Chemistry 882

Lecture Notes 6

Weliky

Partition function q for 1 particle

$$q = \sum_{j=1}^{t} e^{-\varepsilon_j/kT}$$

sum over states

For N identical but distinguishable (individually identifiable) particles (like in a solid)

$$Q = q^N$$

For indistinguishable (interchanging) particles like in a gas or liquid

$$Q = \frac{q^N}{N!}$$

reduction

Can also consider all N particles as a single system with states

$$Q = \sum_{j=1}^{t} e^{-E_j/kT}$$

$$p_j = \frac{e^{-E_j/kT}}{Q}$$

$$\langle \varepsilon \rangle = \frac{U}{N}$$

$$U = \frac{\sum_{j=1}^{t} E_j e^{-E_j/kT}}{Q} = kT^2 \frac{d \ln Q}{dT} = NkT^2 \frac{d \ln q}{dT}$$

$$S = k \ln W = k \sum_{j=1}^{t} -p_j \ln p_j = k \ln Q + kT \frac{d \ln Q}{dT}$$

Helmholtz Free Energy

$$F = U - TS = -kT \ln Q \qquad \left(\frac{\partial F}{\partial T}\right)_{N,V} = -S$$

$$\mu = -kT \left(\frac{\partial \ln Q}{\partial N}\right)_{T,V} = \left(\frac{\partial F}{\partial N}\right)_{T,V}$$

$$p = kT \left(\frac{\partial \ln Q}{\partial V}\right)_{N,T} = -\left(\frac{\partial F}{\partial V}\right)_{T,N}$$

$$G = F + pV$$

constant p

$$\Delta G = \Delta F + p\Delta V + \cancel{V\Delta p}$$

≈ 0 for processes in liquids

$$\Delta G \approx -kT \ln \frac{Q_{final}}{Q_{initial}}$$

Key question in chemistry is

how does μ depend on [ ]

Simplest model for Q is

non-interacting particles restricted

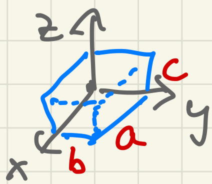
to volume of dimensions a, b, c

$\uparrow \;\; \uparrow \;\; \uparrow$

$x \;\; y \;\; z$

directions

$\equiv$ ideal gas



Start with single particle in volume

From quantum mechanics (solution of time-indep. Schrödinger Eq.)

Planck's constant

$$\varepsilon_{n_x,n_y,n_z} = \frac{h^2}{8m}\underbrace{\left[\frac{n_x^2}{a^2}+\frac{n_y^2}{b^2}+\frac{n_z^2}{c^2}\right]}_{\text{energy in a 3-D box}}$$

quantum numbers $\equiv$ positive integers

particle mass

$$q = \sum_{n_x=1}^{\infty} \sum_{n_y=1}^{\infty} \sum_{n_z=1}^{\infty} e^{-n_x^2 \boxed{h^2/8ma^2kT}} e^{-n_y^2h^2/8mb^2kT} e^{-n_z^2h^2/8mc^2kT}$$

unitless

$e^{g+h} = e^g e^h$

Consider $n_x = 1$, $h^2 \approx 10^{-66}\ J^2\text{-}s^2$, $m \approx 10^{-23}$ kg

$a \approx 10^{-2}$ m

6kDa / mole

$T \approx 300$K

$8 \approx 10$

$kT \approx 10^{-20}$ J

$$\frac{h^2}{10ma^2kT} \approx \frac{10^{-66}\ \cancel{J^2}\text{-}s^2}{(10)(10^{-23}\ kg)(10^{-4}\ m^2)(10^{-20}\ \cancel{J})}$$

$\cancel{J}$

$\approx$ spacing of terms in sum $\approx \frac{10^{-66}}{10^{-46}} \approx 10^{-20}$

