Published in final edited form as:
Biochem Biophys Res Commun. 2011 January 7; 404(1): 335–338. doi:10.1016/j.bbrc.2010.11.119.

Extrusion of the C-terminal Helix in Navel Orangeworm Moth Pheromone-Binding Protein (AtraPBP1) Controls Pheromone Binding†

Wei Xu, **Xianzhong Xu**, **Walter S. Leal**, and **James B. Ames***
Departments of Entomology and Chemistry, University of California, Davis, CA 95616

Abstract

The navel orangeworm, *Amyelois transitella* (Walker), is an agricultural insect pest that can be controlled by disrupting male-female communication with sex pheromones, a technique known as mating disruption. Insect pheromone-binding proteins (PBPs) provide fast transport of hydrophobic pheromones through aqueous sensillar lymph and promote sensitive delivery of pheromones to receptors. Here we present a mutational analysis on a PBP from *Amyelois transitella* (AtraPBP1) to evaluate how the C-terminal helix in this protein controls pheromone binding as a function of pH. Pheromone binds tightly to AtraPBP1 at neutral pH, but the binding is much weaker at pH below 5. Deletion of the entire C-terminal helix (residues 129–142) causes more than 100-fold increase in pheromone binding affinity at pH 5 and only a 1.5-fold increase at pH 7. A similar pH-dependent increase in pheromone binding is also seen for the H80A/H95A double mutant that promotes extrusion of the C-terminal helix by disabling salt bridges at each end of the helix. The single mutants (H80A and H95A) also exhibit pheromone binding at pH below 5, but with ~2-fold weaker affinity. NMR and circular dichroism data demonstrate a large overall structural change in each of these mutants at pH 4.5, indicating an extrusion of the C-terminal helix that profoundly affects the overall structure of the low pH form. Our results confirm that sequestration of the C-terminal helix at low pH as seen in the recent NMR structure may serve to block pheromone binding. We propose that extrusion of these C-terminal residues at neutral pH (or by the mutations in this study) exposes a hydrophobic cleft that promotes high affinity pheromone binding.

Keywords

AtraPBP1; NMR; pheromone-binding protein; *Amyelois transitella*; pheromone; navel orangeworm moth; histidine protonation switch; disulfide bridge

INTRODUCTION

The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), is the most serious insect pest of almonds and pistachios in California, and a major pest of walnuts, figs

†This work was supported by NIH grants EY012347 (J.B.A.) and RR11973 (UC Davis NMR), USDA-AFRI grant 2010-65105-20582 (WSL), NSF grant 0234769 (WSL), and UC Davis NMR facility.

*To whom correspondence should be addressed. Tel: (530) 752-6358. Fax: (530) 752-8995. ames@chem.ucdavis.edu.

and a number of other crops. This agricultural pest is primarily controlled with pyrethroids and insects growth regulators, but alternative methods of control, including sex pheromone-based mating disruption, are sorely needed. A potential way of controlling insect pests is to disrupt detection of sex pheromones. The sex pheromone system of this species has been previously identified [1,2], but some constituents are unstable thus requiring the development of stable alternatives (parapheromones) for practical applications. We aim at employing olfactory proteins to screen potential attractants (parapheromones), an approach termed “reverse chemical ecology” [3]. Previously, we have identified olfactory proteins from the navel orangeworm, including a male antennae-specific pheromone-binding protein, AtraPBP1 [4]. There is growing evidence in the literature suggesting that pheromone-binding proteins (PBPs) contribute to the sensitivity and possibly the selectivity of the insect’s olfactory system [5].

A molecular mechanism for moth PBPs has been proposed based on the PBP from silkworm moth, BmorPBP1, for which a pH-dependent conformational change was shown to be involved in pheromone binding and release [6–8]. Indeed, previous structural studies showed the C-terminal part of PBPs, which is unstructured in pheromone-PBP complex [9] and forms an α-helix at low pH that competes with pheromone for the binding pocket [10–12], thus enabling the delivery of the pheromone in acidic environment similar to that formed by the negatively charged dendrite surfaces of the olfactory receptor neurons [13]. Functional studies also showed that BmorPBP1, when co-expressed with pheromone receptor BmorOR1 in the empty neuron system of *Drosophila melanogaster*, enhanced the response to the pheromone, indicating that OBPs contribute to the inordinate sensitivity of the insect’s olfactory system [5].

