A Structural Investigation of the Male European Corn Borer's Pheromone Binding Protein-3

by

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Abstract

European Corn Borer (*Ostrinia nubilalis*) is an economically impacting moth of the North American agricultural community. First introduced to the United States in 1917, *Ostrinia nubilalis* now causes a yearly sum of approximately $1 billion in lost crops. The most effective method of ending the losses due to *O. nubilalis* lie in blocking the reproduction process of the sexually mature moths. By targeting and inhibiting male *O. nubilalis* pheromone detection system via sensory inhibition, the life cycle of the moth can be disrupted resulting in a decrease of the *O. nubilalis* population. *O. nubilalis* pheromone-binging protein 3 (OnubPBP3) plays a critical role in pheromone detection by the male moth. Understanding the mechanism of pheromone detection and the role of OnubPBP3 in this process, structure, and function of this protein, and its interaction with pheromones is critical for developing an inhibitor. We present here the transformation, overexpression, refolding of the inclusion bodies, purification and the preliminary biophysical analysis of OnubPBP3 through fluorescence spectroscopy, circular dichroism (CD) analysis, and 2D {^{1}H, ^{15}N} heteronuclear single quantum coherence (HSQC) NMR spectroscopy. The data gained suggest that OnubPBP3 is a globular protein, primarily helical, and contains a hydrophobic binding pocket where it is believed to interact with the pheromone.

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Chapter 1

Introduction

The European Corn Borer (*Ostrinia nubilalis),* was first documented in the United States of American near Boston, MA in 1917 (Capinera, 2000). *O. nubilalis* was presumably imported into the country on broom corn from Italy and Hungary (Mason, 1996). The moth quickly established itself, feasting on the many crops of United States' agricultural economy. *O. nubilalis* quickly spread west, making its way to the central United States by 1944 and eventually to the Rocky Mountains (ISU, 2013). The species also spread north and south making its way into Canada and down the Atlantic coast all the way to the gulf coast.

Today, the moth can be found in nearly all places that corn is grown on an industrial scale. This is because *O. nubilalis* is a leading pest to corn along with three hundred other garden-variety crops such as lima beans, bell peppers, tomatoes, etc. (Phelan, 1996). Current averages of approximately $1 billion are lost each year in the United States due to the *O. nubilalis* population (Hutchison, 2010).

Many different techniques have been used to try and reduce the $1 billion loss, which dramatically impacts both small and large American corn producers. For nearly one hundred years, the agricultural community has used a combination of biological and chemical management, along with genetic

mutations of crops, all in order to reduce the damage. Initially, it was suspected that *O. nubilalis* was thriving because it was no longer fighting its native parasites. This lead to the release of over twenty-four species of parasites in the United States (Baker, 1949). In the early 1900s, other research exploring which types of pathogens could lead to diseases in *O. nubilalis* was conducted. Research in chemical defenses also started in the mid 1920s with extensive research of many pesticides. In 1944, the agricultural community gained promising ground when it was discovered that using DDT resulted in roughly a 90% control of *O. nubilalis* (Brindley, 1963). However, in 1972 DDT was canceled due to evidence that it possessed carcinogenic properties and was a hazard to wildlife (EPA, 1990). Lastly, in the late 1900s genetically modified organisms (GMO) gained traction and in 1996 corn that was genetically modified to produce an insecticidal protein were commercially available.

The *Bt* (*Bacillus thuringiensis*) corn was designed to be an economically satisfying resistance to *O. nubilalis* damage. Along with these more defensive actions, smaller, more mechanical practices can be taken. These include practices such as plowing, grazing or even burning the stocks after harvesting in order to ensure the destruction of hibernating larvae. Harvesting early is also beneficial because it reduces the amount of time the moths have to feast on the crop.

Each of these techniques has both positives and negatives. Biological and chemical resistance, though effective in some cases, has the potential to be very time consuming and costly. Also, consumer satisfaction can be greatly

impacted when spraying potentially harmful chemical on products that are going to be consumed. Although GMOs can be cost efficient and resist *O. nubilalis*, they too have lost consumer satisfaction due to the altering of the genome of a product that is readily consumed. Lastly, though mechanical practices can slightly help, they are very timely and not economically beneficial (Vasileiadis, 2011).

This research will explore a potential *O. nubilalis* resistant mechanism, which is both cost and environmentally friendly, and it all starts at the moth's reproductive cycle. The reproduction cycle of the *O. nubilalis* contains two different stages: a larval stage and a sexually mature adult moth stage.

After mating, the female moth lays her eggs on the crop's leaves, stock or on the crop itself. After the eggs hatch, larvae emerge and begin to feed on the crop. While feeding, the larvae create small holes that weaken and damage the various parts of the crop. Once the moths have multiplied their size and weight, they go through a dormant pupation period and then reemerge as sexually mature *O. nubilalis* at which point they begin to mate.

The mating process begins with the male moths locating and seeking out the female moths. They do this by sensing species-specific pheromones that the females release into the air. Pheromone-binding protein 3 (PBP3) in the male moths' antennae binds the hydrophobic pheromone and carries it across the aqueous sensillar lymph to the olfactory receptor neurons (ORN) triggering a neuronal response. PBP3 is unique to the male European Corn Borers and is not found in the females. This is because the males use this olfactory system and the

neural response to follow the pheromone trail upwind to locate the female where the mating process is initiated.

