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Ion-Ion Dual-Electrospray Chemistry

Tyler Schriber

Dr. Franco Basile

Final Honor's Research Paper

Literature Review and Background

The Electrospray Ionization Mass Spectrometry (ESI-MS) technique has been used for years, and it is centered on the ability to spray ionized droplets into a mass spectrometer, while under specific environmental conditions. There are many different techniques that are used in order to properly get a mass spectrometry reading, due to the hardships of getting the proper ions through to the chamber. The charged ions are those that are meant to make it into the final chamber, and this requires for the neutral particles to be caught before entering the mass spectrometer, either by other molecules, or by electrospray positioning.¹ The different possibilities may be necessary in order to try to recreate the results of the experiment, though they are not completely stated within the paper with which our research is based upon.

The ionized droplets, that are sprayed, enter into a heated capillary, where their state is capable of being changed into a gas. The formation, into the gaseous phase, requires the pressures from the repulsive Coulombic forces between the ions, within the droplets, to overpower the surface tension forces of the droplet itself. This finally causes the droplet's surface to separate, which is called Coulombic fission. This is repeated until the point when the ions are no longer in liquid phase, but the gaseous phase.² This is when they are most easily capable of being separated, and analyzed, using the mass spectrometer.

The mass spectrometry performed was done with a Quadrupole Ion Trap. This uses a large burst of electrons, which is increased quickly to shoot in ions that are formed from the electrons. An AC voltage is applied to end-caps on opposite sides of the gate, and through all of this the final ions, that are to be measured, are expelled from the spectrometer in order of mass-to-charge ration, which is common for mass spectrometers.³ Within this process, there was also Collision-induced Dissociation (CID), where the ions are acted upon by a higher potential, at the

same time as the excitation voltage, which causes collisions with buffer gas atoms to enhance the kinetic energy. This is used to separate the ions to make it most possible for a clear fragmentation of the ions.³ This is often coupled with tandem mass spectrometry, where multiple mass differentiations are done one after another. This is seen to substantially increase the fragmented ions that are being amassed within the ion trap.³ With the use of all of these different tools, one should be able to differentiate amino acid sequences to a pretty high specificity.

Single spray electrospray ionization mass spectrometry is used often in order to sequence polypeptides, and thus amino acids. This sequencing is done after chemically digesting polypeptides into smaller peptide sizes that can then be sequenced, using a similar collision-induced dissociation as stated previously. B. S. Gnanesh Kumar, and his team, used chymotrypsin digestion, for the digestion of Jack bean α -mannosidase, which they then sequenced the remaining pieces of, using the electrospray ionization mass spectrometry and CID. The fragmentation from this process allowed for them to see clear amino acid sequencing, but also intrapeptide disulfide bonding within the protein. They used multiple different fragmentations in order to properly get the entire sequence, because there has not yet been a procedure capable of doing large polypeptides the size of entire proteins, even those quite small.⁴ This type of study of protein structure and function is known as proteomics, and is the basis for the entire study.

The final important part of the process is the use of the dual spray technique. This technique was first introduced in the mid 2000s, and is now used for many analytical purposes within the scientific field. Rui Wang used a dual electrospray configuration, but with a extractive electrospray ionization. The process is slightly different, but the reasoning is very similar. The dual spray allows for two different solutions to be charged, and then sprayed into each other,

allowing the separate charged droplets to react. The unknown seems to be what proper charges, and droplet amounts, are necessary for the greatest coalescence between the separate sprays. With a better coalescence between the droplets of the two sprays, the two sets of ions was be more likely to react with each other to give the complex that is wanted.⁵ Our entire research falls on the necessity of this dual spray setup to work properly, making as much of the complex with our peptide as possible, and still being capable of separating the amino acid sequence of the peptide that was introduced.