Our previous studies have suggested that AtraPBP1 undergoes a pH-dependent conformational change [4]. The recent NMR structure of AtraPBP1 [14] reveals two pH-dependent salt bridges involving H80/E132 and H95/E141 at each end of the C-terminal helix (termed histidine protonation switch) that were suggested to control pheromone binding. Here we present a mutational analysis on AtraPBP1 to further understand how the C-terminal helix gates pheromone binding as a function of pH. Deletion of the entire C-terminal helix (residues 129 – 142, called AtraPBP1Δ129N-142V) causes a more than 100-fold increase in pheromone binding affinity at pH 5 and only 1.5 fold increase at pH 7. A similar increase in pheromone binding is seen for the H80A/H95A double mutant and single mutants (H80A and H95A) that destabilize the C-terminal helix by disabling the histidine protonation switch. NMR and circular dichroism data demonstrate large overall structural changes in each of these mutants at low pH, indicating that removal of the C-terminal helix by these mutations profoundly affects the overall structure. We propose that extrusion of the C-terminal helix at neutral pH or by the mutations in this study exposes a hydrophobic site to promote pheromone binding.

Materials and Methods

Expression and Purification of AtraPBP1 Mutants

The following 4 plasmid for expression (pET) vectors were constructed with the QuickChange site-directed mutagenesis kit (Stratagene) [15] by using the pETAtraPBP1 vector [4] as template DNA: pETAtraPBP1H80A (His-80 replaced by Ala), pETAtraPBP1H95A (His-95 replaced by Ala), pETAtraPBP1H80AH95A (His-80 and His-95 replaced by Ala), pETAtraPBP1 129N-142V (C-terminus from Asn-129 to Val-142 deleted). Non-labeled proteins were prepared by a periplasmic expression, which is known to generate properly folded, functional OBPs [4,15]. Uniformly 15N-labeled proteins were expressed in *E. coli* and purified by ion-exchange and gel-filtration chromatography as described previously [14]. Typically, around 10 mg of purified protein was obtained from a

1-liter culture. The identity and integrity of the final protein sample was confirmed by SDS-PAGE and LC-ESI/MS. CD spectra were recorded by using a J-810 spectropolarimeter (Jasco, Easton, MD) with protein in either 20 mM ammonium acetate, pH 7, or 20 mM sodium acetate, pH 5.5 [4].

Cold Pheromone Binding Assays

Binding was measured by separately incubating 5 μg of protein, with 1 μl, 3.2 mM of the major constituent of the sex pheromone, (Z,Z)-11,13-hexadecadienal (Z11,Z13–16Ald), in a 50 μl solution. The unbound and bound protein were separated using an ultracentrifugal device, Z11Z13–16Ald was extracted from the bound protein with hexane after lowering pH, and quantified by gas chromatography, according to a previously reported "cold binding assay" [4,8].

NMR Spectroscopy

Samples of wildtype and the various mutants of AtraPBP1 for NMR analysis consisted of ^{15}N-labeled protein (0.5 mM) [14] dissolved in 0.3 ml of a 95% H_2O/5% ^2H_2O solution containing 10 mM sodium acetate (pH 4.5) or 10 mM sodium phosphate (pH 7.0). All NMR experiments were performed at 25°C on Bruker Avance III 600 MHz spectrometer equipped with a four-channel interface and triple-resonance cryoprobe (TCI) with pulsed field gradients. The ^{15}N-^{1}H HSQC spectra were recorded on a sample of ^{15}N-labeled AtraPBP1 (in 95% H_2O, 5% ^2H_2O). The number of complex points and acquisition times were: 256, 180 ms (^{15}N (F1)); and, 512, 64 ms (^{1}H (F2)).

RESULTS AND DISCUSSION

Pheromone Binding Studies

Previous pheromone binding studies on wildtype AtraPBP1 showed that sex pheromone Z11,Z13–16Ald binds very tightly at high pH, in contrast to no detectable binding at low pH (Fig.1) [4]. A mutant sample of truncated AtraPBP1 protein (called AtraPBP1Δ129N-142V), that eliminates the C-terminal helix, was prepared under the same conditions as wildtype AtraPBP1. The truncation mutant showed about 1.5-fold higher affinity binding to Z11, Z13–16Ald compared to that of wildtype AtraPBP1 at pH 7 (Fig. 1). In stark contrast to the lack of any detectable pheromone binding to wildtype AtraPBP1 at pH 5, the truncation mutant retained its binding to Z11, Z13–16Ald at pH 5 (Fig. 1), although with lower affinity than binding at pH 7. The same phenomenon was observed previously for BmorPBP1 [11]. We then mutated H80 and H95 to examine their role in stabilizing the C-terminal helix via a histidine protonation switch [14]. AtraPBP1H80A/H95A, AtraPBP1H80A and AtraPBP1H95A each retained pheromone binding at low pH like that of the C-terminal truncation mutant, AtraPBP1Δ129N-142V (Fig. 2). At pH 7, the His mutants each showed equal or even higher binding affinity to Z11, Z13–16Ald than native AtraPBP1. In summary, the C-terminal truncation mutant and His mutants all showed relatively high affinity pheromone binding at pH 5 that contrasts with the lack of pheromone binding to wildtype AtraPBP1 at pH 5. The high affinity pheromone binding by each mutant at pH 5 suggests that the C-terminal helix is either destabilized or otherwise absent in these mutants to allow pheromone binding to both conformational forms at high and low pH.

