correction factor, ΔE_{corr}, strongly depends on the value of the preexponential factor, A.[16] The correction factor is quite small for unimolecular reactions of relatively small molecules, for which preexponential factors are in the range $10^{10}-10^{16}$ s^{-1}. However, dissociation of large molecules in the gas phase is often characterized by very low (10^5 s^{-1})[17] or very high (10^{39} s^{-1})[9] values of A. For these extreme values of the preexponential factor Tolman's correction becomes quite significant with the result that the Arrhenius activation energy is strongly correlated with the preexponential factor. This strong correlation can reverse the order of Arrhenius activation energies for different systems relative to the order of the corresponding threshold energies.

Correlation between the Arrhenius parameters is a direct consequence of the relative change in the spacing between vibrational levels of the reactant and the transition state (TS) and is a function of the degree of tightness/looseness of the transition state. Large preexponential factors are associated with reactions proceeding via very loose TS's. In this case the spacing between vibrational levels in the TS is decreased relative to the reactant molecule. This results in a higher average energy of the TS and positive correction factor, meaning that the Arrhenius activation energy is higher than the threshold energy for the reaction. For reactions proceeding via a very tight transition state (low preexponential factors), ΔE_{corr} is negative and the Arrhenius activation energy is lower than the threshold energy.

In this study we apply Tolman's correction to the existing body of Arrhenius parameters for dissociation of proteins and protein−ligand complexes,[9−12] for which experimental preexponential factors are in the range from 10^{16} to 10^{39} s^{-1}. We demonstrate that threshold energies for these systems are very similar even though Arrhenius activation energies determined experimentally are quite different. This implies that application

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10.1021/jp0345093 CCC: $25.00
Published on Web 07/03/2003

of Tolman's correction is of crucial importance for understanding the energetics of dissociation of large molecules.

Method

Tolman's correction factor was calculated using a procedure described earlier.[16] The contribution of each vibrational mode to the average energy, $<E_i>$, is given by the standard expression

$$<E_i> = \frac{h\nu_i}{e^{h\nu_i/k_BT} - 1} \quad (2)$$

where ν_i is the vibrational frequency, k_B and h are Boltzmann's and Planck's constants, respectively, and T is the temperature. Because the average energy is an additive property and we are interested in the difference between the average energy of the reactant and the transition state, we need to consider only transition modes that change in the course of the reaction in our calculations.

The activation entropy ($\Delta S^{\ddagger}$) and the preexponential factor (A) are related through the absolute reaction rate theory expression

$$A = e\frac{k_BT}{h}\exp\left(\frac{\Delta S^{\ddagger}}{R}\right) \quad (3)$$

where R is the molar gas constant. The entropic contribution of each vibrational mode, S_i, is calculated as follows

$$S_i = R\left(\ln q_i + T\frac{d \ln q_i}{dT}\right) \quad (4)$$

where the q_i is the vibrational partition function given by

$$q_i = \frac{1}{1 - e^{h\nu_i/k_BT}} \quad (5)$$

Similarly to the average energy, the value of the activation entropy depends only on the change in the vibrational frequencies of the transition modes and the temperature.

In our simulations we had to assume some model for the TS of protein dissociation. It should be noted that because the unimolecular reaction dynamics of systems with such complexity is largely unexplored, the choice of the TS is not straightforward. In this study we chose the TS rather arbitrarily. We assumed that only relatively "soft" modes change in the course of reaction and kept the transitional modes in a reasonable range (>300 cm^{-1}). Furthermore, because we wanted to model reactions with a wide range of preexponential factors, we had to assume that many vibrational frequencies of the excited ion are affected by dissociation. In most of the calculations presented below, we assumed 1000 cm^{-1} mode for the reaction coordinate and varied the 210 modes of 1200 cm^{-1} each in the reactant to match a specified preexponential factor at 415 K, the average experimental temperature. We also explored the influence of the choice of the transition state on calculated correction factors as detailed in section Factors Affecting Tolman's Correction Factor.

The average energies of the transition modes of the reactant and the transition state were calculated using eq 2. Calculations were performed for each pair of Arrhenius parameters reported in refs 9−12.

Arrhenius Activation Parameters and Threshold Energies

Figure 1a shows a plot of the Arrhenius activation energies for several proteins and protein−ligand complexes reported in the literature[9−12] as a function of log(A). There is a clear linear dependence of E_a on log(A) regardless of the identity of the proteins and protein−ligand complexes. The correlation between E_a and log(A) implies that the relative stability of the species cannot be extracted from the differences in activation energies but rather must be deduced from the differences in threshold energies for reactions.

