Osmotic pressure and protein binding

Igor R. Kuznetsov, KochLab Symposium talk 5/15/09

Today we take a closer look at one of the solution thermodynamics key points from Steve's presentation. Here it is:

$$\frac{\mathrm{d}[ln(k_{\mathrm{off}})]}{\mathrm{d}[\mathrm{osmolal}]} = -\frac{\Delta N_w}{55.6}$$

the change in the protein-DNA unbinding rate with respect to the water activity (in terms of solute osmolal concentration) is proportional to the change in the number of the "excluding" water molecules during dissociation.
Some of us may see how this result comes about right away, but if you are like me, understanding it takes some time. What I am going to do today is show how we can arrive at this important formula, one baby step at a time.

Solution thermodynamics

First we will review the basics of the solution thermodynamics briefly, especially the quantities that are important for this talk: chemical potential μ, Gibbs free energy G, and osmotic pressure P_{osm}. Then we will look at how water activity changes with osmotic pressure. Finally, we'll arrive at the aforementioned dissociation – water activity dependence.

Let us start with conservation of energy. We postulate that the energy is conserved or, more specifically, that the internal energy of the system E is conserved unless heat is added (changing entropy), work is done (changing volume), or more matter is introduced into the system (changing the number of particles). Thus, we write $E = E(S, V, N_i)$. The change in E is given by chain rule as

$$\mathrm{d}E = \left(\frac{\partial E}{\partial S}\right)_{V,\{N_i\}} \mathrm{d}S + \left(\frac{\partial E}{\partial V}\right)_{S,\{N_i\}} \mathrm{d}V + \left(\frac{\partial E}{\partial N_i}\right)_{V,S} \mathrm{d}N_i \,.$$

We call various partial derivatives in the above equation: T, -P, μ:

$$T \equiv \left(\frac{\partial E}{\partial S}\right)_{V,\{N_i\}}, \quad -P \equiv \left(\frac{\partial E}{\partial V}\right)_{S,\{N_i\}}, \quad \mu \equiv \left(\frac{\partial E}{\partial N_i}\right)_{V,S}$$

in other words, the first law of thermodynamics becomes

$$\mathrm{d}E = T\mathrm{d}S - p\mathrm{d}V + \sum_i \mu_i \,\mathrm{d}N_i \,.$$

Since the state variables S, V, and N are all extensive, we can use Euler's homogeneous function theorem and write explicit definition of internal energy as

$$E = TS - pV + \sum_i \mu_i N_i \,.$$

In context of experimental observation, we are interested in things like: what reactions are going to take place; in what direction and to what extent; what will be the final equilibrium state. Examining the internal energy potential differential, we see that when all the state variables are kept constant, the change in E is zero. In other words, the thermodynamic potential "internal energy" has an absolute minimum at equilibrium: when S, V, and N_i of a closed system are held constant, the internal energy E decreases and reaches a minimum value. This, in theory, should determine the direction of all chemical reactions that take place under given conditions, as well as their extent. In practice however, we rarely deal with isentropic processes: for example, diluting salt in water is driven by entropy change.

The internal energy potential therefore is not very convenient when looking at chemical reactions that take place in a lab, where the constant parameters are pressure and temperature. Fortunately, we can use a more suitable thermodynamic potential, which has P and T as state variables. This potential is defined as

$$G = E + PV - TS \quad (= \sum_i \mu_i N_i) \,.$$

Taking a differential, we obtain

$$\mathrm{d}G = \mathrm{d}U + p\,\mathrm{d}V + V\mathrm{d}p - T\mathrm{d}S - S\mathrm{d}T \,,$$

$$\mathrm{d}G = T\mathrm{d}S - p\,\mathrm{d}V + \sum_i \mu_i\,\mathrm{d}N_i + p\,\mathrm{d}V + V\mathrm{d}p - T\mathrm{d}S - S\mathrm{d}T \,,$$

$$\mathrm{d}G = V\mathrm{d}p - S\mathrm{d}T + \sum_i \mu_i\,\mathrm{d}N_i \,,$$

along with a useful identity (Gibs-Duhem corollary)

$$\sum_i \mu_i\,\mathrm{d}N_i = V\mathrm{d}p - S\mathrm{d}T \,.$$

The Gibbs free energy G is minimized when a system reaches equilibrium at constant pressure and temperature. As such, it is a convenient criterion of spontaneity for processes with constant pressure and temperature.