With the mechanisms listed above considered, targeting and disrupting the *O. nubilalis* reproductive cycle by blocking the male moth's pheromone detection system is the most advantageous route of resistance. By inhibiting the males' ability to locate the female moths, mating is blocked, which would result in reduced numbers of larvae and most importantly less crop damage. `

In order to achieve the control of moth population through sensory inhibition, the three-dimensional (3D) structure of *O. nubilalis* pheromone binding protein 3 (OnubPBP3) must be determined and the binding site must be mapped to understand the binding mechanism between the pheromone and the protein. This work details the expression and purification process of the 16.3 kDa OnubPBP3 along with preliminary biophysical analysis of the protein through fluorescence spectroscopy, circular dichroism (CD) analysis, and 2D {^{1}H, ^{15}N} heteronuclear single quantum coherence (HSQC) NMR spectroscopy.

Chapter 2

Materials and Methods

2.1 Cloning, Overexpression and Purification of OnubPBP3

The *OnubPBP3* gene was previously cloned into a pET-22b vector by Mohanty group prior to starting this work with OnubPBP3. Mohanty group verified

the correct orientation of all pET-22b-OnubPBP3 plasmids by DNA sequencing.

The constructed pET-22b-OnubPBP3 plasmids were transformed into *Escherichia coli* origami 2 (Katre, 2009) cells and inoculated onto Lysogeny Broth (LB)-ampicillin-tetracycline agar plates (Bertani, 1951). The cells were grown overnight at 37^{o} C, and then a single cell colony was used to inoculate 30 ml of LB-ampicillin-tetracycline media. The culture was grown overnight to saturation and then used as a starter culture to inoculate the overexpression media. LB-ampicillin-tetracycline was further used when overexpressing unlabeled OnubPBP3, and minimal media, containing 0.4% (w/v) D-[^{13}C] glucose and 0.1% (w/v) $^{15}NH_4Cl$, was used to overexpress double-labeled ^{15}N,^{13}C -OnubPBP3 (Mohanty, 2004). Cells were grown at 37^{o} C to $A_{600\ nm}$ of 0.5- 0.6, then once the optical density (OD) was verified, expression was induced with 1mM isopropyl β–D-1-thiogalactopyranoside (IPTG). The temperature was reduced to 30^{o} C after induction and the cells were further incubated for four hours while expressing unlabeled OnubPBP3 and sixteen hours while expressing ^{15}N,^{13}C-OnubPBP3. The cells were harvested by centrifugation. The cell pellet then underwent a freeze-thaw process in order to weaken the cell wall (one hour in the -80^{o} C, one hour on ice). This process was repeated seven times, then the cell pellet was re-suspended using bacterial protein extraction reagent (B-PER) and lysed by sonication. OnubPBP3 inclusion body (IB) was collected by centrifugation (12,000 rpm, 20 minutes), re-suspended using sonication in B-PER reagent diluted to 10% and pelleted again by centrifugation (12,000 rpm, 20 minutes). The IB was washed once more with 10% B-PER using the same sonication and

pelleting by centrifugation techniques. The IB was denatured and solubilized using Guanidine Hydrochloride (GuHCl) at 6M and, then the protein was refolded into its native form by slowly removing GuHCl using step dialysis (Fig. 1) (Zhang, 2009).

Refolded OnubPBP3 was purified first by anion exchange chromatography using a resin containing diethyl-aminoethyl groups (DEAE) (General Electric, Boston, MA). OnubPBP3 was then purified by gel-filtration on a HiLoad™ 26/60 Superdex™ 75 column (General Electric, Boston MA). Through SDS-PAGE and size exclusion chromatography measurements, it was verified that the pure, refolded OnubPBP3 was in a monomeric form.

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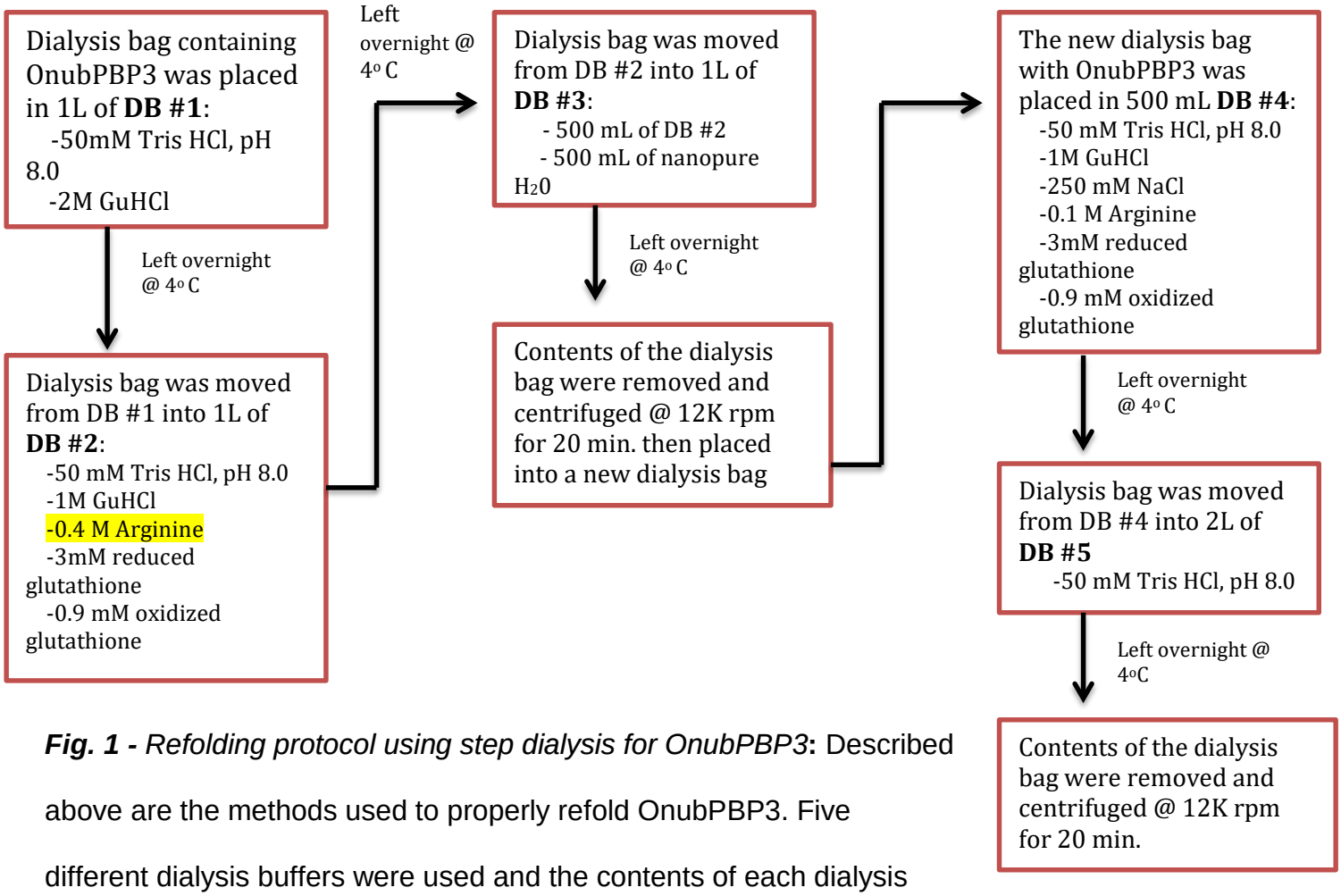

