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Report:

Oestrogens exert diverse effects on growth, development and differentiation of a wide range of tissues. The physiological effects of oestrogens are mediated by a ligand-inducible transcription factor termed the oestrogen receptor (ER) [1]. ER is an important target for chemotherapeutic drugs against certain reproductive cancers and for the treatment of osteoporosis and hormone replacement in menopausal women. A wide variety of synthetic compounds can bind to and either activate or inhibit ER depending on their effects on the receptor's two activation functions (AF1 and AF2). The balance between receptor agonism and antagonism elicited by such ER ligands are central to their therapeutic effects. Improved knowledge of the structural events that underlie these processes will undoubtedly facilitate the rational design of better drugs for the treatment of oestrogen-related conditions.

ER is a member of a large family of nuclear receptor transcription factors with a characteristic modular structural organisation with distinct domains associated with transactivation, DNA binding and hormone binding [1]. The C-terminal ligand-binding domain (LBD) is multifunctional and, in addition to harbouring a ligand recognition site, contains regions for receptor dimerisation and ligand-dependent (AF-2) transactivation. Hormone binding to ER-LBD induces a conformational change in the receptor that initiates a series of events that culminate in the activation or repression of responsive genes. The precise mechanism whereby ER affects gene transcription is poorly understood but appears to be mediated by numerous nuclear factors that are recruited by the DNA-bound receptor [2].

Our previous structural studies have demonstrated that the LBD undergoes structural alterations in the presence of different classes of ER ligand [3,4]. Accordingly, receptor agonists and partial- (AF2) antagonists each induce distinctive orientations in the LBD's carboxy-terminal helix (H12) — a region that contains the core of the receptor's ligand-dependent transactivation function (AF2). Only ER agonists elicit a

receptor conformation that results in the formation of a specific binding site for the consensus LxxLL motif of NR coactivators (unpublished data; [5]).

As part of our on-going studies on ER, we have solved the structures of several ER-ligand complexes during the review period:

A. Partial agonist ERβ complex: We have determined the structure of human ERβ-LBD in complex with the phyto-oestrogen genistein (GEN) at 1.8Å resolution [4]. Phyto-oestrogens are a diverse group of oestrogenic compounds produced by plants primarily as bactericidal and fungicidal agents. The presence of such compounds in the human diet appears to be beneficial and may even confer reduced risk to hormone-dependent breast and prostate cancer, heart disease and alleviate symptoms associated with the menopause. GEN binds to both ER isoforms with moderate affinity but exhibits a preference for ERβ acting as a partial agonist.

The hERβ-GEN complex was crystallised using the vapour diffusion technique at 18°C using PEG 6000 and sodium chloride as precipitants [4]. The resultant hexagonal rods belong to space group $P6_122$ and have unit cell dimensions of a=b=63.12Å c=250.23Å with one LBD molecule per asymmetric unit. Data were collected to 1.8Å resolution from a single crystal on station ID14-EH4. A total of 358818 observations were recorded and subsequently reduced to a unique set of 28523 reflections (99.7% data coverage between 60Å and 1.8Å) with a $R_{merge}(I)$ of 0.049. The structure of the complex was solved by molecular replacement (*AMoRe*) and refined with *REFMAC* using all available data. The final model has a R_{cryst} of 21.9 and R_{free} of 25.5.

The partial agonist, GEN, is completely buried within the hydrophobic core of the protein and binds in a manner similar to that observed for ER's endogenous hormone, 17β-oestradiol. However, unlike ER agonists, GEN binding to hERβ-LBD does not elicit the characteristic positioning of the C-terminal transactivation helix (H12) over the binding cavity. Instead H12 lies in a similar orientation to that induced by ER AF2 antagonists so that it occludes the coactivator binding site. While the origins of GEN's destabilising influence on H12 are unclear, such a sub-optimal alignment of the transactivation helix is consistent with this ligand's partial agonist character in ERβ. Presumably, the preferential occupation of the coactivator binding cleft by H12 in the GEN complex sets up a direct competition for this site with ER coactivators. Consequently, potential coactivators must first displace H12 into an 'agonist-like' conformation prior to binding.

