Melatonin binding by plant PR-10 proteins

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Melatonin holds a strong place as an animal hormone but its significance in the plant kingdom has been recognized only very recently. The physiological role of melatonin as a phytohormone is still under investigation and its binding partners are largely unknown. We have studied plant Pathogenesis-Related proteins of class 10 (PR-10) as potential melatonin binders. PR-10 proteins, with their canonical fold characterized by a large cavity formed between a β-sheet and an α-helix, have gained increasing recognition as versatile phytohormone binders. In the present study we demonstrate, by crystal structure determination, melatonin binding by two PR-10 proteins: Hyp-1 from St John's wort (*Hypericum perforatum*) and LlPR-10.2B from yellow lupine (*Lupinus luteus*). Hyp-1 binds melatonin in three unusual binding sites, two of which are internal chambers (1, 2), while the third one (3) is formed as an invagination of the protein surface. The electron density in site 2 does not allow unambiguous modeling of a melatonin molecule but suggests a melatonin degradation product. This pattern of ligand occupation is reproducible in repeated crystallization/structure determination experiments and is the same as in an Hyp-1/ANS complex. Among a number of potential natural mediators tested (including the cytokinin phytohormone *trans*-zeatin), melatonin was the only one to form a crystalline complex with Hyp-1. On incubation with melatonin, LlPR-10.2B also bound three ligand molecules. Two of them are bound within the capacious internal cavity, while a very well defined electron density near the cavity entrance suggests an unknown molecule, probably a product of melatonin dimerization. In a cocrystallization experiment with an equimolar mixture of melatonin and *trans*-zeatin, LlPR-10.2B formed a complex in which one of the melatonin binding sites is occupied by *trans*-zeatin, while the binding of melatonin at the second site and binding of the unknown ligand are unchanged. This unusual complex, when compared with the previously described PR-10/*trans*-zeatin complexes, provides an interesting insight into the involvement of PR-10 proteins in phytohormone regulation in plants, and implicates the PR-10 proteins as low-affinity melatonin binders under the conditions of elevated melatonin concentration.