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# Letter to the Editor: $^{1}H$, $^{13}C$ and $^{15}N$ backbone assignments of the pheromone binding protein from the silk moth *Antheraea polyphemus* (ApolPBP)

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## Biological context

The pheromone binding protein from the silk moth *Antheraea polyphemus* is located in the sensillum lymph of antennal hair of the male moth species. It is believed to be a carrier protein, responsible for the transport of volatile, hydrophobic sex pheromones to the chemosensory membranes of olfactory neurons through a G-protein coupled receptor mediated signal transduction process (Boekhoff et al., 1990). It has been reported that *Bombyx mori* PBP (BmPBP) undergoes a pH dependent conformational change (Damberger et al., 2000; Horst et al., 2001a,b; Lee et al., 2002) between two forms: $BmPBP^{A}$ (acidic form, below pH 5.0) and $BmPBP^{B}$ (basic form, above pH 6.0). Similar to BmPBP, for ApolPBP we observe a conformational transition between pH 5.0 and 6.0, with reduction of regular secondary structure at lower pH, as indicated by NMR and CD titrations between pH 4.0 and 8.0. In order to investigate the generality of the pH transition and conformational behavior observed for BmPBP we have initiated NMR studies on ApolPBP, and here we report complete backbone assignments at pH 6.3.

## Methods and results

Recombinant ApolPBP, consisting of 142 residues (67% identity with the 142-residue BmPBP), was expressed in *E. coli*, purified and refolded according to the first of the two protocols published (Prestwich, 1993). NMR samples contained 1 mM uniformly $^{15}N$- or $^{15}N/^{13}C$-labeled ApolPBP (95% $H_2O$/5% $D_2O$) in 50 mM phosphate buffer (pH 6.33) with 1 mM EDTA and 0.1% $NaN_3$. All NMR data were collected at 35 °C on Bruker DMX500, DMX600 and DRX800 spectrometers equipped with x,y,z-shielded gradient triple resonance probes. The following experiments were used for the sequential assignment of $^{1}H_N$, $^{1}H_\alpha$, $^{15}N$, $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}CO$ resonances: 2D $\{^{15}N,^{1}H\}$-HSQC (Figure 1A), 2D $\{^{13}C,^{1}H\}$-HMQC, 3D HNCA, 3D HNCO, 3D HNCACB, 3D CC(CO)NH, 3D CBCA(CO)NH, 3D HCACO, 3D $^{15}N$- and $^{13}C$-edited NOESY. Data were processed and analyzed using NMRPipe (Delaglio et al., 1995) and NMRView (Johnson and Blevins, 1994). 2D $\{^{15}N,^{1}H\}$-HSQC spectra collected over the range of 5 to 40 °C do not show significant conformational changes or denaturation. All six cysteinyl residues are in the oxidized state as indicated by their $^{13}C_\beta$ chemical shifts. The overall secondary structure of ApolPBP as determined from the CSI index is similar to that adopted by the different characterized forms of BmPBP (Figure 1B). It resembles closely $BmPBP^{B}$ and the bombykol-BmPBP complex (Sandler et al., 2000), as judged by the absence of the C-terminal helix $\alpha_7$, only present in $BmPBP^{A}$. The CSI of ApolPBP also shows the presence of a longer N-terminal $\alpha_1$ helix similar to that seen in $BmPBP^{B}$ and the complex. However, there are differences in the position of the helix $\alpha_1$ and the length of the helices between ApolPBP and $BmPBP^{B}$. Apart from sequence differences, this could possibly be due to the

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differences in the binding pocket, which dictates ligand recognition and specificity. Despite high sequence similarity, PBPs are ligand specific (Du et al., 1995).