Structural Analysis by NMR and Circular Dichroism

To verify that the mutants in this study are structurally intact, we recorded ^{15}N-^{1}H HSQC NMR spectra of each mutant (Fig. 3). The peaks in the spectra represent main chain and side-chain amide groups that serve as fingerprints of overall conformation. The NMR spectra of each mutant at pH 7.0 are fairly similar to that of wildtype, confirming that the

mutants all adopt a similar native tertiary structure. The ^{15}N-^{1}H HSQC spectra also indicate that the mutants are properly folded at low pH. However, the NMR spectra of the mutants at pH 4.5 are somewhat different from the spectrum of wildtype AtraPBP1 at pH 4.5. These spectral differences at pH 4.5 suggest that the C-terminal helix is destabilized by the His mutants. The unstructured C-terminus in these mutants at pH 4.5 leads to the exposure of hydrophobic residues in the protein core that have been implicated in pheromone binding [11,12] and perhaps explains why these mutants bind to pheromone at pH 4.5.

Near-UV circular dichroism (CD) spectra of AtraPBP1Δ129N-142V at high and low pH were somewhat different from that of wildtype AtraPBP1 (Fig. 4). The wildtype AtraPBP1 near-UV CD spectrum showed a maximum at 254 nm and three minima at 269, 278 and 287 nm. However, the near-UV spectrum of AtraPBP1Δ129N-142V showed a different result with a maximum at 292nm and minimum at 268 nm (Fig. 4). These spectral differences suggest there may be local structural changes near aromatic side-chain groups caused by the C-terminal truncation. Indeed, W37 and F76 make close contacts with the C-terminal helix in the NMR structure of AtraPBP1. The near UV CD spectra of AtraPBP1H80AH95A, AtraPBP1H80A and AtraPBP1H95A are all very similar to that of AtraPBP1Δ129N-142V, but very different from that of wildtype AtraPBP1. The spectral similarity between His mutants and C-terminal truncation mutant suggests that the C-terminal helix must be destabilized in the His mutants like the unstructured C-terminus seen in the structure of BmorPBP1 at pH 7.0 [11,12].

pH-dependent Extrusion of C-terminal Helix

The recent NMR structure of AtraPBP1 at pH 4.5 [14] shows a C-terminal helix (sequestered inside a protein cavity) that is attached to the protein core by salt bridges formed by H80/E132 and H95/E141 at each end of the helix. The C-terminal helix inside AtraPBP1 also interacts with residues that are implicated to interact with pheromone [11,12]. H80 and H95 were both measured to have side-chain pKa values near 5.5 [14]. Protonation of the H80 and H95 imidazole side-chains is expected to abolish the two salt bridges (H80/E132 and H95/E141) that in turn destabilizes the C-terminal helix at pH 7 (termed histidine protonation switch). Indeed, the structure of BmorPBP1 at pH 7 shows an unstructured and exposed C-terminus [11,12]. Mutations that substitute uncharged residues in the histidine protonation switch (H95A, D132N, and E141A) dramatically affect the pH-dependent binding of bombykol pheromone to BmorPBP1, demonstrating the functional importance of the switch [15]. In this study, we show that removal of the C-terminal helix promotes pheromone binding to the low pH form of AtraPBP1. Also, mutations of H80 and H95 disable salt bridges at the two ends of the C--terminal helix and allow pheromone binding at pH 4.5. We propose that sequestration of the C-terminal helix serves to block pheromone binding at low pH. Deprotonation of H80 and H95 at neutral pH abolishes salt bridges at the two ends of the C-terminal helix that promote detachment and extrusion of the helix from the protein core. This extrusion of the unstructured C-terminus allows exposure hydrophobic residues in the protein core that in turn interact with bound pheromone.

>pH-dependent pheromone binding to moth pheromone binding protein (AtraPBP1).

>C-terminal helix blocks pheromone binding at low pH.

>C-terminal deletion promotes pheromone binding at low pH.

>H80A and H95A mutants promote pheromone binding at low pH.