Closely spaced terms in sum $\Rightarrow$ approximate sum as integral

$$q \approx \int_0^{\infty} e^{-n_x^2h^2/8ma^2kT}\, dn_x \int_0^{\infty} e^{-n_y^2h^2/8mb^2kT}\, dn_y \int_0^{\infty} e^{-n_z^2h^2/8mc^2kT}\, dn_z$$

abc

$$\approx \frac{V}{\Lambda^3} \qquad \Lambda = \sqrt{\frac{h^2}{2\pi mkT}}$$

$$\frac{1}{\Lambda^3} \equiv \text{quantum concentration}$$

$$\equiv \text{concentration of thermally populated states}$$

$$q = \frac{V}{\Lambda^3}$$

$\equiv$ number of thermally populated states

For $T \simeq 300\ K$

$m \simeq 2 \times 10^{-23}\ kg \simeq 12\ kDa$

$kT \simeq 5 \times 10^{-21}\ J$

$h^2 \simeq 4 \times 10^{-67}\ J^2\text{-}s^2$

$$\frac{1}{\Lambda^3} \simeq \left(\frac{2\pi m k T}{h^2}\right)^{3/2} \simeq \left(\frac{6\,(2\times10^{-23}\ \cancel{kg})(5\times10^{-21}\ \cancel{J})}{4\times10^{-67}\ \cancel{J}\ \cancel{kg}\text{-}m^2}\right)^{3/2}$$

$$\simeq \left(\frac{1.5\times10^{22}}{m^2}\right)^{3/2} \simeq \frac{2\times10^{33}}{m^3} \simeq 3\times10^{6}\ M$$

How does this compare to typical [solute]

For N particles,

$$Q = \frac{q^N}{N!} = \frac{\left(\frac{V}{\Lambda^3}\right)^N}{N!}$$

$$U = NkT^2\left(\frac{d\ln q}{dT}\right) = \frac{3NkT}{2}$$

$$\langle \varepsilon \rangle = \frac{U}{N} = \frac{3}{2}kT$$

$$S = k\ln Q + kT\frac{d\ln Q}{dT} =$$ Sackur-Tetrode Equation

$$Nk\left\{\ln\left(\frac{V}{\Lambda^3 N}\right) + \frac{5}{2}\right\} = Nk\left\{\ln\left(\frac{1}{\Lambda^3 c}\right) + \frac{5}{2}\right\}$$

$$c = \frac{N}{V}$$

$$F = U - TS = NkT\ln\left(\frac{N}{e\,\Lambda^{-3}V}\right)$$

$e \approx 2.7$ number

$\ln(e) = 1$

$$p = -\left(\frac{\partial F}{\partial V}\right)_{N,T} = \frac{NkT}{V} = ckT$$

$$\mu = \left(\frac{\partial F}{\partial N}\right)_{V,T} = kT\ln\left(\frac{c}{\Lambda^3}\right)$$

Consider a reference concentration c^0 (often but not always 1M)

$$\mu = kT \ln\left\{\left(\frac{c^0}{\Lambda^3}\right)\left(\frac{c}{c^0}\right)\right\}$$

$$= \underbrace{kT \ln\left(\frac{c^0}{\Lambda^3}\right)}_{\mu^0} + kT \ln\left(\frac{c}{c^0}\right)$$

$R = N_A k$

molar units

$$\mu^0 + RT \ln\left(\frac{c}{c_0}\right)$$

$$\Delta\mu = \mu(c_2) - \mu(c_1) = RT \ln\left(\frac{c_2}{c_1}\right)$$

The model is developed for non-interacting "ideal gas" particles

The model is often used for solutes in liquid solutions ⇒ under what conditions is this sensible and why is it sensible for these conditions?

