

The Kinetic Method: Silver Ion Affinities of Amino Acids

Purpose: Determine the relative experimental and molecular mechanics calculated gas phase Ag^+ affinity for tyrosine and glutamine.

Prelab:

Determine the masses of the Ag^+ bound dimer of tyrosine and glutamine as well as the masses of the corresponding Ag^+ bound amino acids. Look up the isotope pattern for Ag (<http://www.webelements.com/> or <http://www.colby.edu/chemistry/NMR/IsoClus.html>).

Introduction

Carboxylate anions associate with metal ions in the gas phase and in solution.¹ Understanding these ion-ion interactions is important for understanding charge-charge type molecular recognition mechanisms. In the gas phase anions interact directly with the metal ion. In solution, the solvent plays a very important role in determining the strength of these charge-charge interactions, both in terms of the enthalpy and the entropy of the ion binding. Often in unraveling solvent influences in molecular recognition, it is useful to compare the strengths of the interaction in solution with the gas phase interactions. In this experiment we will determine the gas phase relative binding affinity of the two amino acids tyrosine and glutamine for Ag^+ ions, Figure 1.

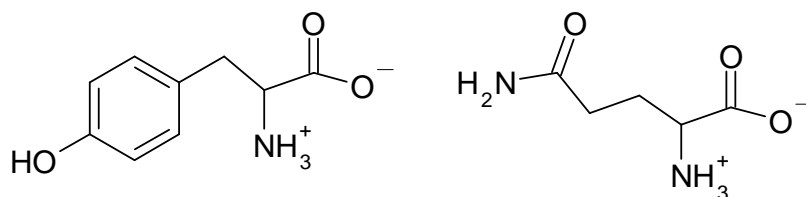


Figure 1. Tyrosine (left) and Glutamine (right).

In solution, amino acids can associate with alkali, alkaline earth, and quaternary ammonium ions. Such interactions are called ion-pairing. In this respect it might seem strange to use Ag^+ for this experiment. However, Li^+ , Na^+ , and Ag^+ affinities are often found to parallel each other.² Ag^+ ion affinities are, however, stronger and easier to study.¹ When the Ag^+ affinity trends do differ from the alkali and alkaline earth metals, then specific d-orbital interactions may be involved. Orbital interactions are interesting when considering metal ion interactions in metallo-enzymes (a majority of enzymes are metallo-enzymes). In addition, Ag^+ ions are fairly commonly used to generate ions for electrospray ionization of proteins. So Ag^+ ion adducts play an important analytical role.

Competitive Ag^+ affinities

In this experiment we will determine the relative Ag^+ affinity for tyrosine and glutamine. We will form the Ag^+ bound dimer in the gas phase and then determine the branching ratio for the formation of Ag^+ bound monomers. The goal is to determine the difference in binding enthalpy for the two amino acids, Figure 2. The advantage of determining relative binding affinities is that any experimental offsets or errors will cancel out giving more accurate values than if the reactions were followed separately.

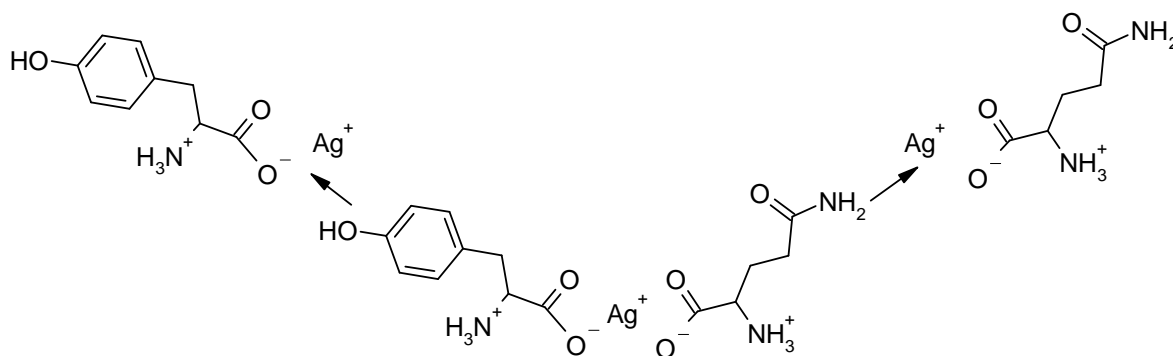


Figure 2. Relative Ag^+ affinities from a competitive reaction.