Fig. 1 - *Refolding protocol using step dialysis for OnubPBP3***:** Described above are the methods used to properly refold OnubPBP3. Five different dialysis buffers were used and the contents of each dialysis buffer are included.

2.2 Delipidation of OnubPBP3

The dilapidation of natively folded, unlabeled OnubPBP3 was achieved using the protocol of Katre *et al.* (Katre, 2009). Following gel-filtration chromatography, the fractions containing pure OnubPBP3 were concentrated to 1 ml using a Millipore ultrafiltration concentrator (capacity 15 ml, molecular

weight cutoff of 3,000). The 1 ml sample was loaded into a pre-prepared Lipidex^{TM} column, equilibrated prior with 50 mM sodium citrate buffer pH 4.5 (Buffer C), and was continuously shaken at room temperature overnight. The sample was drained out of this first column into a second column, which was also pre-equilibrated with Buffer C. The first column was washed with 1 ml of Buffer C and continuously shaken for one hour. After the hour, the wash was drained into the second column. The delipidation process was repeated with the second Lipidex^{TM} column to ensure proper dilapidation of the unlabeled OnubPBP3. After delipidation, the sample was further concentrated in order to increase the protein concentration of the sample.

2.3 Fluorescence Spectroscopy

The fluorescence experiment was carried out on a Cary Eclipse Fluorescence Spectrophotometer (Varian, Palo Alto, CA) using a quartz cuvette with a path length of 1-cm. All samples were prepared with 20 mM phosphate buffer, pH 6.5, with 0.25% methanol at 22^{o} C and were conducted three times in order verify reproducibility.

The binding of the fluorescent ligand *N*-phenyl-1-naphthylamine (NPN) to delipidated OnubPBP3 carried out at pH 6.5 and the fluorescent signal given off by NPN at various wavelengths was monitored. Small aliquots of 20 mM NPN in methanol were incrementally added to a 2 ml sample of 1 *u*M of delipidated OnubPBP3. The final concentration of NPN in the sample at each data collection point ranged from 0-15 *u*M. After each addition of NPN, the sample was

incubated for 10 min. Then the fluorescence data was collected at the excitation wavelength of 350 nm, and emission of 370-600 nm. Control samples with phosphate buffer and previously used concentrations of NPN were analyzed in order to correct the fluorescent signal. The dissociation constant (K_D) was calculated from the nonlinear regression using the program Origin 6.1.

2.4 Desalting of OnubPBP3

Desalting of natively folded, unlabeled OnubPBP3 was carried out using a CentriPure MINI Spin Column (Desalt Z-50, Princeton Separations Inc., Freehold, NJ) at pH 4.5, 5.5 and 6.5. The new, dry column was washed by centrifuging the column for 2 minutes at 800 rpm with 650 *u*l of 20mM sodium phosphate buffer at various pH. The column was moved to a clean collecting tube, and 200 *u*l of pure, unlabeled OnubPBP3 was placed in the column. The column was centrifuged again for 2 minutes at 800 rpm. The column was removed from the collecting tube, and the desalted protein in the collecting tube was stored at 4^{o} C.

2.5 Circular Dichroism Spectroscopy

All circular dichroism (CD) experiments were conducted with a Jasco J-810 automatic recording spectropolarimeter in our lab (Smita Mohanty) in the Department of Chemistry at Oklahoma State University (Stillwater, OK) using rectangular quartz cells with a 0.05 cm path length. The experiment was

conducted at room temperature using a 0.05 cm rectangular quartz cell. Each sample was prepared to a final protein concentration of 15.3 *u*M of desalted unlabeled OnubPBP3 in 20 mM sodium phosphate buffer, pH of 4.5, 5.5, or 6.5. At each of these pH values, the data were averaged over 5 scans with a response time of 4s and a scan speed of 50 nm/min. All values were corrected using a control for each pH, which contained no OnubPBP3. The experimental values were corrected by subtracting the blank spectra samples. The CD ellipticity values were then normalized (mean molar ellipticity per residue) by standard method. The secondary structures of OnubPBP3 at pH 4.5-6.5 were evaluated using CDSSTR, CONTINLL and SELCON3 programs incorporated in a CDPro software package. These values were used to create a graphical representation of the secondary structures (Fig. 4).

