Structure–Function Relationship of PBAN/MRCH

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1. Introduction

Pheromone biosynthesis activating neuropeptides (PBANs) (Raina and Klun 1984) are C-terminally amidated neuropeptides that regulate sex pheromone biosynthesis in moths. To date, four different PBAN molecules were isolated and their primary structures have been determined. The first PBAN molecule (33 amino acids) was isolated from *Helicoverpa zea* (Hez-PBAN; Raina et al. 1989a). Two other molecules were isolated from *Bombyx mori*, Bom-PBAN-I (33 amino acids), and Bom-PBAN-II (34 amino acids) (Kitamura et al. 1989, 1990). A fourth molecule (33 amino acids) was recently isolated from *Lymantria dispar* (Lyd-PBAN, Masler et al. 1994). Hez-PBAN shares about 80% homology with Bom-PBAN and Lyd-PBAN. The C-terminal pentapeptide (Phe-Xxx-Pro-Arg-Leu-NH$_2$, Xxx = Ser) of all four PBAN molecules is identical and shares homology with the C-terminal pentapeptide of the pyrokinins, a family of myotropic peptides isolated from *Leucophea maderae* and *Locusta migratoria* (Xxx = Ser, Gly, Thr, Val; Schoofs et al. 1993). This C-terminal part is also homologous to the C-terminal region of two other neuropeptides: *Pseudaletia separata* pheromontropin (Pss-PT, 18 amino acids) (Matsumoto et al. 1992a) and *B. mori* diapause hormone (Bom-DH) (Imai et al. 1991). The PBAN gene of *H. zea* (Davis et al. 1992b; Ma et al. 1994) and the c-DNA of *B. mori* PBAN (Kawano et al. 1992; Sato et al. 1993) have recently been cloned. In both moths the gene sequences contain, in addition to the PBAN sequence, peptides having the common C-terminal pentapeptide (Phe-Xxx-Pro-Arg-Leu-NH$_2$) (Sato et al. 1993; Ma et al. 1994).

The presence of PBAN has been demonstrated in a variety of moths as well as nonlepidopteran species, and its mode of action has been studied in many laboratories (for reviews, see Raina 1993 and Altstein et al. 1993). These studies have revealed that the neuropeptide is present in larvae and in adult male and
female brain–subesophageal ganglia. The neuropeptide was also found in the corpora cardiaca–corpora allata complex of adult female moths as well as in the thoracic and abdominal ganglia. Natural and synthetic PBAN were found to activate pheromone biosynthesis in homologous and heterologous moth species during photophase and scotophase, and the activity of the neuropeptide was found to be mediated by the cyclic nucleotide adenosine 3’5’-monophosphate (cAMP) and dependent on the presence of Ca\(^{2+}\). Some aspects, related to the potential target organ of PBAN, its route of transport, its mode of action on the pheromone biosynthetic pathway in the gland, and the involvement of additional peptide and nonpeptide hormonal factors in its activity, are controversial and are under investigation in various laboratories (Raina, 1993; Altstein et al., 1993).

Identification of the amino acid sequence of Hez-PBAN (Raina et al. 1989a) and Bom-PBAN (Kitamura et al. 1989, 1990) enabled structure–activity analysis using synthetic PBAN and fragments derived from its sequence. Studies performed in several laboratories using various species of Heliothinae and \(B.\) \(mori\) revealed that the C-terminal region is important for the onset of biological activity whereas the N-terminal is not (Kitamura et al. 1989; Gazit et al. 1990; Raina and Kempe 1990, 1992; Kuniyoshi et al. 1991, 1992a). Within this region, the C-terminal pentapeptide was found to be the minimal sequence required for any pheromonotropic activity. Structure–function studies were also performed using other insect neuropeptides containing the PBAN pentapeptide C-terminal sequence (the pyrokinin peptide family, Bom-DH, Pss-PT). All of the above peptides stimulated pheromone biosynthesis and confirmed the importance of the C-terminal region for the onset of the pheromonotropic activity (Fónagy et al. 1992d; Kuniyoshi et al. 1992b; Nachman et al. 1993b; Schoofs et al. 1993; Abernathy et al. 1995).

Although the C-terminal region was found to be essential, the activity of the C-terminal pentapeptide itself was much lower than that of the full-length PBAN (Raina and Kempe 1990, 1992; Kuniyoshi et al. 1991, 1992a). Substitution of \(L\)-amino acids with \(D\)-amino acids in the pentapeptide, along with blockage of its N-terminus, yielded high activities, which were in some cases higher than those of PBAN 1-33NH\(_2\) (Kuniyoshi et al. 1992a; Raina and Kempe 1992). Furthermore, the N-terminus, which was not active by itself, was found to be essential for full potency. These results indicated that proteolytic enzymes might interfere with the bioassay and suggested that the presence of sequences within the PBAN molecule play a protective role against the enzymatic degradation \emph{in vivo}. To test this hypothesis and to determine which sequences have a conformational role and which protect the peptide against proteolytic degradation, we performed a detailed structure function analysis using PBAN-derived peptides in the presence and absence of protease inhibitors.

Matsumoto et al. (1990) purified and revealed the primary structure of a neuropeptide from heads of adult \(B.\) \(mori\) which induces cuticular melanization. The peptide was termed \emph{melanization and reddish coloration hormone} (Bom-