The Kinetic Method

One of the most embarrassing moments in a chemist's life happens if you are caught using kinetic arguments to determine a thermodynamic parameter (say at a national ACS meeting). Doing so brings immediate rebuke and consternation. Repeatedly in multiple courses you are taught that kinetics and thermodynamics are really separate considerations. For example, fast reactions can be thermodynamically unfavorable (e.g. weak acid dissociation). Slow reactions can be very thermodynamically favorable (the reaction of hydrogen and oxygen without a catalyst). In fact the only point where kinetics and thermodynamics interrelate is that the equilibrium constant for a chemical reaction is the ratio of the forward and reverse rate constants. Kinetics and thermodynamic arguments just can't be mixed.

But what happens if the thermodynamic parameters that you need to measure are not accessible by available methods, but you can make kinetics measurements? When can kinetic measurements be used to determine thermodynamic energies? Essentially never. However, the Kinetic Method is a long-standing and widely used technique that does just this: the method uses kinetic measurements to infer thermodynamic energies. The Kinetic Method is widely used in mass spectrometry for the determination of relative energies and enthalpies of binding. In our case the kinetic method will be used to determine the relative enthalpy for the unimolecular decomposition of the Ag^+ bound dimer for two amino acids.

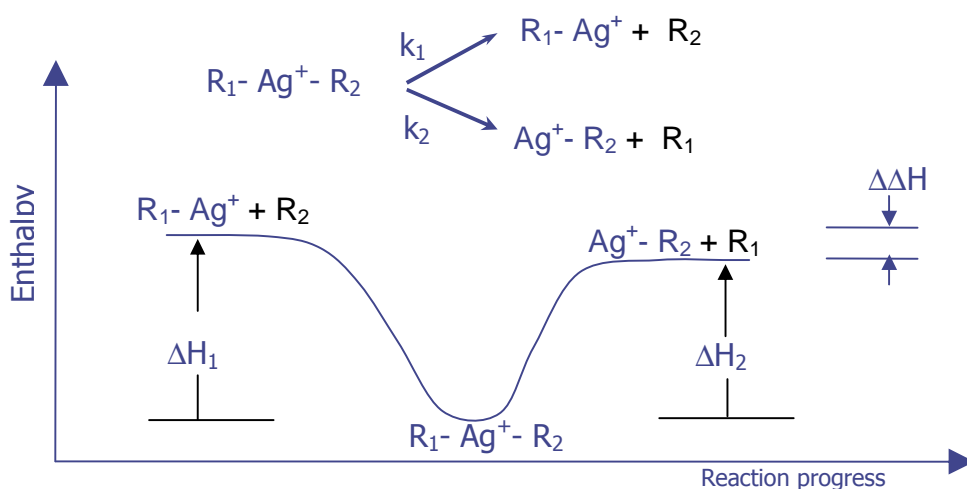


Figure 3. The kinetic method for determining thermodynamic parameters from competitive kinetics measurements.

The branching ratio is given by the intensity of the mass spectral peaks for the two products, I_1 and I_2 , in the collisionally induced dissociation of the Ag^+ bound dimer. The branching ratio is the ratio of the rate constants for the competitive dissociation, Figure 3:

$$\text{Branching ratio} = \ln I_2/I_1 = \ln k_2/k_1 \quad (1)$$

Assume the reactions follow the Arrhenius rate law:

$$k = A e^{-E_a/RT} \quad \text{or} \quad \ln k = \ln A - E_a/RT \quad (2)$$

where E_a is the activation energy and A is the pre-exponential factor. The branching ratio for the two reactions gives

$$\ln I_2/I_1 = \ln k_2/k_1 = \ln A_2/A_1 - (E_{a2} - E_{a1})/RT \quad (3)$$

If we assume that the pre-exponential factors are the same for both reactions the first term will be zero. Then we assume the enthalpies for the reactions are equal to the activation energies, $\Delta H_1 = E_{a1}$, $\Delta H_2 = E_{a2}$ and then the relative enthalpy for the two reactions is defined as $\Delta\Delta H = (\Delta H_1 - \Delta H_2) = (E_{a2} - E_{a1})$, as shown in Figure 3. Substituting into equation 3 gives

$$\ln I_2/I_1 = \ln k_2/k_1 = -\Delta\Delta H/RT \quad (4)$$

In our case, $\Delta\Delta H$ is the difference in Ag^+ binding affinity for the two amino acids. Then since $\Delta H = \Delta U + \Delta PV$ and assuming each product is an ideal gas with $\Delta PV = \Delta nRT$. However, $\Delta n = 0$ since both products are just a single gas phase species, giving $\Delta H = \Delta U$. The relative binding affinity is very easily calculated just from the product peak intensities in a single five-minute experiment.