2.6 Nuclear Magnetic Resonance Spectroscopy

The NMR experiment was carried out at 35 ^{o}C, using the Varian INOVA 600 MHz spectrometer equipped with a triple resonance ^{1}H/^{15}N/^{13}C probe at the Statewide Shared NMR Facility housed in the Department of Chemistry, Oklahoma State University (Stillwater, OK). The NMR sample used for 2D {^{1}H, ^{15}N} heteronuclear single quantum coherence (HSQC) spectra consisted of 300 *u*l of 0.8 mM of natively folded double-labeled ^{15}N^{13}C OnubPBP3 in 50 mM sodium phosphate buffer, pH 6.5, containing 1 mM EDTA, 0.01% NaN_3 , and 5% D_2O in a Shigemi tube. 2D {^{1}H, ^{15}N} (HSQC) spectra were collected for

OnubPBP3 at 37 ^{o}C, then the data was processed using NMR pipe and analyzed by Sparky (Delaglio, 1995 & Tonelli, 2015)

Chapter 3

Results and Discussion

As discussed previously, OnubPBP3, along with many other pheromone binding proteins (PBP) in other moth species, binds species-specific pheromones in order to carry out their reproductive cycle. Even though many of these PBPs have very similar protein sequences, and more importantly, the residues that are thought to be involved in protein folding are found in the same positions, they specifically bind the pheromones associated with their species (Mohanty, 2004). It is because of this, that the full 3D structure and mechanism of interaction between the PBP and pheromone of each species must be determined. These results will assist in the work to discover the conformational features involved in the pheromone binding and structure of OnubPBP3.

3.1 Fluorescence Spectroscopy

After expressing and purifying OnubPBP3, the protein's activity was tested using fluorescence spectroscopy. In its native form, OnubPBP3 is a soluble protein that acts as a hydrophilic transporter to hydrophobic sex pheromone in the antennae of the male moths. The understanding of the

mechanism in which the pheromone binds with OnubPBP3 is crucial in disrupting *O. nubilalis* mating cycle. It is believed that the protein's tertiary structure forms a hydrophobic binding pocket directly in the center of OnubPBP3. To test if our protein contains the hydrophobic pocket and also its binding activity, the hydrophobic probe, NPN, was used as a synthetic standard in representation of *O. nubilalis'* native sex pheromones. While NPN is in an aqueous environment it produces no fluorescent signal, but when the ligand is bound inside the hydrophobic pocket it emits a strong fluorescent signal (Loh, 1984). As explained in the methods, delipidated OnubPBP3 was titrated with increasing concentrations in a NPN solution, and the fluorescent signal produced was measured at each concentration (Fig 2). The spectroscopy confirmed the presence of the hydrophobic pocket and a direct positive relationship between the concentration of OnubPBP3 present in solution and the fluorescent signal produced by the NPN probe.

After plotting the relationship between the concentration of the NPN probe and the intensity of the fluorescent signal (Fig. 3), the linear equilibrium dissociation constant (K_d) was calculated. This K_d value was determined to be 3.385 uM, with an error of ± 0.56 uM. This value indicated that the affinity of OnubPBP3 to the NPN ligand is very strong and bound tightly to one another.

