

The Kinetic Method: Silver Ion Affinities of Amino Acids

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

Prelab:

Determine the masses of the Ag^+ bound dimer of tyrosine and glutamine, the Ag^+ bound dimer of tyrosine and asparagine, as well as the masses of the corresponding Ag^+ bound single amino acids. Look up the isotope pattern for Ag (<http://www.webelements.com/> or <http://www.colby.edu/chemistry/NMR/IsoClus.html>). Get the instructions for the Direct Infusion Mode for the Agilent Ion Trap SL (on the departmental Instrumentation page).

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 three amino acids tyrosine, glutamine, and asparagine for Ag^+ ions, Figure 1.

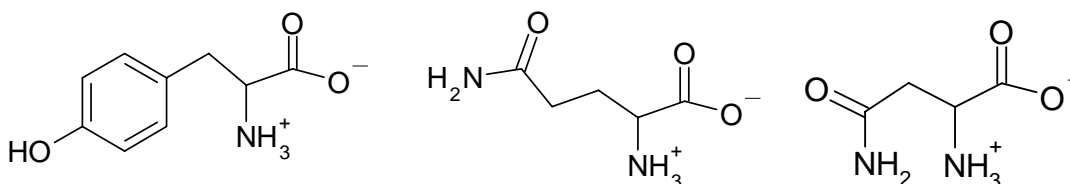


Figure 1. Tyrosine (left), glutamine (center), and asparagine (right).

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

First, we will determine the relative Ag^+ affinity for tyrosine, tyr, and glutamine, gln. 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.

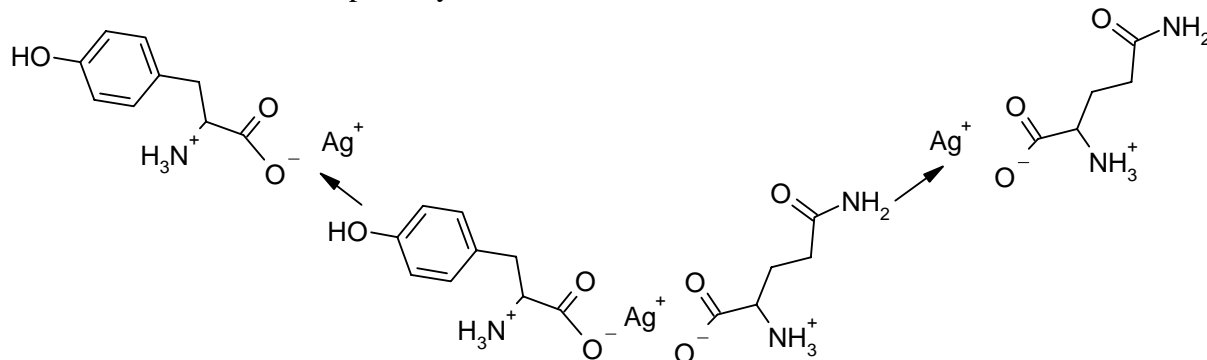


Figure 2. Relative Ag^+ affinities from a competitive reaction.

The second determination will be for the relative Ag^+ affinity for tyrosine, tyr, and asparagine, asn. From the tyr-gln and tyr-asn pairs the relative affinity of the three together can be determined. For example if the affinities are in the order tyr<gln and asn<tyr, then the overall order is asn<tyr<gln.

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.

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.