Where most of the studies done have been using a single electrospray, the work the was done relied on a front-end dual spray system, similar to above, where both of the sprays were coming from different angles, with respect to the capillary entry to the mass spectrometer, as well as with different charges, one of each positive and negative charge. We employed that same chemical structures stated above, most importantly the microdroplet chemistry, and gas phase ion/ion chemistry. Victoria Cotham, and her group, used this technique in order to properly form a bioconjugation of a peptide and chromogenic anions. This allows for the formation of ion complexes to occur directly before the CID, and mass spectrometry occur. The charge on the final peptide complex is still positive, due to a greater charge on the original peptide, and this allows for the ion trap mass spectrometry to still occur properly. It is important to get the correct amount of energy, in order to overcome the requirements for a nucleophilic attack between the N-terminus of the peptide and the 4-formyl-1,3-benzenedisulfonic acid (FBDSA) that were used. These reacted together directly as they enter the heated cylinder, and should be completely reacted as they move into the mass spectrometry chamber.⁶

If we are able to recreate this work we should also be able to expand from it. We used a slightly different technique than the ultraviolet dissociation that was previously used⁶, and

instead use the microwave technology that Dr. Basile has within his laboratory setting. We also may be using different peptides and anions than the peptide and FBDSA⁶. The actual substances used seem to be less important than the ability to have a nucleophilic attack on the anions from the peptide's N-terminus, however. While this earlier work has only been able to sequence small numbers of peptides, using our resources, we should be able to sequence amino acids that are of a much greater length. With our LC machine, and our microwave system, we should also be able to do these sequencings at a more rapid pace. The work done by the Cotham group is a good starting point, which I hope that we can show great advancement from by the end of our research.

Building the Board

From the beginning, this research required a lot of building and designing to try to recreate the original experiment. The only thing that we had was the skeleton of our platform. It was the bare minimum. Based on the Cotham article we made the greatest angle possible between a centered sprayer and the side sprayer. We used a combination of plastic and metal parts in order for conduction of electricity and insulation of the capillary. The capillaries had to be run into metal fittings, in order for the solutions to come in contact with the charged metal, and gain that charge. On the other side of the fittings the capillaries were made into synthetic sprayer tips. The tips could only be a small distance from the inlet of the instrument, so finding the proper distance to place the fitting was important. The distance, as well as the angle, was necessary to figure out for the side sprayer. The LCQ instrument has a positive charge source, which was attached to a wire through a converter that was already a part of our board. For the negative charge there was no previous mechanism on the board, and we used an external source for that charge. A converter for this was machined in the University of Wyoming Physical

Sciences Machine Shop. Once the converters were placed onto the boards, the wires were run to the fittings and the building seemed to be complete. Seemed is the important term, because we were far from ready.

Tinkering

There are far more holes in the platform than are being used. This is because of the things that I talked about in the previous section. The original places that we assumed would allow for analysis did not work. We ended up having to move the sprayers closer to the inlet in order to increase the precision of the sprayer. The movement of the capillary tips was enough at the increased distance for the solutions to miss the inlet at times. Once they were moved closer, the precision of the analysis increased. The closeness of these would cause an issue later, but much of the initial instrumental analysis was done with this set-up.

Making Solutions

Each sprayer's solution had to be made multiple times throughout the research, because they had their own expirations. Each solution was made the same way every time, in order for validity. The peptide was made in five-milliliter solutions of one-to-one water to acetonitrile, as well as one tenth percent Formic Acid. The peptide used was Angiotensin II, which has a formula weight of one thousand forty-six and a half grams per mol. Five milligrams were used in each solution. The other solution, of FBDSA, was made to be ten milliliters. This solution was made at a one-to-one ratio of water to methanol. Three milligrams of the molecular weight two hundred sixty-six grams per mole FBDSA were used in these solutions.

Initial Tuning

Before any actual experimentation can be done, a tuning file must be made. The initial tuning file for each of the sprayers is actually when we realized that the sprayers needed to be

moved. A tuning file is necessary in order to optimize any data that is recorded afterwards. The first tuning files were run for the straight on sprayer, using the peptide solution. When these came out good, the side sprayer was tuned. This was also done with the peptide solution. These tuning files were run to make sure the sprayers were working properly, initially, but also to set up the software for the experimental chemical reaction.

First Attempt

The first attempt at the reaction was finally accomplished after more than two months of building the board. We had each sprayer running the separate solutions, and they had their different charges. When they were run we could not see the results that we expected, the bonding of the molecules. We could not understand why there seemed to be nothing going into the inlet, but then it was noticed that there was arcing of electricity between the positive and negative capillary tips. Dr. Basile was asked for help on what to do about the arcing, and the lack of results, and he told us first to make sure that the instrument was working properly. When we ran diagnostics on the instrument it was learned that the arcing between the glass capillaries had caused a short within the instrument, and that a fuse had been blown.