2

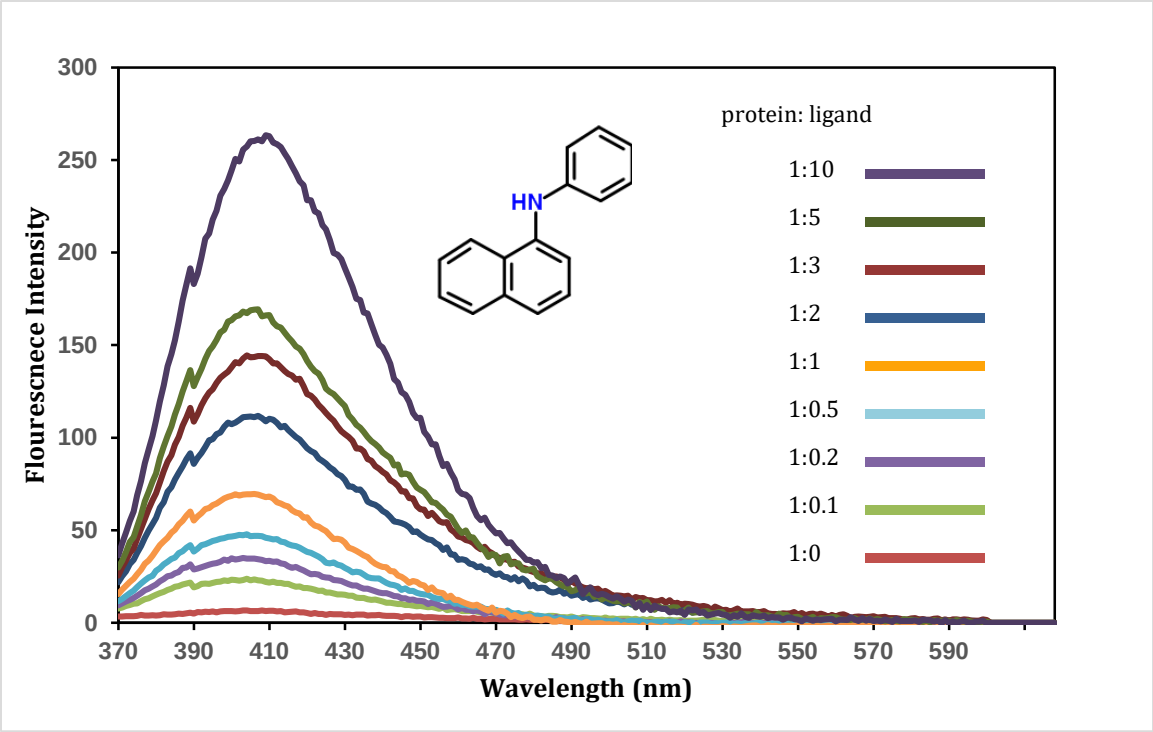

Fig 2 - *Fluorescence Binding study of OnubPBP3 to N-phenyl-1-naphthylamine (NPN):* The fluorescence emission of NPN bound to OnubPBP3 at various concentration ratios of protein (OnubPBP3): Ligand (NPN) is represented. As seen in the figure, the concentration of NPN and fluorescent signal display a positive direct relationship. The increase of fluorescence intensity was measured from 370 nm to 600 nm.

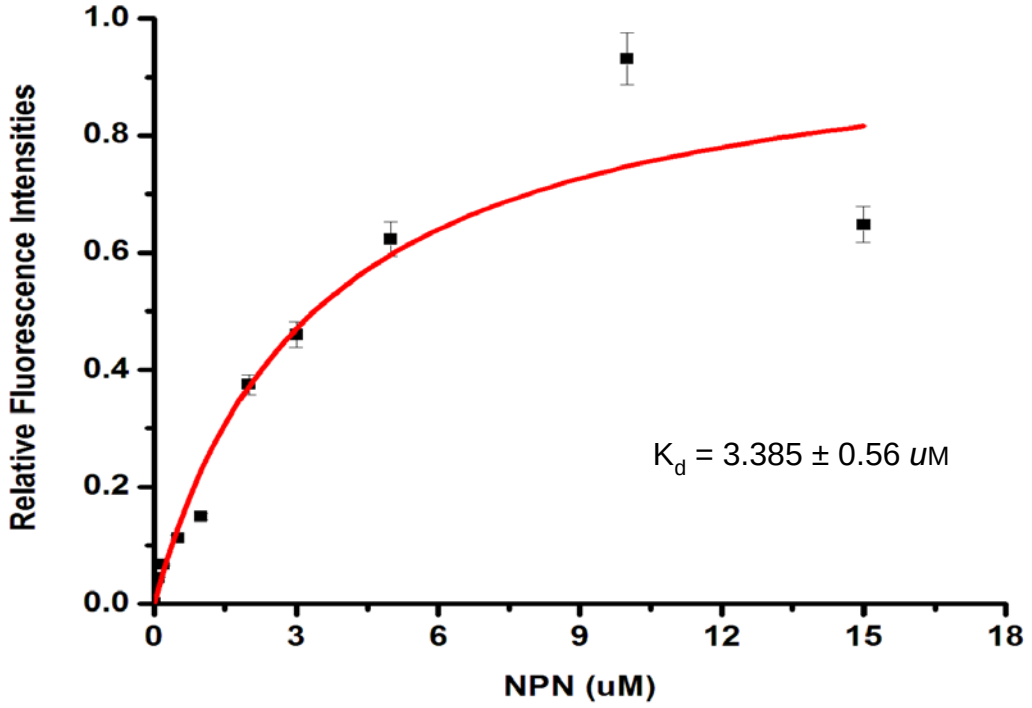

Fig. 3 - *Plot of the relative fluorescence intensity measured compared to N-phenyl-1-naphthylamine (NPN) concentration:* The graph demonstrates an increase in fluorescent signal at NPN increases from 0 *u*M to 10 *u*M. After 10 *u*M the binding protein reaches saturation and a decrease in fluorescent signal is seen at 15 *u*M NPN.

[3]

3.2 Circular Dichroism Spectroscopy

The secondary structure of OnubPBP3 plays a vital role in the pheromone –protein interaction mechanisms and is highly regulated by the pH gradient across the sensillar lymph in the *O. nubilalis* olfactory system. The amount of random coils, α-helixes, and β-sheets can give important insight in regards to the secondary structure and mechanisms of OnubPBP3. For PBPs at high pH, the C-

terminus tail is outside of the hydrophobic pocket, which allows pheromone to bind tightly inside the pocket. Once the protein travels across the sensillar lymph to the olfactory receptor, where the pH is acidic, the conformation of the protein changes and the C-terminus occupies the hydrophobic pocket releasing the ligand (Katre, 2009). Circular dichroism (CD) spectroscopy was used to analyze the change in secondary structure at near-UV (180-250 nm) with a change in pH from 6.5 to 5.5 and finally to 4.5 of unlabeled protein. At each of the pH levels, the mean residual ellipticity (deg.cm2/dmol) was compared. The near-UC CD scans at three pH levels were overlaid in order to visually compare how altering the pH induces conformational change/s in the secondary structure of OnubPBP3 (Fig. 4)

The visual representation shows very similar structures for pH 4.5 and 5.5. However, at pH 6.5 a noticeable change in the secondary structure is observed. Although the fractional representation of the secondary structures demonstrates that at pH 6.5, there is a greater degree of α-helix compared to pH 4.5 and 5.5, there is also a difference in the total helical values for pH 4.5 and 5.5 (Table 1). This could be a result of the decrease in hydrogen bond formation when the pH is at 4.5, 5.5 or 6.5. When the pH is lower, there are more protons readily available in solution to bind and stabilize the carbonyl and amino groups. When the pH is higher, there are fewer free protons, so the carbonyl and amino groups will more readily form hydrogen bonds with each other. Since the carbonyl-amino hydrogen bonds help to stabilize α-helixes, this could give reason to the higher percentage of α-helixes at pH 6.5 compared to pH 4.5 and 5.5 (Myers, 1996).