>Extrusion of C-terminal helix controls pH-dependent pheromone binding.

ABBREVIATIONS

PBP	pheromone-binding protein
ApolPBP1	*Antheraea polyphemus* pheromone-binding protein-1
AtraPBP1	*Amyelois transitella* pheromone binding protein-1
BmorPBP1	*Bombyx mori* pheromone-binding protein-1
HSQC	heteronuclear single quantum coherence
IPTG	isopropyl β-D-1-thiogalactoside
SDS PAGE	sodium dodecylsulfate polyacrylamide gel electrophoresis
SEC	size exclusion chromatography

Acknowledgments

We are grateful to Jerry Dallas for help with NMR experiments.

REFERENCES

1. Coffelt JA, Vick KW, Sonnet PE, Doolittle RE. J. Chem. Ecol. 1979; 5:955–966.
2. Leal WS, Parra-Pedrazzoli AL, Kaissling KE, Morgan TI, Zalom FG, Pesak DJ, Dundulis EA, Burks CS, Higbee BS. Naturwissenschaften. 2005; 92:139–146. [PubMed: 15668783]
3. Leal WS. Top. Curr. Chem. 2005; 240:1–36.
4. Leal WS, Ishida Y, Pelletier J, Xu W, Rayo J, Xu X, Ames JB. PloS ONE. 2009; 4:e7235. [PubMed: 19789654]
5. Syed Z, Ishida Y, Taylor K, Kimbrell DA, Leal WS. Proc. Natl. Acad. Sci. U S A. 2006; 103:16538–16543. [PubMed: 17060610]
6. Wojtasek H, Leal WS. J. Biol. Chem. 1999; 274:30950–30956. [PubMed: 10521490]
7. Damberger FF, Nikonova L, Horst R, Peng G, Leal WS, Wuthrich K. Protein Sci. 2000; 9:1038–1041. [PubMed: 10850815]
8. Leal WS, Chen AM, Ishida Y, Chiang VP, Erickson ML, Morgan TI, Tsuruda JM. Proc. Natl. Acad. Sci. U S A. 2005; 102:5386–5391. [PubMed: 15784736]
9. Sandler BH, Nikonova L, Leal WS, Clardy J. Chem. Biol. 2000; 7:143–151. [PubMed: 10662696]
10. Horst R, Damberger FF, Luginbuhl P, Guntert P, Peng G, Nikonova L, Leal WS, Wuthrich K. Proc. Natl. Acad. Sci. U S A. 2001; 98:14374–14379. [PubMed: 11724947]
11. Damberger FF, Ishida Y, Leal WS, Wuthrich K. J. Mol. Biol. 2007; 373:811–819. [PubMed: 17884092]
12. Lautenschlager C, Leal WS, Clardy J. Structure. 2007; 15:1148–1154. [PubMed: 17850754]
13. Keil TA. Tissue Cell. 1984; 16:705–717. [PubMed: 6515640]
14. Xu X, Xu W, Rayo J, Ishida Y, Leal WS, Ames JB. Biochemistry. 2010; 49:1469–1476. [PubMed: 20088570]
15. Xu W, Leal WS. Biochem Biophys Res Commun. 2008; 373:559–564. [PubMed: 18503757]