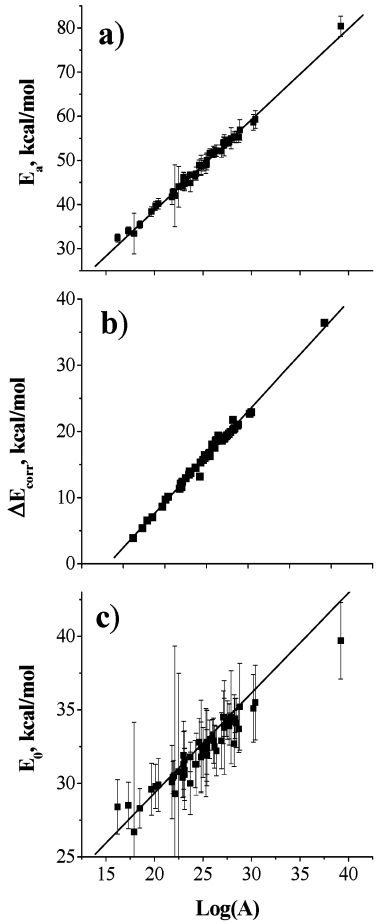

Figure 1. Arrhenius activation energy from refs 9−12 (a), Tolman's correction factor (b), and threshold energy (c) as a function of log(A) for reaction. Lines are linear fits through data points.

As discussed earlier, threshold energies can be obtained by subtracting the correction factor, ΔE_{corr}, from the corresponding Arrhenius activation energy. The dependence of Tolman's correction factor on log(A) is plotted in Figure 1b. The correction factor increases almost linearly with log(A). ΔE_{corr} ranges from 4 to 24 kcal/mol for most of the systems and reaches a value of 40.7 kcal/mol for the largest log(A) of 39.2. This demonstrates that the correction factor becomes very large for large values of the preexponential factor.

Figure 1c shows the dependence of threshold energies on log(A). Although some correlation between log(A) and E_0 remains, we reach the remarkable conclusion that most of threshold energies fall in a narrow range from 29 to 35 kcal/ mol. The only exemption is the threshold energy of 39.7 kcal/mol obtained for the reaction with the highest E_a (80.4 kcal/mol) and log(A) of 39.2. Experimental Arrhenius parameters from ref 9 and the corresponding calculated values of Tolman's correction, threshold energies, and activation entropies are summarized in Table 1. The entire compilation is given in the Supporting Information. Uncertainties in threshold energies were obtained by propagating errors in E_a and log(A).

TABLE 1. Arrhenius Parameters (E_a and log(A)) for Dissociation of Proteins and Protein−Ligand Complexes Reported in Ref 9; Calculated Tolman's Corrections (ΔE_{corr}), Threshold Energies (E_0), and Activation Entropies ($\Delta S^{\ddagger}$) at 415 K

log(A)	E_a	T, K	ΔE_{corr}	E_0	$\Delta S^{\ddagger}$
18.5 ± 0.4	35.4 ± 0.9	413	7.1	28.3 ± 1.3	23.5 ± 0.5
19.7 ± 0.6	38.4 ± 1.1	413	8.8	29.6 ± 1.8	29.0 ± 0.9
17.3 ± 0.5	34.0 ± 0.9	413	5.5	28.5 ±1.6	18.0 ± 0.5
16.2 ± 0.6	32.4 ± 0.9	413	4.0	28.4 ± 1.8	12.9 ± 0.5
23.7 ± 0.6	44.9 ± 2.0	423	14.9	30.0 ± 2.1	47.3 ± 1.2
23.0 ± 1.1	46.2 ± 1.1	428	14.3	31.9 ± 2.3	44.1 ± 2.1
24.8 ± 0.9	48.6 ± 1.8	428	16.8	31.8 ± 2.4	52.3 ± 1.9
23.0 ± 0.8	45.6 ± 1.6	428	14.3	31.3 ± 2.2	44.1 ± 1.5
26.2 ± 0.6	51.8 ± 1.1	433	19.3	32.5 ± 1.5	58.7 ± 1.3
25.7 ± 0.5	51.6 ± 1.0	433	18.6	33.0 ± 1.3	56.4 ± 1.1
26.1 ± 0.6	52.0 ± 1.2	433	19.1	32.9 ± 1.5	58.2 ± 1.3
39.2 ± 1.2	80.4 ± 2.3	463	40.7	39.7 ± 2.6	118.2 ± 3.6
30.2 ± 1.0	58.6 ± 1.8	423	23.5	35.1 ± 2.3	77.0 ± 2.5
28.8 ± 1.2	56.9 ± 2.3	423	21.7	35.2 ± 2.9	70.6 ± 2.9
30.4 ± 1.1	59.3 ± 2.0	423	23.8	35.5 ± 2.5	77.9 ± 2.8

E_a, ΔE_{corr}, E_0 are in kcal/mol; $\Delta S^{\ddagger}$ is in cal/(mol K).