## Extent of assignments and data deposition

Assignment of $^{15}$N, $^{1}$HN, $^{13}C_{\alpha}$, $^{13}C_{\beta}$ and $^{13}$CO backbone resonances was completed for all residues except S1, L8, S9 and N10, for which only the $^{13}C_{\alpha}$, $^{13}C_{\beta}$ and $^{13}$CO were determined. The assignment of $^{1}H_{\alpha}$ resonances was completed for 138 residues. The amino acid side-chain assignments of non-labile hydrogens are 91% complete.

The assigned $^{1}$H, $^{13}$C and $^{15}$N chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5689).

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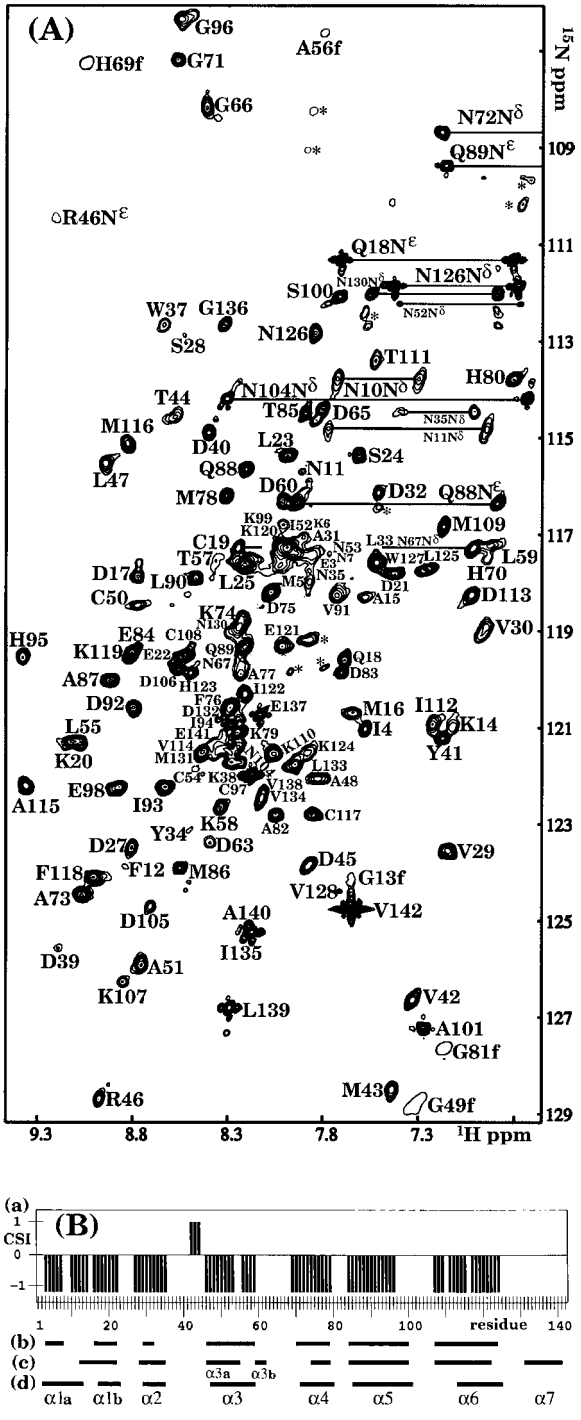


*Figure 1.* (A) {$^{15}$N,$^{1}$H}-HSQC spectrum of uniformly $^{15}$N/$^{13}$C-enriched ApolPBP. Asn and Gln side-chain $NH_2$ peaks are marked by horizontal lines. Unassigned signals – by asterisks. Folded peaks are shown as single contour and marked with 'f'. The backbone resonance of N72 and three side-chain resonances are outside the region shown. (B) The consensus CSI index ($H_{\alpha}$, $C_{\alpha}$, $C_{\beta}$ and CO) for ApolPBP (a). Indexes of +1, 0 and −1 indicate β-sheet, random coil and α-helical structure respectively. For comparison, alpha helical regions of BmPBP basic form (b), BmPBP acidic form (c) and the X-ray structure of BmPBP-bombykol complex (d) are shown here as thick horizontal lines.