Also, note that the chemical potential is now conveniently defined as a partial derivative of G with respect to N_i :

$$\mu \equiv \left(\frac{\partial G}{\partial N_i}\right)_{T,P} \,.$$

Transitional entropy of solution, free energy, chemical potential, osmotic pressure

Consider a binary solution - a mixture of 2 kinds of molecules. Assume that before mixing both substances had equal temperature and pressure. Then the

spontaneous process of mixing is driven purely by entropy: the only state variable that changes is entropy S, and so the change in G is going to come from increase in entropy of mixing alone (rearrangement of molecules). Before mixing, the free energy of constituents is given by

$$G^0 = (E + PV - TS)^0 = \mu^0 N_i^0 \,,$$

while after the mixing it is

$$G = \sum_i \mu_i^0 N_i^0 - T\Delta S \,.$$

To figure out the change in Gibbs free energy we need to know the change in S due to rearrangement. If we start from two substances (say water "w" and solute "s") perfectly separated, and end up with a perfectly mixed solution at equilibrium, the change will be given by Boltsman's entropy formula

$$S = k_B ln W \,,$$

where W is the number of ways N_w molecules of water and N_s molecules of solvent can be rearranged on a lattice of the size $N = N_w + N_s$. The number of such permutations, taking into account that N_w molecules and N_s molecules are identical among themselves, is given by

$$W = \frac{N!}{N_w! N_s!} \,.$$

Using Stirling's formula for large N ln $N! \approx NlnN + N - 1$, we obtain

$$\Delta S = -k_B \left(N_w ln(\frac{N_w}{N}) + N_s ln(\frac{N_s}{N}) \right) \,.$$

The free energy of a binary mixture is given by

$$G = G_0 - T\Delta S = \mu_w^0 N_w^0 + \mu_s^0 N_s^0 + k_B T \left(N_w ln(\frac{N_w}{N}) + N_s ln(\frac{N_s}{N}) \right) \,.$$

From here we calculate the chemical potentials of solvent by taking partial derivatives of G with respect to N_w and N_s:

$$\mu_s = \mu_s^0 + k_B T ln(x_s) \,,$$

$$\mu_w = \mu_w^0 + k_B T ln(1 - x_s) \,,$$

where x_s is a mole fraction of the solvent, and $x_s + x_w = 1$.

Let us examine these results. The equilibration of the chemical potential is the driving force behind the molecular diffusion, just like the temperature equilibration is the driving force behind the heat transfer. If we try to apply the above formulation to the case when two solutions of different solvent concentration are brought in contact across a semi-permeable membrane, we seem to be

“in trouble". Imagine two tanks, one with salt solution and the other with pure water, separated by a membrane that is only permeable to water (just like RBC membrane). Chemical potential of water in the solution is lower than that in pure water, so more water molecules are going to be transported in the direction of the lower μ than in the opposite direction. But no matter how long this continues, the solt concentration in solution is never going to go to zero, and as a result the entropic parts of the chemical potential on the two sides of the membrane will never balance. Is the osmoses going to drive all of the available pure water into the solution, desperately trying to equilibrate μ? Not really, because the enthalpic part of the chemical potential μ_0 depends on pressure:

$$\mu_w(T,P) = \mu_w^0(T,P) - P_{\rm osm} v_w = \mu_w^0(T,P_0) + P v_w - P_{\rm osm} v_w \,.$$

The hydrostatic pressure in the tank with water and solt rises to counter the osmosis across the semi-permiable membrane (the enthropic logarithmic term). Hence we can talk of the "Osmotic pressure", defining it as $P_{\rm osm} = (\mu - \mu_o)/v_w$.