From this experiment, we conclude that OnubPBP3 is a highly helical protein at neutral pH and, as the pH drops, the percentage of α-helixes also decreases.

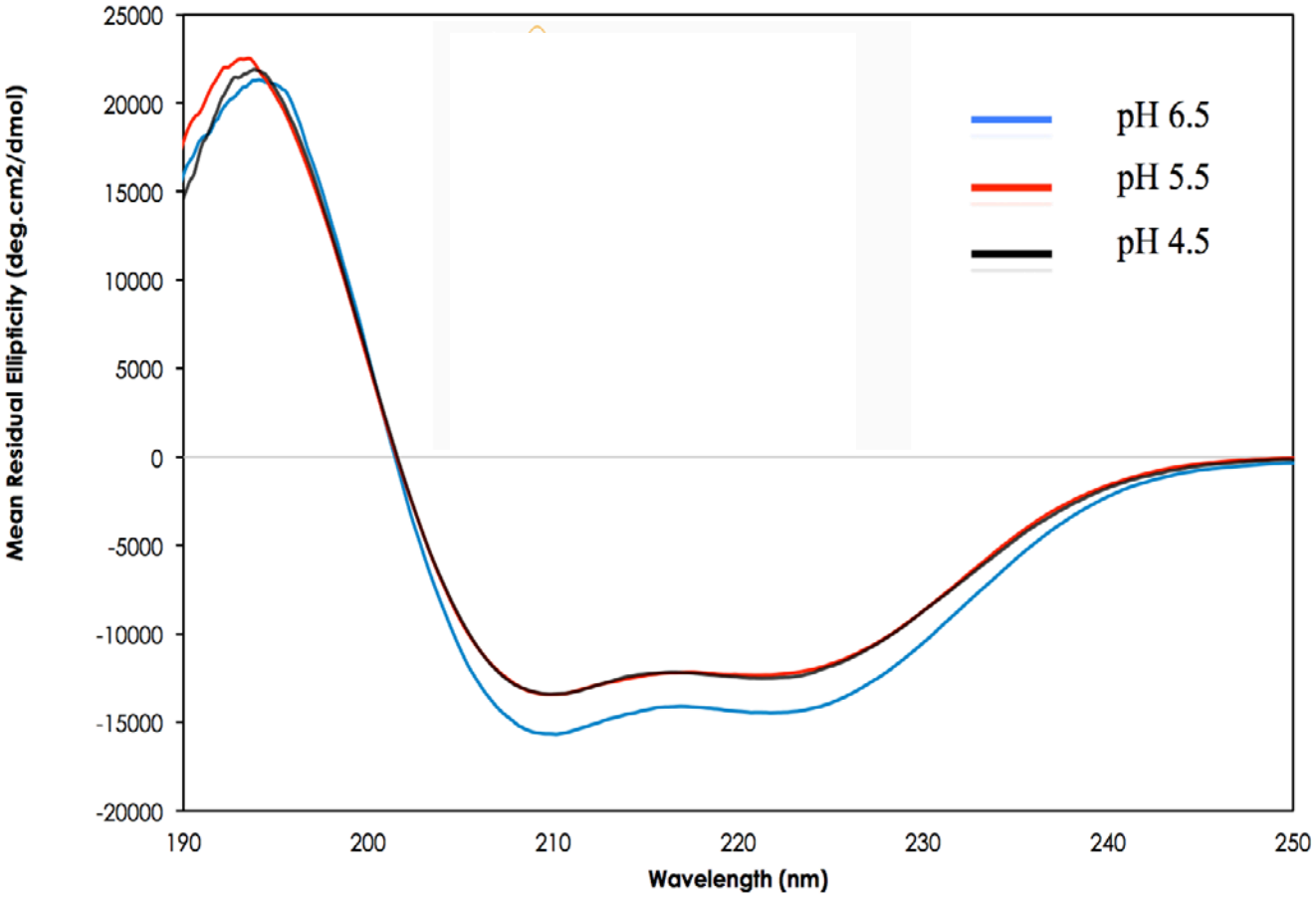

Fig. 4 - *Circular dichroism spectroscopy study of OnubPBP3*: This experiment was performed at three different pH levels. At each pH, the mean residual ellipticity (deg · cm^2 /dmol) was measured at wavelengths from 190-250 nm. The data from each experiment was graphed is order to visually compare the secondary structure of OnubPBP3 at various pH (pH 6.5 is represented in blue, pH 5.5 is represented in red, and pH 4.5 is represented in black).

4

	H(r)	H(d)	S(r)	S(d)	Trn	Unrd	Helix Total (%)
pH 4.5	0.255	0.194	-0.013	0.026	0.219	0.281	44.9
pH 5.5	0.242	0.182	0.016	0.035	0.218	0.281	42.4
pH 6.5	0.274	0.202	0.003	0.03	0.243	0.242	47.6

Table 1 - *Comparison of the Secondary Structure of OnubPBP3*: Values for the fractions of the various secondary structures of OnubPBP3 at pH 4.5 – 6.5 is given in this table. The total fraction of α-helix structures is calculated [H(r) + H(d)] and given as a percentage for each pH.

5

3.3 Nuclear Magnetic Resonance Spectroscopy

In order to investigate the conformation of OnubPBP3, nuclear magnetic resonance spectroscopy (NMR) was used. 2D {^{1}H, ^{15}N}-heteronuclear single quantum coherence (HSQC) NMR data was collected (Fig. 5). This region is very sensitive to conformational changes and can detect the slightest alterations in protein conformation due to change in pH, ligand or temperature. Thus, the 2D HSQC experiment depicts the fingerprint of a protein. In the 2D {^{1}H, ^{15}N}- HSQC of ^{15}N, ^{13}C-OnubPBP3, the individual peaks are well dispersed which indicates that the protein is folded well.