Initial Solution

This was not a huge problem, but the diagnostics do not tell which of the fuses is blown, or that it is a fuse at all. The instrument manual had to be read to learn that it was a fuse problem, and then it was required to open up the instrument to look at the fuses on the inside. The blown fuse was noticeable, a new one was ordered, and then it was replaced. This was a much longer process than we hoped, because the exact fuse was not specified, and we needed to order the new fuse.

Second Attempt

After the fuse was replaced, we attempted the chemical reaction again. It was obvious that the arcing between the capillaries was a problem, so the negative charge from the external source was ramped, rather than starting at three kilovolts. Every time that the voltage started to get high, the arcing would start again, and the voltage would be shut off. It was decided that the angle of the two sprayers might change the arcing, so the left sprayer was moved, from straight on from the inlet, to at a greater angle. More holes had to be put into the platform, but there was now a greater angle between the sprayers. After making the moves, the experiment was again tried, but the arcing still occurred. When this happened, the original paper was more closely read in order to make sure nothing had been missed.

Missing the Small-Stuff

In only a single sentence within the paper, it is discussed that a sheath gas was used within the original Cotham experiment. A nitrogen gas was supposed to have been used to nebulize the solutions. These nebulizer gases are used within the electrospray technique to help propel the solutions into the inlet with greater efficiency. The use of this gas will be enough to keep the capillaries from arcing, because it will hold some of the charge, as well as be extra propellant towards the mass spectrometer inlet. The gas requires a completely different plumbing set-up, which had to be added to the electrospray platform. T-fittings were placed near the end of each of the capillaries, so that the gas would go out and around the capillary. This required a change in much of how the platform was arranged, because it doubled the number of fittings around the capillaries in the small space. A picture of the platform is attached at the end of the report. It has become much more crazy than originally planned, but it is the most simple design that we could make for what was necessary.

The Move

After a semester off from the research, the lab added a brand new freezer that could hold all of the molecules at negative eighty degrees Celsius. While this freezer was an amazing addition to the lab, allowing for a deep freeze of all of the molecules and peptides, it ran from the same outlet as the LCQ instrument that I had been using for my research. When I attempted the experiment again, the instrument would not turn on, which I assumed was an instrument problem. The real problem was over use of the single outlet. The instrument was now blowing the breaker because the freezer was added. Instead of rearranging the entire lab, the platform was moved to the other LCQ instrument. This was an easy move, but it did not solve all of the problems.

Gas Again

The thought was that, once the move was made, it was going to be smooth sailing through the reaction. Chemistry research never works out that way, though. More tuning files were made for each of the sprayers, to make sure it was still working properly after the switch. They again took some time to get perfect, because they had shifted, but within a couple attempts they were again efficiently analyzing the peptide. Again, though, there was a problem with the sheath gas. The LCQ that the platform was moved to was not properly relaying the nitrogen through the instrument to the platform. This setback led to a great deal of troubleshooting. The first thing checked was the plumbing bringing the gas to the instrument, and then from the instrument to the platform. After finding out that it was getting stopped within the instrument, the next place checked was the solenoid valves. Using an external battery, the valves worked properly, but they did not open when the software told them. This told us that it was not the valves, but a problem with the circuitry of the instrument. The next step would seem to be

finding the circuit board that is malfunctioning. Instead, it was decided that it would be easier to circumvent the instrument completely. My time with the Basile Undergraduate Research Group ended with this decision, running out of time in school before I could get the instrument to work for me.

Need For Continuation

I was not able to get all of the instrumentation to work for me before graduating, but I think that if everything gets set up properly, this research could be instrumental in allowing for more efficient protein analysis. The ability to increase mass spectrometer, and CID, ability to larger molecules has applications in multiple fields. The microwave peptide-breakdown technology that the University of Wyoming has, accompanied with this technique, would be very useful, and I believe that my research is very close to reaching that end point. There have been many setbacks in the work that I have done, but I think there is a strong base for another student, or myself, to build off of, and reach the goal that I have been striving for over the last eighteen months. From this point, the only thing left is optimizing the instrumentation, and changing around the settings until the original paper is recreated within our lab. I hope that the work that I have put in is continued, because this peptide analysis could increase the ability to analyze foreign substances, infectious materials, and any other similarly structured molecule.

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