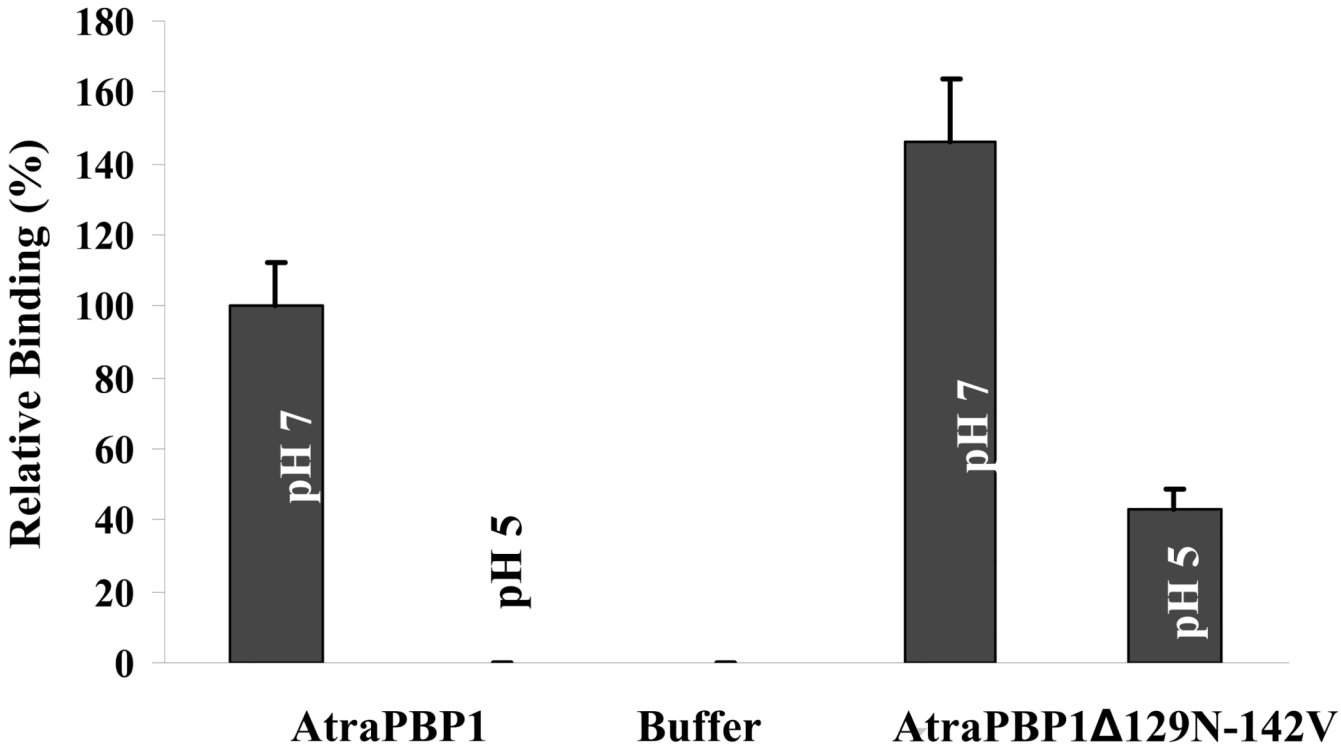


Figure 1.

Binding of the major constituent of the navel orangeworm sex pheromone, Z11,Z13–16Ald, to a pheromone-binding protein expressed in the pheromone-detecting sensilla in male antennae, AtraPBP1 and its C-terminal truncated form, AtraPBP1Δ129N-142V. The native conformation does not bind Z11Z13–16Ald at low pH, but binding in acidic conditions is somewhat retained in the truncated protein thus suggesting that the C-terminus plays a role in pheromone binding and release.