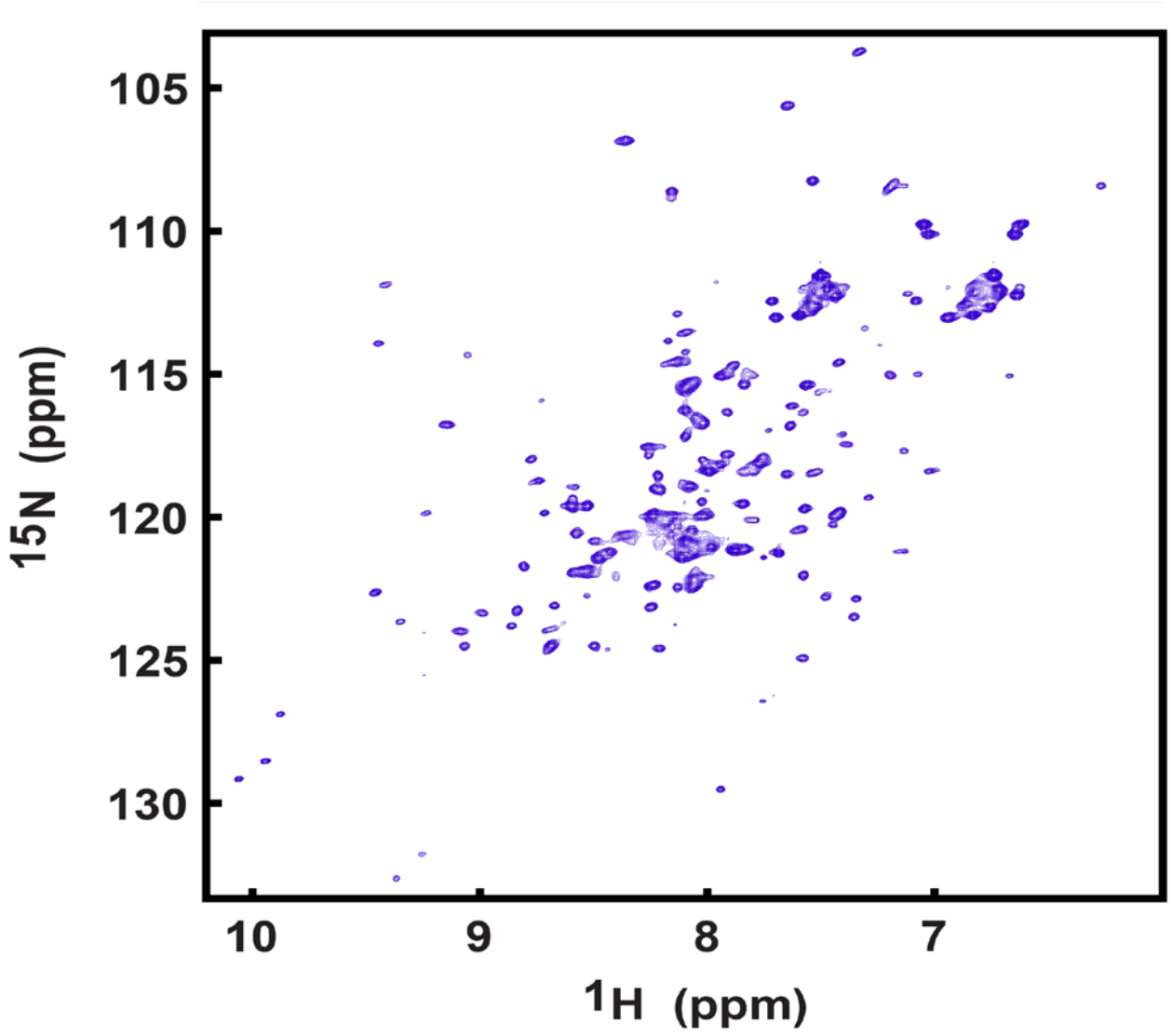

Fig. 5 - *2D [^{1}H, ^{15}N] HSQC spectra of ^{15}N^{13}C double labeled OnubPBP3 in 50 mM sodium phosphate buffer, pH 6.5 containing 5% D2O, 1mM EDTA, and 0.01% sodium azide*: Each peak represents the interactions of a single nitrogen in the protein back-bone with its bound amide hydrogen. This figure is representative of the finger-print or thumb-print region of OnubPBP3.

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Chapter 4

Conclusion

Still today, *Ostrinia nubilalis* causes the North American agricultural community millions, and even billions of dollars in crops lost to the moths' destruction. Along with their continued economical dent, there is yet a way to stop these pests and traditional methods of management have remained economically and socially unsatisfying. However, by completely understanding the biomechanics behind the olfactory system used by male *O. nubilalis* to locate the female, it is believed that the reproduction system of the species could be disrupted. This would lead to the decreased destruction of hundreds of garden-variety crops and a decrease in the economic impact of *O. nubilalis.*

Along with the biomechanics of the pheromone binding system, the full 3D structure and function of *O. nubilalis* pheromone binding protein 3 (OnubPBP3) must be detailed, which will require a combination of approaches. This work presents expression and purification protocols along with ligand binding analysis through fluorescence spectroscopy, the secondary structure composition of OnubPBP3 by circular dichroism, and mapping of the three-dimensional structure using 2D {^{1}H, ^{15}N}- HSQC NMR spectra. These finding verified that near neutral pH, native OnubPBP3 is a soluble, globular protein containing a hydrophobic pocket that readily binds and holds hydrophobic ligands. The protein consists

mostly of α-helixes in its secondary structure but does undergo conformational changes to the secondary structure when pH is altered.