Protein-DNA binding

In simple terms, a protein-DNA association reaction can be written as

$$P + D <-> PD \,.$$

The chemical potential is given by

$$\mu_i = \mu_i^0 + k_B T ln(x_i) \,.$$

At chemical equilibrium, we get

$$\mu_{\rm PD} = \mu_P + \mu_D \,,$$

so the association constant can be calculated as

$$K = \frac{k_{\rm off}}{k_{\rm on}} = \frac{[x_{\rm PD}]}{[x_P][x_D]} = exp\left(\frac{\mu_{\rm PD}^0 - \mu_P^0 - \mu_D^0}{kT}\right) = \exp\left(\frac{-\Delta G}{KT}\right) \,,$$

where ΔG is change in free energy going from PD to P and D.
Imagine we introduce an osmotic agent (say PEG) in the water. Following the argument of [2], we observe that the unbinding state differs from binding state in the amount of molecules of water that are excluded, i.e. the amount of molecules unable to solvate PEG. It is analogous to the case of the semi-permeable membrane: in order to unbind, the system PD has to draw additional water molecules against the chemical potential gradient (against the osmotic pressure). The additional work to overcome the osmotic pressure resistance is equal to the difference in chemical potentials multiplied by the number of additional water molecules. This work can also be expressed as osmotic stress multiplied by the volume of additional water molecules that need to be drawn.

The additional work requirement will make the dissociation events less frequent. In other words, the association constant depends on osmotic stress

$$K = K(P_{\rm osm})\,.$$

Since $\Delta G = -k_B T ln K$ (from above), and G has an additional osmotic pressure contribution $P_{\rm osm}\Delta V_w$, we have

$$\frac{{\rm d}(lnK)}{{\rm d}P_{\rm osm}} = -\frac{\Delta V_w}{k_B T}\,.$$

Osmotic pressure is usually measured in osmolal units, so that

$$P_{\rm osm} = (k_B T/\nu_w)({\rm osmolality}/55.6)\,,$$

where ν_w is the molecular volume of water, and 55.6 is the molarity of pure water. Taken together, these numbers give an osmotic pressure of 24 atm per osmolal at 20 °. We can rewrite the above expression as

$$\frac{{\rm d}(lnK)}{{\rm d[osmolarity]}} = -\frac{\Delta N_w}{55.6}\,.$$

I don't see how we can expand Parsegian's derivation to case when the dissociation reaction is not at equilibrium. In order to arrive at the result from [1] that Steve used, we will need to resort to the phenomenological Arrhenius type reaction rate for protein unbinding (this is from talking to Evan). Thus we would postulate that the off-rate is given by

$$k_{\rm off} = k_0 exp\left(\frac{-\Delta G}{kT}\right)\,,$$

where ΔG is the potential energy barrier that needs to be overcome in order for the protein to unbind. If we were to pull on the molecule (say with optical tweezers), the potential energy barrier height ΔG would reduce, and the reaction off-rate would increase accordingly. The osmotic stress on the other hand leads to the increase of the barrier height commensurate with the product of the additional excluding water volume and the osmotic pressure. Differentiating the Arrhenius law above, we obtain the desired

$$\frac{{\rm d}[ln(k_{\rm off})]}{{\rm d[osmolal]}} = -\frac{\Delta N_w}{55.6}\,.$$

References

[1] Sidorova, N.Y. and D. C. Rau, Biopolymers, **53(5)**, 363–368 (2000)

[2] Parsegian,V.A., Rand, P.R. and D.C. Rau, Meth. Enz., **259**, 43-94 (1995)