The important assumptions are, however, that the pre-exponential factors are equal and that $\Delta U = E_{a2} - E_{a1}$. Using absolute reaction rate theory, the pre-exponential factor of a reaction is related to the entropy of activation, ΔS^{\ddagger} , by:

$$A = \frac{kT}{h} \left(\frac{eRT}{P^\ddagger} \right) e^{\Delta S^{\ddagger}/R} \quad (5)$$

The assumption that the pre-exponential factors are equal corresponds to both reactions having the same entropy of activation.² This requirement is difficult to verify without a complete kinetics study, which is difficult in the gas phase. This equal entropy of activation requirement is only met if the normal vibrational mode of the bound dimer that corresponds to the reaction coordinate leads to either of the reaction products. Another way of saying this is that both activated complexes need to have similar vibrational normal modes. In other words, the reaction progress to both products has to be very similar and not involve any significant rearrangements. This requirement is rarely met. The assumption that $\Delta U = E_{a2} - E_{a1}$ requires that both reactions have essentially zero activation energy for the back reactions so that the activation energy is essentially equal to the internal energy for the reaction.² This requirement is reflected in the diagram in Figure 3 (no maximum in either of the product channels). Even though this requirement sounds unlikely, in the gas phase this lack of backwards activation energy is probably not that uncommon, but it is difficult to verify.

When can kinetic measurements be used to determine thermodynamic energies? Rigorously, probably never.² But, the Kinetic Method is very commonly used for cases where the information is not available in any other way. However, by doing this laboratory, you will hopefully become more comfortable with the differences between kinetic and thermodynamic measurements and have a deeper understanding of why the two aspects of reactivity are so disjoint. The results are also helpful in understanding ion-pairing interactions, when the gas phase results are compared to solution measurements.

Collisionally Induced Dissociation (CID)

The Kinetic Method is designed for use with unimolecular dissociation. The Ag^+ bound dimer is stable in the gas phase and can be isolated in a mass analyzer called an ion trap. Think of the ion trap as a bottle for gas phase ions. The energy for the unimolecular dissociation is provided by increasing the kinetic energy of the ions in the trap by applying a radio-frequency field. The trap has a background buffer gas of helium at a low pressure. The increased kinetic energy of the ions causes more frequent and more energetic collisions with helium atoms that provide the energy to dissociate the dimer.

Procedure

Prepare a solution of 1×10^{-4} M tyrosine, 1×10^{-4} M glutamine, and 1×10^{-4} M AgNO_3 in 20% methanol/80% water. Use only HPLC reagent grade methanol and Reagent grade water (Milli-Q). Use the instructions for the Direct Infusion Mode for the Agilent Ion Trap SL (on the departmental Instrumentation page) to determine the mass spectrum of the mixture. Set the syringe pump to 20 $\mu\text{L}/\text{min}$. Use a capillary voltage of 4 kV and a Compound Stability of 10-20% to avoid dissociating the Ag bound dimer. Isolate and fragment the Ag^+ dimer and determine the ion intensities and branching ratio (see instructions below). Use replicate measurements to determine the uncertainty of your results. Use two different collision energies (Ampl values) in the range of 0.2 to 0.5 and compare the results. Glutamine readily loses H_2O , so two peaks will appear for Ag^+ bound glutamine. Add the intensities of the two glutamine peaks to determine the branching ratio.

MS/MS Instructions

1. To do CID and MS/MS, start by clicking on the MS(n) tab in the control section.
2. Click on the "mouse maximum cursor" icon in the top icon bar. This icon shows a MS peak with a red dot above the peak and a black arrow. Click on the peak that you want to fragment. A small, white, vertical arrow should appear on the chosen peak.
3. Click right just to the right of the chosen peak and choose Isolate/Fragment.
4. In the MS(n) window, the mass of the chosen peak should be listed in the first "Isolation mass" dialog box. The strength of the CID is determined by the value in the Ampl dialog box at the right hand side of this same line. Ampl is short for the amplitude of the added radiofrequency. Change the Ampl value, typically in the range of 0.2-0.8 to obtain the desired level of fragmentation. A typical setting would be large enough to decrease the isolated mass peak to about 20% of its starting value, but not so large as to completely remove the isolated mass peak. The CID collision energy necessary to break up a non-covalently bound complex is significantly less than normally required to produce fragment ions in conventional CID applications. So the Ampl value you choose will be in the lower part of the 0.2-0.8 range.
5. Click on the All Off button when finished with MS/MS.

Calculations and Report

Use the branching ratio to calculate the difference in Ag^+ binding affinities for tyrosine and glutamine. We don't know the effective temperature in the source, so report your results as $\Delta\Delta H/RT$. In similar literature based experiments the effective source temperature is often about 900 K. Calculate $\Delta\Delta H$ assuming a 900K source temperature. Use replicate trials to determine the uncertainty in your final results.