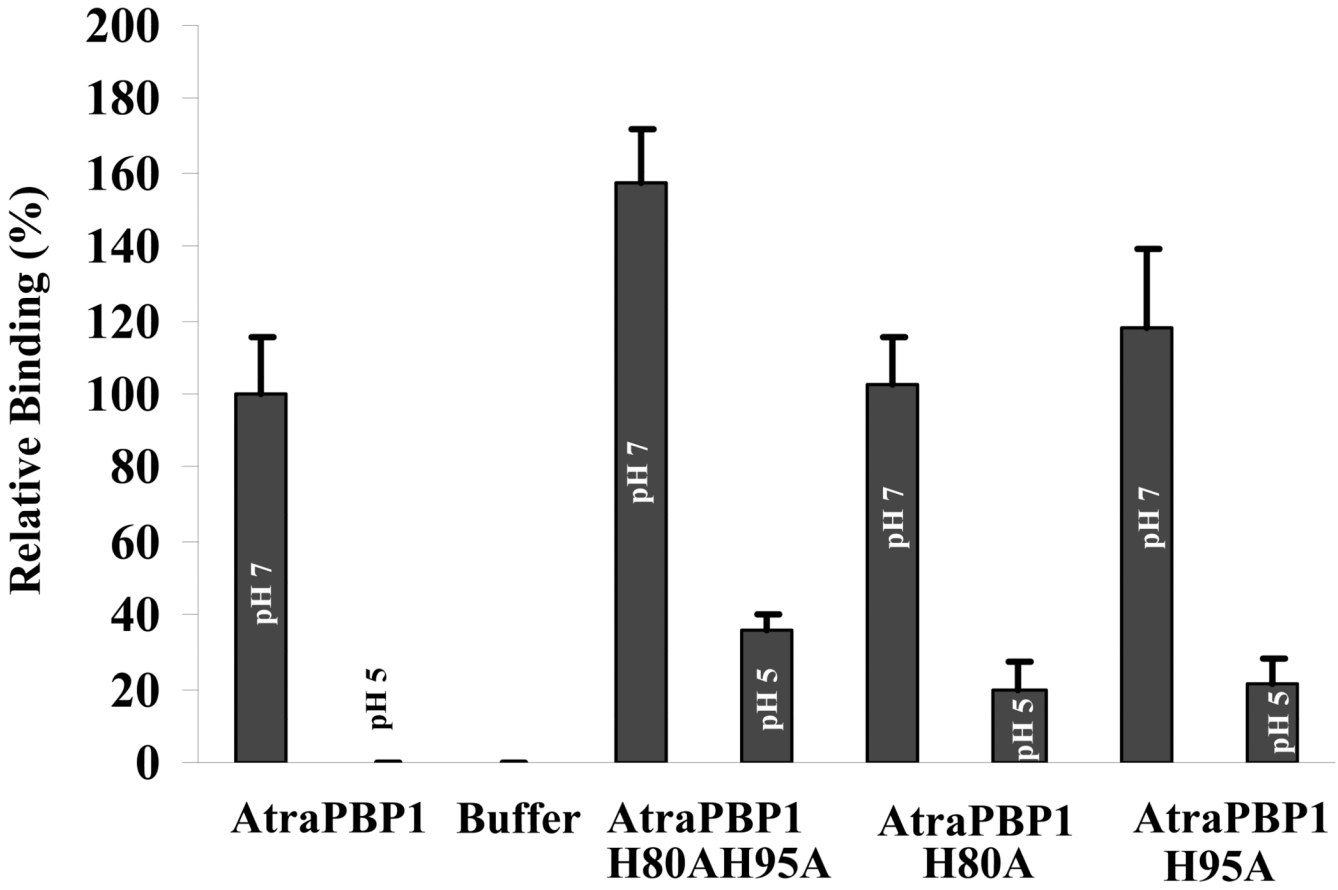
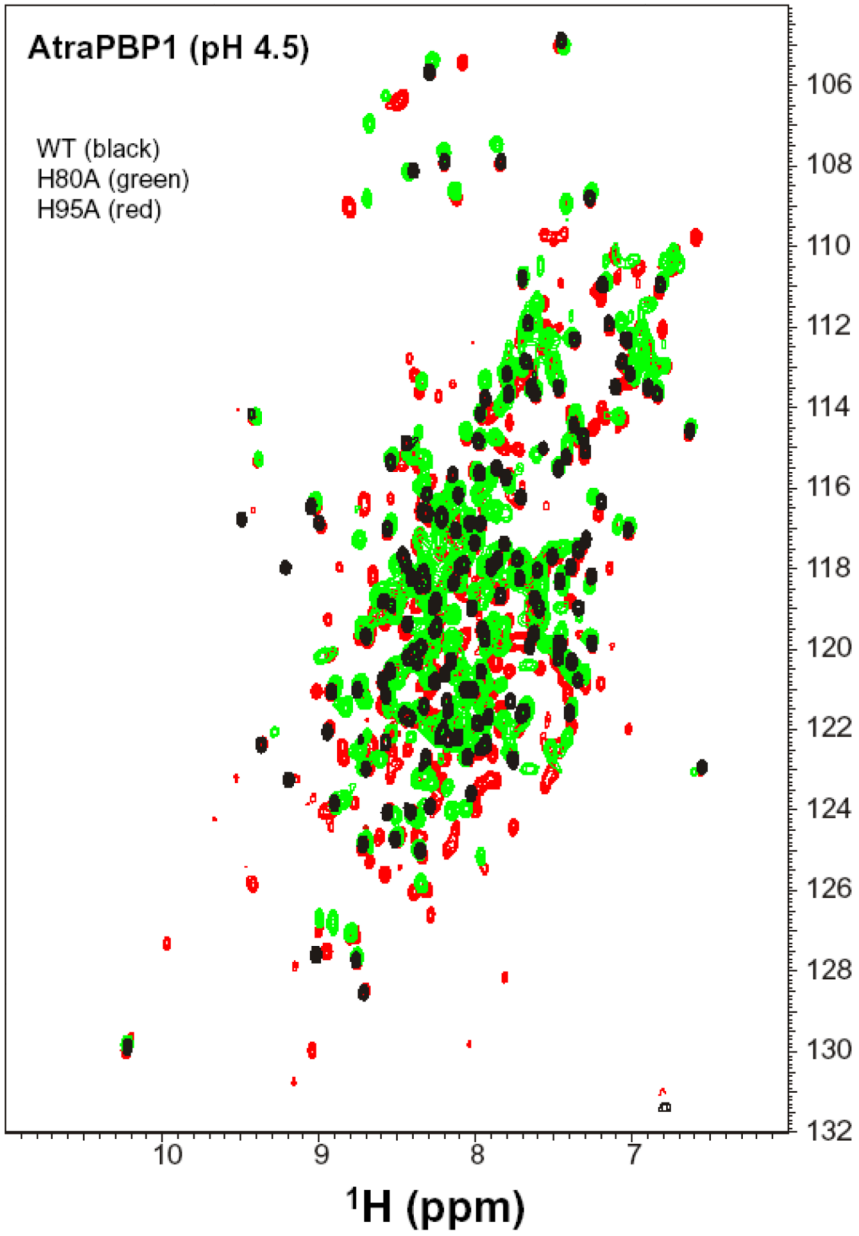


Figure 2.

