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# Letter to the Editor: NMR assignment of the A form of the pheromone-binding protein of *Bombyx mori*

Reto Horst[a], Fred Damberger[a], Guihong Peng[b], Larisa Nikonova[b], Walter S. Leal[b,c] & Kurt Wüthrich[a,*]

[a]*Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule, CH-8093 Zürich, Switzerland;* [b]*National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305-8634, Japan;* [c]*Department of Entomology, University of California at Davis, 95616 Davis, CA, U.S.A.*

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## Biological context

The pheromone-binding protein from *Bombyx mori* (BmPBP) is present at high concentration in the lymph of insect olfactory sensilla, and transports a hydrophobic pheromone from the periphery of the sensillum to the olfactory receptors. BmPBP undergoes a pH-dependent conformational change between two forms A ('acidic form', observed at pH below 5.0) and B ('basic form', observed at pH above 6.0), which appears to have a direct relation to its biological function (Damberger et al., 2000). In the crystal structure of the B form complexed with the pheromone, the hydrophobic ligand is completely shielded from the solvent (Sandler et al., 2000). We have started NMR structure determinations of the A form and the ligand-free B form in order to obtain further insight into structure–function correlations of BmPBP. Here we report complete sequence-specific assignments for the A form of BmPBP.

## Methods and experiments

Recombinant BmPBP was expressed in *E. coli*. Details of the protein purification have been published elsewhere (Damberger et al., 2000). The NMR sample contained 10 mg of uniformly $^{13}C/^{15}N$-labeled BmPBP, which had been lyophilized from $H_2O$, in 0.5 ml of 50 mM potassium phosphate in 95% $H_2O$/5% $D_2O$ at pH 4.5 with 2 mM $NaN_3$.

*To whom correspondence should be addressed. Fax: +41 1 633 11 51.

NMR measurements were performed at 20 °C on a Bruker DRX 600 spectrometer. Proton chemical shifts are referenced to internal 3-(trimethyl-silyl)propane-1,1,2,2,3,3-$d_6$-sulfonic acid, sodium salt (DSS). $^{13}C$ and $^{15}N$ chemical shifts are referenced indirectly to DSS, using the absolute frequency ratios. Sequence-specific assignments (Wüthrich, 1986) of the polypeptide backbone resonances were initially obtained using 2D [$^{15}N$,$^1H$]-HSQC, 3D HNCA, 3D HNCACB, 3D CBCA(CO)NH and 3D HNCO spectra (Bax and Grzesiek, 1993), and residual gaps and ambiguities were resolved using sequential NOEs measured in 2D homonuclear and 3D heteronuclear-resolved [$^1H$,$^1H$]-NOESY spectra (Wüthrich, 1986). The chemical shifts of the $\alpha CH$–$\beta CH_n$ fragments provided the starting points for nearly complete $^1H$ and $^{13}C$ assignments of all $CH_n$ moieties in non-aromatic side-chains, using 2D ct-[$^{13}C$,$^1H$]-HSQC, 3D H(C)CH-TOCSY and 3D (H)CCH-COSY experiments (Gehring and Ekiel, 1998). $^1H$ spin systems of the aromatic rings of Trp, Tyr and Phe were identified using 3D TROSY-(H)CCH-COSY (Pervushin et al., 1998) and a TROSY version of the proton-relayed 2D [$^{13}C$,$^1H$]-COSY experiment (Zerbe et al., 1996), i.e., 2D $^1H$-TOCSY-relayed ct-[$^{13}C$,$^1H$]-TROSY (to be described elsewhere). Sequence-specific assignments of aromatic side chains were obtained using NOEs between the aromatic protons and the $\beta CH_2$ group or the $\alpha$-proton (Wüthrich, 1986), using 3D $^{13}C$-resolved [$^1H$,$^1H$]-NOESY. Stereospecific assignments for the isopropyl methyls of Val and Leu were obtained using biosynthetically directed fractional $^{13}C$-labeling (Senn et al., 1989) and 2D [$^{13}C$,$^1H$]-COSY. Methionine methyl