Apply μ to understand chemical reactions + transport processes

$$aA + bB \rightarrow xX + yY$$

stoichiometric coefficients

reactant species

product species

Sometimes in chemical or transport processes [ ]'s are held constant as are T, p

a mole A + b mole B → x mole X + y mole Y

c_A, c_B, c_X, c_Y constant

$$\Delta G = x\mu_X + y\mu_Y - a\mu_A - b\mu_B$$

$$= x\{\mu_X^0 + RT\ln c_X\} + y\{\mu_Y^0 + RT\ln c_Y\} - a\{\mu_A^0 + RT\ln c_A\} - b\{\mu_B^0 + RT\ln c_B\}$$

$$= x\mu_X^0 + y\mu_Y^0 - a\mu_A^0 - b\mu_B^0 + RT\ln\frac{c_X^x c_Y^y}{c_A^a c_B^b}$$

ΔG^0 (reaction at standard [ ]'s)

reaction quotient Q (not partition function)

Equilibrium

Condition for $\Delta G = 0$ (no further reaction)

$$\Delta G^o = -RT \ln \boxed{\frac{C_X^{x} C_Y^{y}}{C_A^{a} C_B^{b}}}$$

$K_{eq} \equiv$ equilibrium constant

Sometimes reactions are allowed to proceed to equilibrium and [ ]'s of reactants and products are measured to determine K_{eq}. If dependence of K_{eq} on T is also measured

$\Delta G^o = \Delta H^o - T\Delta S^o$

$$\ln K_{eq} = \frac{1}{R}\left\{\Delta S^o - \frac{\Delta H^o}{T}\right\}$$

often a plot of $\ln K_{eq}$ vs. $\frac{1}{T}$ is linear

$\Rightarrow$ approximate ΔH^o is $-$slope $\times R$; ΔS^o is intercept $\times R$

Folded Protein ⇌ Unfolded Protein

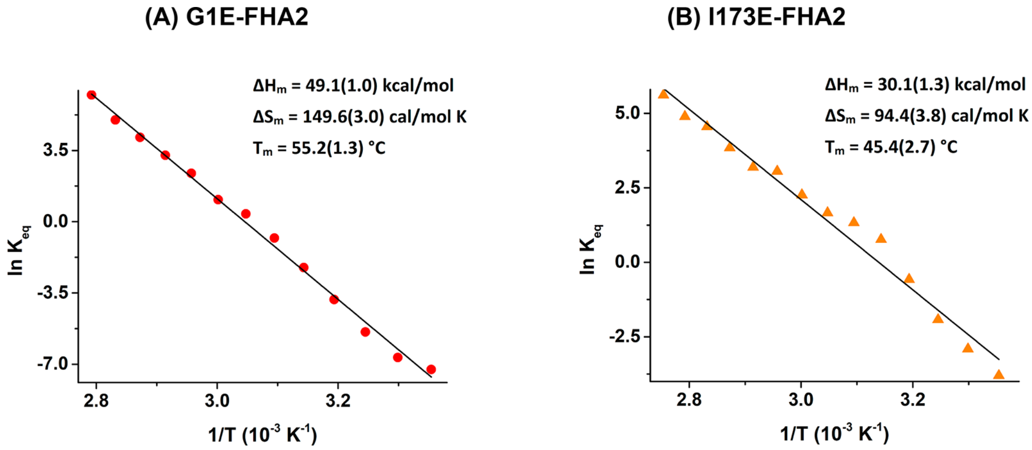


Figure 7. van't Hoff plots of the unfolding ln K_{eq} vs $1/T$ of the (A) G1E-FHA2 and (B) I173E-FHA2 proteins based on θ_{222} data for the temperature range around T_m. Best-fit parameters are given with uncertainties in parentheses.