Binding of Z11Z13–16Ald to AtraPBP1, AtraPBP1H80AH95A, AtraPBP1H80A and AtraPBP1H95A. Unlike native AtraPBP1, all these mutated proteins having both or only single histidine (His80 or His95) replaced by alanine retained binding to Z11Z13–16Ald at low pH. This result confirms two pH-dependent salt bridges involving H80 and H95 that control pheromone binding. The mutation of these two histidines destabilizes of the C-terminal helix and profoundly affects the pheromone binding affinity and overall structure of the low pH form.

3A



3B

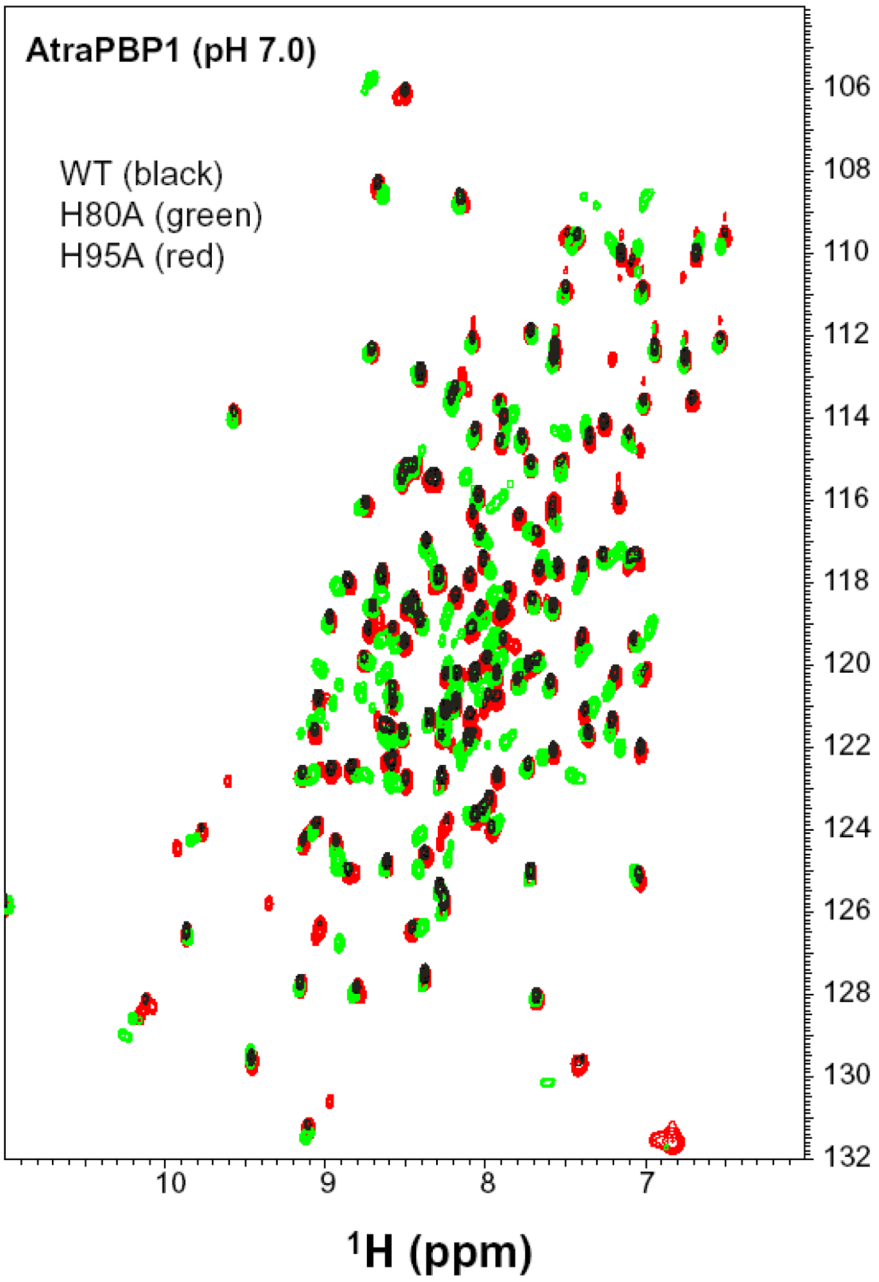


Figure 3.
2D ^{15}N-^{1}H HSQC spectra of ^{15}N-labeled AtraPBP1 at pH 4.5 (A) and pH 7.0 (B). Spectra are superimposed for wildtype (black), H80A (green) and H95A (red). All spectra were recorded at 600 MHz ^{1}H frequency and 298 K.