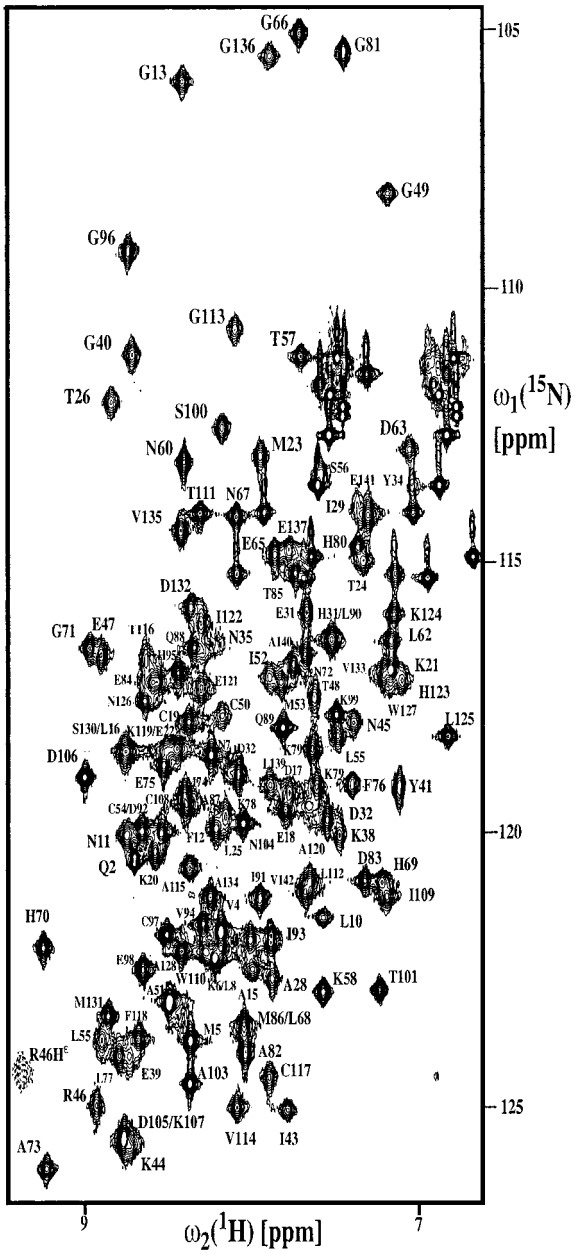


*Figure 1.* [$^1$H,$^{15}$N]-COSY spectrum of uniformly $^{15}$N-enriched BmPBP in 95% $H_2O$/5% $D_2O$ (protein concentration = 1 mM, T = 293 K, pH = 4.5). Backbone resonance assignments are indicated by the one-letter amino acid code and the sequence number. The backbone resonance of Ser 9 and the side-chain resonances of Trp 110 and Trp 127 are outside of the region shown.

groups were assigned in the course of the structure determination using 3D$^{13}$C-resolved [$^1$H,$^1$H]-NOESY. The NMR spectra were processed using the program PROSA (Güntert et al., 1992), and the spectral analysis was supported with the XEASY software package (Bartels et al., 1995).

## Extent of assignments and data deposition

All $^1$H, $^{15}$N and $^{13}$C polypeptide backbone resonances were assigned except for Ser 1, for which only the $C^{\alpha}H$ group was assigned. The amino acid side-chain assignments of non-labile hydrogens are complete except for $CH_3^{\varepsilon}$ of Met 5 and Met 23, and $C^{\zeta}H^{\zeta}$ of Phe 118. For Val 4, only one proton resonance and one carbon resonance were observed for the isopropyl methyl groups. The labile side-chain protons of Asn, Gln, Trp and Arg were completely assigned, except for $N^{\varepsilon 1}H^{\varepsilon 1}$ of Trp 37 and $H^{\eta}$ of Arg 46, whereby individual proton assignments were obtained for all 13 $NH_2$ groups of Asn and Gln. The $^1$H, $^{13}$C and $^{15}$N chemical shifts have been deposited in the BioMagResBank (http:/www.bmrb.wisc.edu) under the BMRB accession number 4849.

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