Chapter 13
The Structure and Function of Ecdysone Receptors

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Abstract The ligand-binding properties of recombinant ecdysone receptor EcR-USP heterodimeric ligand-binding domains (LBDs) from four insect orders are described for a range of ecdysteroids and for a dibenzoylhydrazine (DBH) insecticide (tebufenozide). Much of the order selectivity of the insecticide in the field is reproduced by the affinity of tebufenozide for the recombinant LBDs in the laboratory. Crystal structures are presented for the LBDs of ecdysone receptors from the pest insects Heliothis virescens, Bemisia tabaci and Tribolium castaneum in complex with ponasterone A, as well as of the H. virescens LBD in complex with 20-hydroxyecdysone and BYI06830 (a DBH insecticide). Comparison of ecdysteroid- and BYI06830-bound structures of the H. virescens LBD illustrates the way in which this remarkable protein can adapt its binding pocket to very different ligand chemistries. Finally, comparison of the ligand-binding pockets of H. virescens, B. tabaci and T. castaneum ecdysone receptors begins to provide insights at an atomic level of detail into the insect order selectivity of the DBH insecticides.

Keywords Ecdysone receptor • ligand binding domain • 20-hydroxyecdysone • ponasterone A • tebufenozide • dibenzoylhydrazine • X-ray crystal structure

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13.1 Introduction

Insect metamorphosis has fascinated mankind since ancient times (Peck, 1970). The puffing of specific sites on the giant polytene chromosomes of *Drosophila melanogaster* at the end of larval life and preceding metamorphosis exemplifies the response of the genome to a rise in the titre of the moulting hormone 20-hydroxyecdysone (20E) in the hemolymph (Becker, 1962). Ashburner et al. (1973) proposed a formal model to explain control of the transcription of the vast network of genes whose activity is induced by the hormone and is manifested by puffing at over a hundred chromosomal sites. The first step in this model is the binding of 20E to a postulated receptor protein, the existence of which was suggested by the binding of tritiated hormone to *D. melanogaster* salivary gland chromosomal proteins (Emmerich, 1972) and by analogy to mammalian steroid receptors. The fact that the thiol-specific reagent, N-ethylmaleimide, inhibited the response to 20E was adduced as evidence strengthening the analogy, as this reagent had been shown to block the interaction of mammalian steroid hormones with their receptors (Ashburner, 1972). Ashburner’s experiments further demonstrated that pre-incubation with increasing concentrations of 20E appeared to protect the molecular target against reaction with N-ethylmaleimide.

The existence of the hypothetical ‘ecdysone receptor’ was put on a firm experimental basis by rigorous hormone binding studies employing tritiated ponasterone A ([3H]-ponA), a radiolabelled phyto-ecdysteroid that binds to the receptor more tightly than 20E (Yund et al., 1978). Despite the realization that the receptor was present only in trace amounts (10^3 molecules per cell), there then followed heroic biochemical attempts at its isolation, which at best yielded very small amounts of enriched/purified protein (Landon et al., 1988; Luo et al., 1991). A major advance was provided by the cloning of the *EcR* gene (Koelle et al., 1991) and the realization that the ecdysone receptor is actually a heterodimer of the EcR protein and the ultraspiracle protein product (USP) of the *usp* gene (Thomas et al., 1993; Yao et al., 1993). USP is an insect orthologue of the vertebrate RXR protein. In keeping with the central role played by 20E in insect development, mutations in the *EcR* gene are generally lethal even before the completion of embryonic development (Bender et al., 1997).

DNA sequencing and conceptual translation revealed that both EcR and USP proteins are members of the nuclear receptor family and display the characteristic domain structure of an N-terminal A/B domain (transcription activation), a C domain (DNA binding, very highly conserved), a D region (linker, shown to contain nuclear localisation signals in many nuclear receptors), an E domain (ligand-binding, moderately conserved), and in some cases a distinct F domain (Moras and Gronemeyer, 1998). Cloned DNA encoding EcRs and USPs led to the availability of recombinant EcR and USP proteins, as well as the corresponding ecdysone receptor heterodimers. There has been considerable interest in possible ligands for the USP protein in its own right, some of which may have biological significance (Billas et al., 2001; Jones et al., 2006).

In addition to their natural role in coordinating and controlling the expression of networks of genes in arthropod development and reproduction, ecdysone receptors have been employed as targets for environmentally-friendly insecticides and also in the control of transgenes (e.g. therapeutic genes) in mammalian cells.