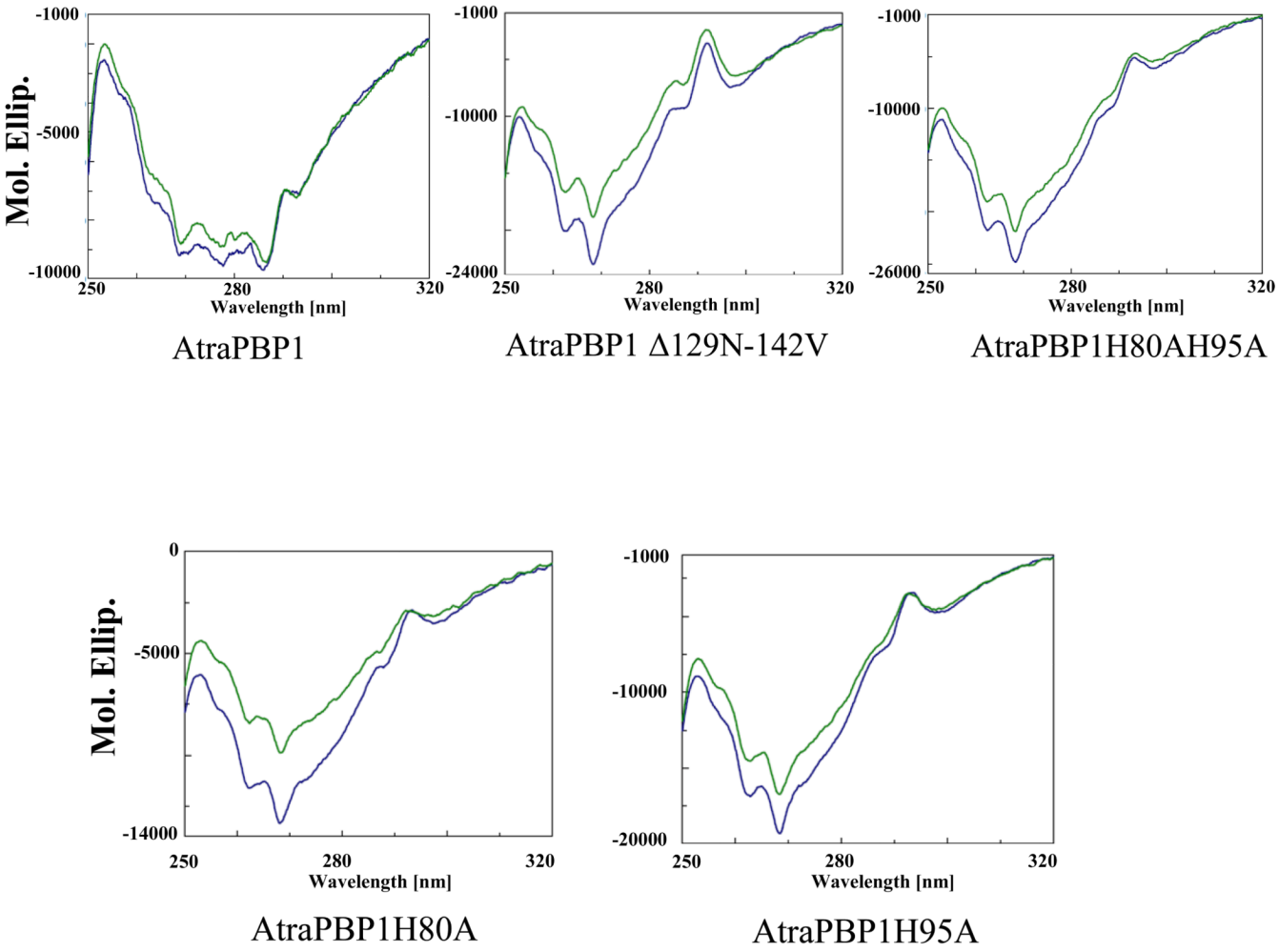


Figure 4.
Near-UV circular dichroism analysis of AtraPBP1, AtraPBP1H80AH95A, AtraPBP1H80A and AtraPBP1H95A at pH 7 (blue) and pH 5.5 (green).