Use MOE and the CHARMM2.2 molecular force field for gas phase calculations of the binding energies for Na^+ and the two amino acids. Make sure to choose the force-field options for gas phase calculations. Do two sets of calculations one with the force-field charges and a second set with charges using the Gasteiger (PEOE) method (you might want to read the section in the Molecular Mechanics Introduction on Partial Atomic Charges). Compare your molecular mechanics calculated results with the experimental values and the literature values for the Ag^+ ion affinities.¹ (As a quick check on your molecular mechanics conformations, the Na^+ to oxygen distance should be near 2.6 Å for both oxygens in the carboxyl group.)

Review your earlier exercises in molecular mechanics to refresh your memory on how to interpret molecular mechanics calculations. Use your molecular mechanics calculations to determine why the one amino acid binds more strongly than the other. Focus on the forces involved. What is the chemical significance of this experiment?

Follow up: Ion Association in Solution

The interaction of a cation with an anion in aqueous solution is called ion-pairing, Figure 4. In outer-sphere ion-pairing the ions share their secondary solvation spheres. In solvent-separated ion-pairing a single layer of solvent molecules separates the two ions. In other words, the ions share their primary solvation spheres. Alkali-metal carboxylate interactions are an example of solvent-separated ion-pairing. Stronger ionic interactions result in contact ion-pairing, where no solvent separates the ion-pair. The gas-phase ion-pairs in this exercise most closely resemble contact ion-pairing. The strength of ion-pairing is primarily dependent on the charge to size ratio of the ions and not on any specific chemical (i.e. orbital) interactions.

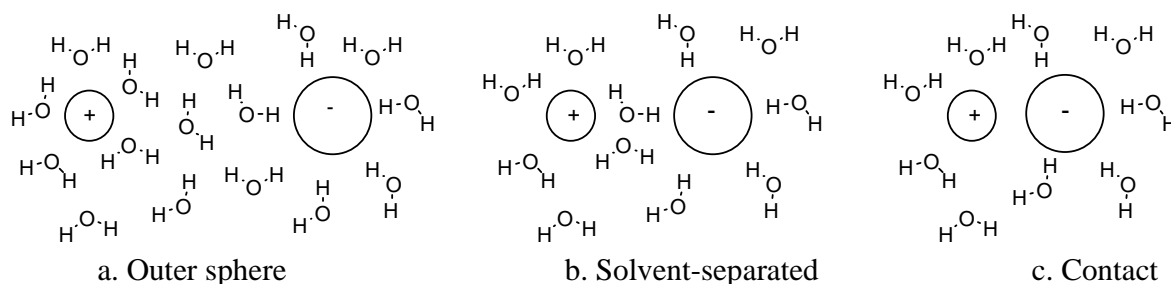
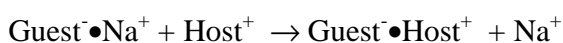


Figure 4. Ion-pairing interactions. Solvent-separated ion-pairs have shared primary solvation spheres.

On the other hand, the interaction of transition metals with carboxylates is usually strong and is best described as traditional Lewis acid-base metal complexation. This type of "ion-pairing" is an especially strong example of contact ion pairing. Ion pairing therefore takes on a range of

interaction energies, with outer-sphere ion-pairing the weakest, contact ion-pairing intermediate in strength, and Lewis acid-base complexation the strongest. The strongest Lewis acid-base interactions are mediated through specific metal d-orbital interactions with the carboxylate acting as a ligand. In the gas phase, ion-ion interactions are necessarily of the contact type, but the ion affinities can be used as an intrinsic measure of the ability of an anion to interact with cations through Coulombic forces.

Solvent-separated ion-pairing can play a role in molecular recognition. The effect on molecular recognition depends on whether the ion-pairing occurs in the free guest or host or in the guest-host complex. Ion-pairing in the guest-host complex can enhance guest-host binding. On the other hand, if either the free guest or host experiences ion-pairing, then ion-pairing can compete with guest-host binding. For example, if the free guest is ion-paired and the ion-paired counter ion is displaced upon binding, then ion-pairing decreases the guest-host affinity:



The relevance of this experiment depends on your point of view. Organic and physical chemists interested in molecular recognition want the trends in Ag^+ ion affinities to parallel the affinities for Li^+ and Na^+ . Then the relative binding affinities can be used to look for the intrinsic ability of different ions to interact through Coulomb interactions. Inorganic chemists and mass spectroscopists want Ag^+ ion affinities to be strikingly different from the alkali and alkaline earth metals so that relative binding affinities can be used to understand specific strong Lewis acid-base orbital based interactions.

Literature Cited:

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3. P. B. Armentrout, "Is the Kinetic Method a Thermodynamic Method," *J. Mass Spect.*, **1999**, *34*, 74-78.