This work laid a framework for detailed NMR characterization of the OnubPBP3. Various 3- and 4-dimensional NMR experimental data will be collected in the future for the resonance assignments of each residue of OnubPBP3 before the calculation of the atomic resolution 3-dimensional structure. A detailed structure-function study of OnubPBP3 and its interaction with pheromone at the molecular level could have far reaching impacts to insect control through the development of pheromone mimics that can specifically inhibit *Ostrinia nubilalis* pheromone detection.

References

Capinera, John L. “European Corn Borer - Ostrinia Nubilalis (Hubner).” *Africanized Honey Bee - Apis Mellifera Scutellata Lepeletier*, University of Florida, Sept. 2000, entnemdept.ufl.edu/creatures/field/e_corn_borer.htm.

Mason, C. E., et al. “The European Corn Borer | The European Corn Borer.” *Biological Agents* | *The European Corn Borer*, Iowa State University, North Central Regional Extension Publication , 1996, www.ent.iastate.edu/pest/cornborer/insect.

Department of Entomology. “The European Corn Borer.” *The European Corn Borer* | *The European Corn Borer*, 2013, www.ent.iastate.edu/pest/cornborer/insect.

Phelan, P. L., et al. “Soil-Management History and Host Preference by Ostrinia Nubilalis: Evidence for Plant Mineral Balance Mediating Insectâ□“Plant Interactions.” *Environmental Entomology*, vol. 25, no. 6, 1996, pp. 1329–1336., doi:10.1093/ee/25.6.1329.

Ranum, Peter, et al. “Global Maize Production, Utilization, and Consumption.” *Annals of the New York Academy of Sciences*, vol. 1312, no. 1, 2014, pp. 105–112., doi:10.1111/nyas.12396.

Hutchison W, Burkness E, Mitchell P, Moon R, Leslie T, Fleischer S et al., Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330:222–225 (2010).

Baker, W. A., Bradley, W. G., and Clark, C. A. Biological control of the European corn borer in the United States. U. S. D.1pt. Agr. Tech. Bull. No. 983, 185 pp. (1949)

Brindley, T. A., and F. F. Dicke. “Significant Developments in European Corn Borer Research.” *Annual Review of Entomology*, vol. 8, no. 1, 1963, pp. 155–176., doi:10.1146/annurev.en.08.010163.001103.

Suspended, canceled, and restricted use pesticides; EPA-20T-1002; U. S. Environmental Protection Agency,Office of Pesticide Programs, U. S. Government Printing Office: Washington, DC, 1990.

Vasileiadis, V.p., et al. “Crop Protection in European Maize-Based Cropping Systems: Current Practices and Recommendations for Innovative Integrated Pest Management.” *Agricultural Systems*, vol. 104, no. 7, 2011, pp. 533–540., doi:10.1016/j.agsy.2011.04.002.

Bertani, G. (1951). STUDIES ON LYSOGENESIS I. : The Mode of Phage Liberation by Lysogenic Escherichia coli. *Journal of Bacteriology*, *62*(3), 293–300.

Mohanty, Smita, et al. “Erratum to ‘The Solution NMR Structure of Antheraea Polyphemus PBP Provides New Insight into Pheromone Recognition by Pheromone-Binding Proteins’ [J. Mol. Biol. (2004) 337, 443–451].” *Journal of Molecular Biology*, vol. 338, no. 5, Jan. 2004, p. 1037., doi:10.1016/j.jmb.2004.03.052.

Zhang, T.; Xu, X.; Shen, L.; Feng, Y.; Yang, Z.; Shen, Y.; Wang, J.; Jin, W.; Wang, X., Modeling of protein refolding from inclusion bodies. Acta biochimica et biophysica SInica 2009, gmp098.

Katre, Uma V., et al. “Ligand Binding Turns Moth Pheromone-Binding Protein into a PH Sensor.” *Journal of Biological Chemistry*, vol. 284, no. 46, 16 Sept. 2009, pp. 32167–32177., doi:10.1074/jbc.m109.013383.

Sreerama, Narasimha, and Robert W. Woody. “Estimation of Protein Secondary Structure from Circular Dichroism Spectra: Comparison of CONTIN, SELCON, and CDSSTR Methods with an Expanded Reference Set.” *Analytical Biochemistry*, vol. 287, no. 2, 2000, pp. 252–260., doi:10.1006/abio.2000.4880.

Delaglio, Frank, et al. “NMRPipe: A Multidimensional Spectral Processing System Based on UNIX Pipes.” *Journal of Biomolecular NMR*, vol. 6, no. 3, 1995, doi:10.1007/bf00197809.

NMRFAM-SPARKY: enhanced software for biomolecular NMR spectroscopy. Lee W, Tonelli M, Markley JL. Bioinformatics. 2015 Apr 15; 31(8):1325-7

Myers, J.k., and C.n. Pace. “Hydrogen Bonding Stabilizes Globular Proteins.” *Biophysical Journal*, vol. 71, no. 4, 1996, pp. 2033–2039., doi:10.1016/s0006-3495(96)79401-8.

Loh, B, et al. “Use of the Fluorescent Probe 1-N-Phenylnaphthylamine to Study the Interactions of Aminoglycoside Antibiotics with the Outer Membrane of Pseudomonas Aeruginosa.” *Antimicrobial Agents and Chemotherapy*, vol. 26, no. 4, 1984, pp. 546–551., doi:10.1128/aac.26.4.546.