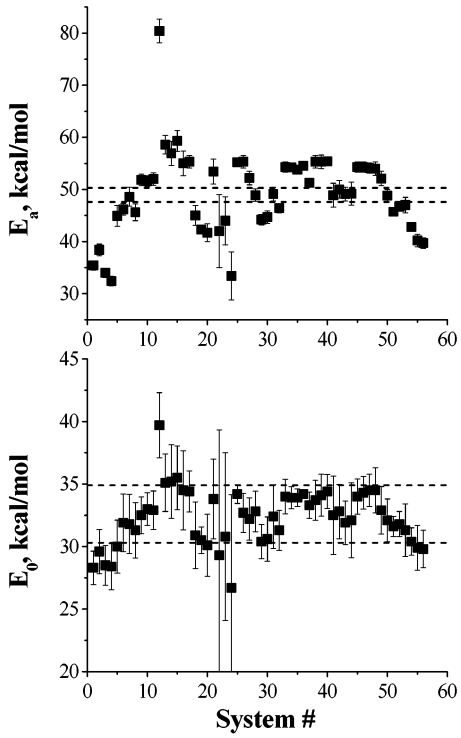

Figure 2. Compilation of values of Arrhenius energies (top panel) and corresponding threshold energies (bottom panel) for all proteins and protein−ligand complexes studied to date using BIRD. The entire compilation is also given as a table in the Supporting Information. Numbering of systems follows the numbering used in the Supporting Information.

Arrhenius activation energies and the corresponding threshold energies for dissociation of all 56 proteins and protein−ligand complexes reported in the literature[9−12] are shown in Figure 2. Dashed lines show the band centered at the average over the entire dataset with the half width given by the average standard deviation. It is clear that most of Arrhenius activation energies are distinctly different from the average value, while most of the threshold energies are the same as the average energy within the indicated error bars. It can be therefore concluded that dissociation of these proteins and protein−ligand complexes is characterized by the same threshold energy of 32.5 ± 2.5 kcal/mol. However, activation entropies for these reactions deduced from the corresponding preexponential factors are quite different (see Table 1), ranging from 13 cal/(mol K) for the lowest log(A) of 16.2 to 118 cal/(mol K) for the highest log(A) of 39.2. This implies that entropy is the major driving force for these reactions.

By converting activation energies into threshold energies we reach quite different conclusions from those reached in the direct comparison of Arrhenius activation energies.[9−12] On fundamental grounds comparing threshold energies is the only way to address the relative stability of species, for which dissociation is characterized by very high or very low preexponential factors, because in this case the Arrhenius activation energy contains the contribution associated with reaction entropy.

Factors Affecting Tolman's Correction Factor

It should be noted that the absolute value of Tolman's correction factor depends on the choice of transition modes that change in the course of the reaction. Because gas-phase unimolecular dissociation of molecules of this size is largely unexplored, nothing is known on the number of modes involved in the dissociation process. It was suggested that softening of numerous vibrational modes during dissociation of protein−ligand complexes is responsible for large preexponential factors.[9] As discussed earlier, we assumed 1000 cm^{-1} for the reaction coordinate and varied the other 210 modes of 1200 cm^{-1} each in the reactant to match a specified preexponential factor. Varying the number of transitional modes and their frequencies results in somewhat different absolute values of ΔE_{corr}. However, the general trends in threshold energies are preserved for any reasonable choice of mode frequencies.

Figure 3 shows the influence of the choice of the transition state on ΔE_{corr}. Reducing the number of transitional modes from 210 to 140 and 70 while keeping the frequency of the modes in the reactant the same (1200 cm^{-1}) results in a smaller slope of the curve of ΔE_{corr} vs log(A) (Figure 3a). Lowering the value of the characteristic frequency of transitional modes to 600 cm^{-1} (Figure 3b) has a similar effect on ΔE_{corr}, although the offset of the curve is also different. The slope of the curve of ΔE_{corr} vs log(A) becomes larger if 10 out of 210 frequencies are reduced to 500 cm^{-1} and converted into internal rotations of reaction products with characteristic frequency of 50 cm^{-1} (Figure 3c). Although the absolute value of ΔE_{corr} is lower in this case, the relative change in ΔE_{corr} between the low and high values of log(A) is larger than in the case when all 210 transitional modes are the same (1200 cm^{-1}).

Tolman's correction factor is also temperature-dependent. The dependence is linear with the slope increasing with log(A) (see Figure 4). For low values of the preexponential factor, ΔE_{corr} decreases (becomes more negative) with increase in temperature. For high preexponential factors, ΔE_{corr} increases with temperature. The scatter in the values of ΔE_{corr} in Figures 1b and 3 results from the error in assignment of the temperature to each pair of Arrhenius factors based on published Arrhenius plots.