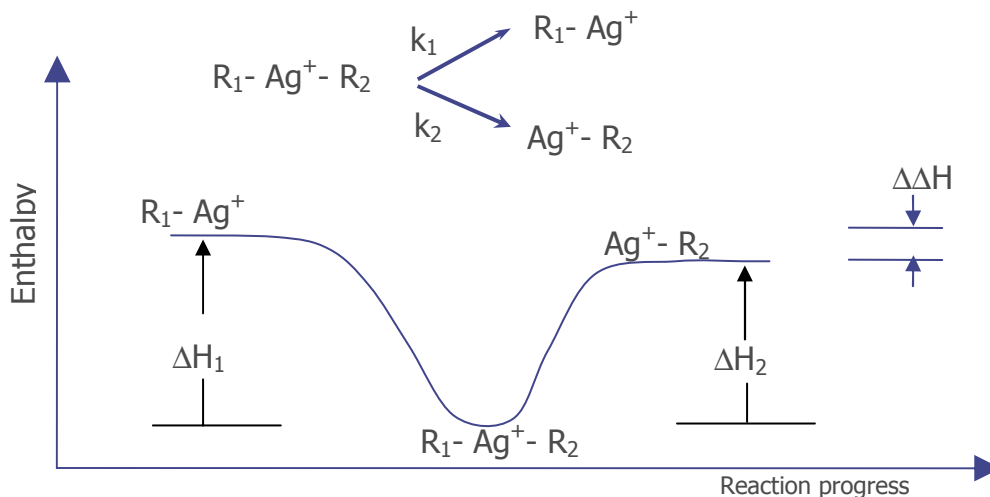


Figure 3. The kinetic method for determining thermodynamic parameters from competitive kinetics measurements.

The branching ratio for the two reactions gives

$$\ln I_2/I_1 = \ln k_2/k_1 = \ln A_2/A_1 - (Ea_2 - Ea_1)/RT \quad (3)$$

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, $\Delta H_1 = Ea_1$, $\Delta H_2 = Ea_2$ and then the relative enthalpy for the two reactions is defined as $\Delta\Delta H = (\Delta H_1 - \Delta H_2) = (Ea_2 - Ea_1)$, 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 $\Delta U = Ea_2 - Ea_1$. Using absolute reaction rate theory, the pre-exponential factor of a reaction is related to the entropy of activation, ΔS^{\ddagger} , by:

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

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 filtered 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.

Then repeat your measurements at the same concentrations for tyrosine and asparagine.

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

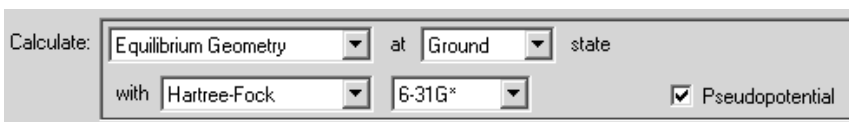
radiofrequency. Change the Ampl value, typically in the range of 0.2-0.8 to obtain the desired level of fragmentation. A typical setting would be large enough to decrease the isolated mass peak to about 20% of its starting value, but not so large as to completely remove the isolated mass peak. The CID 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.

Molecular Orbital Calculations

Use Spartan at the HF/6-31G* level for calculations of the relative binding energies for Ag⁺ and the three amino acids. Use effective core potentials for the Ag atom. Instructions for these calculations follow.

The wavefunctions for silver are not available at the semi-empirical level. In fact, it might be argued that the semi-empirical level is not well suited to calculations with heavy transition or post-transition metals. Hartree-Fock or correlated methods are necessary. For this lab exercise, Hartree-Fock (HF) will be time consuming enough; ideally however, B3LYP is a much better match for metal complexes. To do calculations with silver in Spartan, 6-31G* or higher is necessary. HF calculations are normally all-electron methods. That is, the 1s,2s,2p, and other core levels are treated explicitly. However, for heavy elements like silver, all-electron methods require many orbitals. For these calculations we will use effective core potentials to approximate the inner core electrons and then only the valence orbitals will be treated explicitly. In Spartan you specify the use of effective core potentials by selecting the pseudopotential check box:



To save time in these calculations we need to be strategic in doing geometry optimizations. It is much more efficient to do several step-wise minimizations at increasingly more sophisticated levels, rather than simply building the molecule and minimizing at the final desired level. In particular, we normally minimize first with molecular mechanics, then PM3, then HF/3-21G(*), and then at the desired final level (HF/6-31G* in this case). In addition, we need to be careful for the silver complexes, since you can't do silver at PM3 or 3-21G. In this case, for example start by using the builder's peptide mode and choosing one of your amino acids:

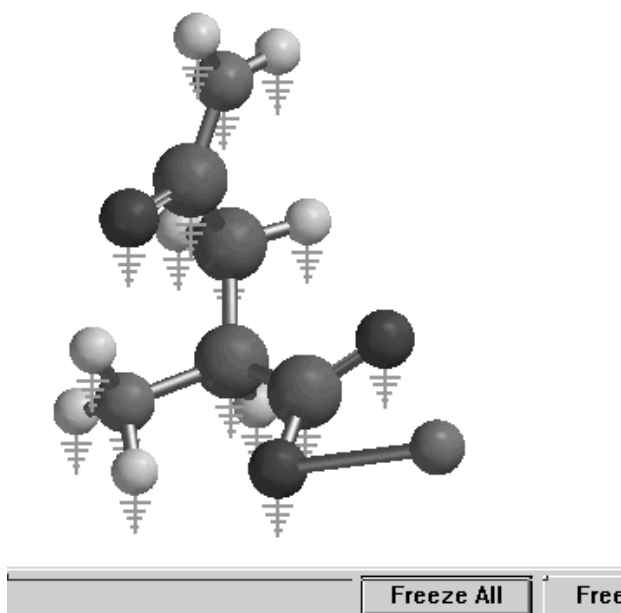


Terminate the amino terminus as NH_4^+ and the carboxy terminus in the acid form, $-\text{COOH}$. Do the molecular mechanics minimization by clicking on the



button. Then minimize successively at the PM3, HF/3-21G(*), and then finally at the HF/6-31G* level. Now enter the expert portion of the builder and substitute an Ag for the carboxyl hydrogen. You can also, if you like, use the Geometry menu and Measure Distance option to set the O-Ag bond length to about 2.45Å.

Another time saving technique is to minimize just the part of the molecule that you have changed first and then minimize the complete molecule. The Freeze Atoms option is used to fix the position of some of the atoms in your molecule while allowing others to move. Pull down the Geometry menu and choose Freeze Center. New buttons will appear at the bottom of the screen, see the figure below. Click on the Freeze All button. Purple flags will appear on all the atoms indicating that they are frozen. Then click on the Ag atom. The purple flag should be removed indicating that the Ag atom is free to move:



Then when you set up the equilibrium geometry run, check the Frozen Atoms check box:

Calculate: Equilibrium Geometry at Ground state
with Hartree-Fock 6-31G* Pseudopotential

Start from: Initial geometry.

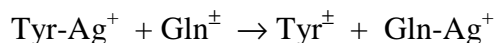
Subject to: Constraints Frozen Atoms Symmetry Total Charge: Cation

Remember to finish with an unconstrained minimization to make sure the atoms in the amino acid can adjust to the presence of the metal ion. In other words, set up a geometry optimization with the frozen atoms checkbox cleared.

One final note is that the structure has a “bond” showing between the O and Ag atoms. This bond isn’t really a bond; it just appears as a convenience for building the structure. Remember

that in the molecular orbital program input file, no information about bond connectivities is included, only the x, y, z positions of the atoms are given.

Energetics comparisons are best done as differences when using molecular orbital methods. For example, for your calculations to compare the relative stability of the gln and tyr Ag complexes, calculate the difference in energy for the reaction:



And similarly for the tyr and asn complexes. By doing the comparison this way, any errors in the calculations will have a chance to cancel out.

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 experiments in the literature 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. Repeat your calculations for tyrosine and asparagine. Rank tyrosine, asparagine, and glutamine in order of increasing binding affinity for Ag^+ .

Compare your molecular orbital calculated results with the experimental values and the literature values for the Ag^+ ion affinities.¹ 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.

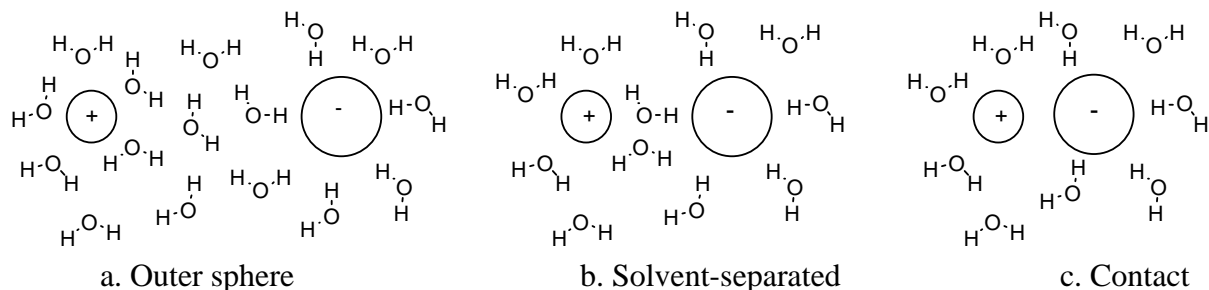
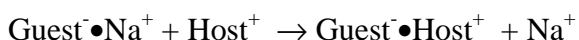


Figure 4. Ion-pairing interactions. Solvent-separated ion-pairs have shared primary solvation spheres.

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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