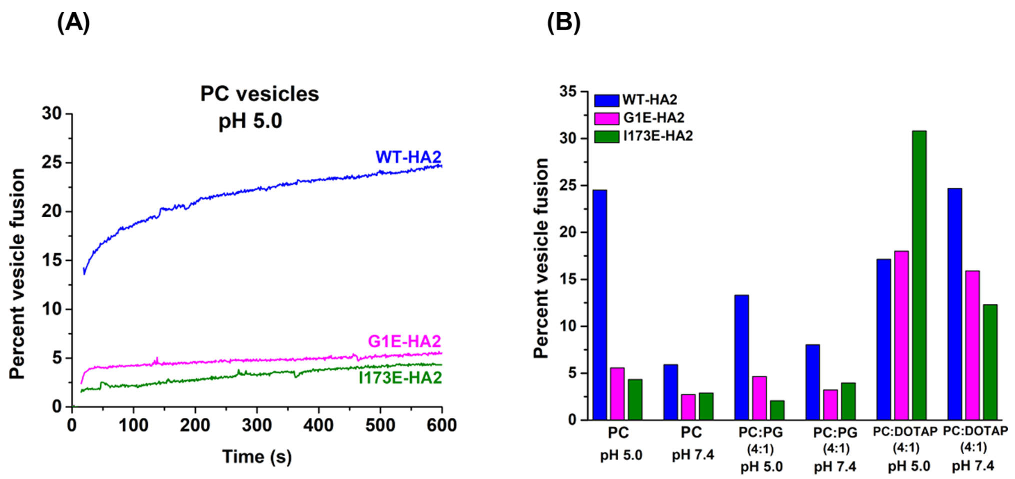


Figure 8. HA2-induced vesicle fusion with a 1:300 protein:lipid ratio. (A) Time courses of POPC vesicle fusion at pH 5.0. (B) Percent vesicle fusion at 600 s after addition of protein for different lipid compositions and pH's. Each bar represents the average of three replicates. There is typically a ±1% variation in percent vesicle fusion among the replicates. See Figure S7 for data using different preparations of proteins and vesicles.

energies at pH 5.0 vs pH 7.4. Detailed interpretations of the fusion extents are presented in the Discussion.

DISCUSSION

This study describes a structural and functional comparison between full-length WT-HA2 and the truncated construct lacking the TM, FHA2, and the G1E and I173E point mutants that are known to inhibit HA-mediated fusion. Significant findings of the present study include (1) a predominant trimer fraction in SRC detergent at pH 7.4 for all protein constructs versus mixtures of the trimer, monomer, and oligomers/aggregates in DM detergent, (2) similar helicities of WT and G1E proteins versus the reduced helicity of I173E proteins, (3) hyper-thermostable WT-FHA2 and HA2 and less stable FHA2 and HA2 mutants with respective reductions in T_m of ∼40 and ∼15 °C, and (4) efficient HA2-induced vesicle fusion of neutral and anionic vesicles at pH 5.0 for WT-HA2 versus a reduced level of fusion with mutants.

Models of Protein Structure and Stability. The CD spectra and analyses of Figure 4 and Table 1 support a 65% average helicity for WT- and G1E-FHA2 and a 58% average helicity for WT- and G1E-HA2. The proteins are well-folded, based on reasonable agreements between these average helicities and the fractions of α-helical residues calculated for a model in which the only α-helical residues are those in high-resolution structures of the FP in detergent and the SE in aqueous solution, and in the TM [residues 2−12, 14−22, 38−105, 110−128, 146−153, and 186−210 (Figure 1B)].[8,16] The calculated helical fractions from this model are 115/193 residues for FHA2 and 140/235 residues for HA2. The helicities of WT-FHA2 and HA2 determined in this study are similar to the helicities reported in some earlier studies.[26,31,41] However, the 65% helicity for WT- and G1E-FHA2 in this study is higher than the helicities of ∼25 and ∼35%, respectively, from a 2011 study.[28] The origin of this discrepancy is not known, but we note that our high and their low helicities correlate with the presence and absence, respectively, of detergent in the samples. Our study also shows that the I173E mutants exhibit only 45% helicities, and a model explaining this reduced helicity is presented below.

The hyperthermostabilities of WT-FHA2 and HA2 are evidenced by the CD spectra and θ_{222} versus T plots (Figures 5 and 6) and the accompanying van't Hoff analyses for determining T_m values (Table 1). The T_m of ≈90 °C for FHA2 and HA2 and the T_m values of ≈80 °C for $HA2_{20-185}$ (SE) and >85 °C for $HA2_{20-211}$ (SE+TM) support a major contribution to stability by the SE, with smaller contributions from the FP and TM, where the latter two HA2 domains are

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