Threshold energies derived in this work are based on a simple harmonic correction for the relative spacing between the vibrational states of the activated molecule and the transition state. The remaining correlation between E_0 and log(A) shown in Figure 1c could be indicative of imperfections in the correction procedure. In particular, this could be the reason for a deviation of the threshold energy calculated for the reaction with the largest preexponential factor from the remaining data. More accurate values of ΔE_{corr} can be obtained from the knowledge of the transition-state frequencies of dissociating complexes, which are currently not available.

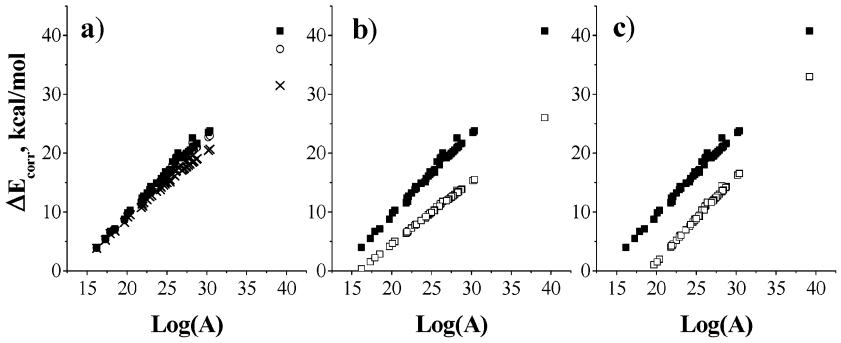

Figure 3. Dependence of Tolman's correction factor on the choice of the transition state. Filled squares represent calculation where 210 frequencies of 1200 cm^{-1} each were used as transitional modes. (a) Influence of the number of transitional modes on ΔE_{corr}: open circles correspond to 140 modes and crosses correspond to 70 modes. Open squares in panel b correspond to 210 transitional modes of 600 cm^{-1} each. Open squares in panel c represent calculation of 200 modes of 1200 cm^{-1} and 10 other modes of 500 cm^{-1} that are converted into internal rotations of reaction products with characteristic frequency of 50 cm^{-1}.

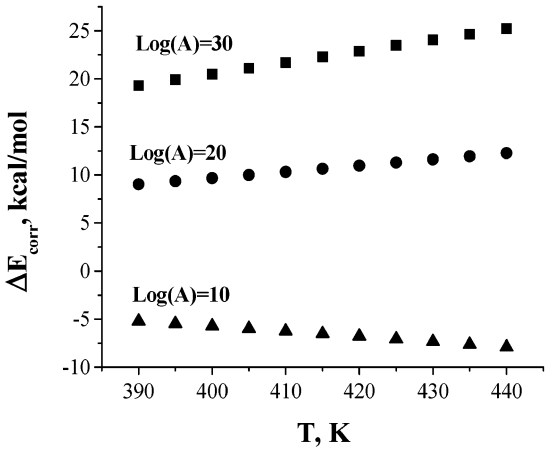

Figure 4. Temperature dependence of ΔE_{corr} for different preexponential factors.

Summary

Dissociation of proteins in the gas phase is commonly characterized by very high values of preexponential factors, which is indicative of softening of a large number of modes in the course of reaction. In this case, the Arrhenius activation energy reflects not only the energetics of dissociation, but also an entropic contribution. Consequently, Arrhenius activation parameters, A and E_a, become strongly correlated.

In this study the existing thermal data were reanalyzed using Tolman theorem to obtain threshold energies from the Arrhenius parameters. We found that Tolman's correction depends on the choice of the transition state. Although our present lack of detailed knowledge about transition states for dissociation of such complex systems makes it impossible to calculate precise Tolman theorem corrections, the conclusion is inescapable that this ensemble of reactions is characterized by very similar dissociation energies. It also follows that the principal driving force for these reactions is entropy. Further, for thermal dissociation reactions of complex molecules and adducts, Arrhenius activation energies must be corrected to separate energetic and entropic effects. Tolman's theorem defines the procedure for doing this.

Acknowledgment. This work was performed at the W. R. Wiley Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the U.S. Department of Energy's Office of Biological and Environmental Research and located at the Pacific Northwest National Laboratory. PNNL is operated by Battelle for the U.S. Department of Energy. This research was carried out within the project 40457 supported by the Office of Basic Energy Sciences of the U.S. Department of Energy. The authors are gratefully thankful to Professor Chava Lifshitz for critical reading of the manuscript and very helpful discussions.

Supporting Information Available: Table containing all Arrhenius parameters used in the figures, corresponding Tolman's correction factors, and threshold energies. This material is available free of charge via the Internet at http://pubs.acs.org.

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