B. ERα / NR-box peptide complex: A variety of nuclear factors termed coactivators serve as intermediaries between liganded NRs and the general transcription machinery [2]. p160 coactivators, such as transcriptional intermediary factor-2 (TIF2), are recruited by NRs in a ligand- and AF2-dependent through a distinctive common signature motif termed the NR-box which comprises the core consensus sequence LxxLL (where L is leucine and x is any amino acid).

We have solved the structure of oestradiol-liganded human ERα-LBD in complex a 12mer peptide derived from the NR-box III region of TIF2 at 2.4Å resolution. The ER / peptide complex crystals are monoclinic (a= 56.31Å b= 90.54Å c= 59.23Å β=109.83°) and contain one ERα homodimer per asymmetric unit. Data between 25 and 2.4Å (95% complete; $\langle I/\sigma I \rangle$ 7; $R_{merge}(I)$ 0.097) were collected on station ID14-EH4.

The NR-box III module binds in a helical conformation along the coactivator binding site — a shallow, hydrophobic groove on the surface of ER-LBD that is formed by residues from helices H3, H4, H5 and H12. As with other ER-coactivator complexes [5], the peptide conformation is stabilised by a 'charge clamp' interaction whereby N- and C-capping interactions are provided by a glutamic acid residue from H12 and a lysine located at the C-terminal end of H3 respectively.

Surprisingly the box III peptide binds in a different orientation to that observed in all previous NR-box / NR-LBD complexes. Both the hydrophobic pockets along the binding groove are occupied by leucine sidechains of the coactivator peptide. However, the peptide has 'corkscrewed' along the binding site by one residue. Consequently, both leucines of the LxxLL motif that are typically buried in other NR-box complexes are rotated out of the binding site altogether and exposed to solvent. As a result, the box III

interacting motif comprises LxxYL rather than the more typical LxxLL. The physiological significance of this altered binding mode is not clear but it illustrates the general principle that the binding groove of ERα can accommodate other sequence motifs apart from the characteristic LxxLL module. It appears, at least in the case of ERα, that the coactivator binding groove can accommodate several different large hydrophobics at the +4 position of the consensus motif.

C. ERβ / Full antagonist complex: The structure of rat ERβ-LBD in complex with the full (AF1/AF2) antagonist ICI 164,384 has been determined at 2.3Å resolution. This complex represents the first structural example of ER in the presence of a pure antioestrogen.

Crystals, grown using the hanging-drop vapour diffusion technique with PEG 2000 monomethylether as a precipitant, belong to space group $I2_12_12_1$ with cell dimensions *a*=59.37Å *b*=81.4Å *c*=520.97Å. However, these crystals contain five LBD molecules per asymmetric unit and are highly pseudo-symmetric. Although we were able to solve this structure using *AMoRe*, there is considerable disorder in certain regions of the asymmetric unit that precludes successful refinement. Fortuitously, brief exposure of the native crystals to chloromercuribenzene sulphonic acid (PCMBS) results in a fivefold reduction in the long *c* cell edge whilst maintaining the same space group (*a*=60.38Å *b*=82.67Å *c*=106.34Å). These PCMBS-treated crystals contain a single LBD monomer per asymmetric unit and diffract to 2.3Å on station ID14-EH4. A total of 185069 observations were recorded and subsequently reduced to a unique set of 12201 reflections (98.2% data coverage between 20Å and 2.3Å) with a $R_{merge}(I)$ of 0.06. The structure has been solved and refined (R_{cryst} 22.0 / R_{free} of 25.1).

The resultant ER-LBD conformation is fundamentally different from that observed in the presence of other classes of ER ligand. The full antagonist binds to ER so as to completely abrogate the interaction between the H12 transactivation helix and the remainder of the LBD. In addition, ICI 164,384 perturbs the arrangement of monomers within the receptor homodimer so that the extent of the dimer interface is significantly reduced and more accessible to solvent. The observation that the full antagonist promotes an orientation of ER's carboxy-terminal helix that is distinct from that seen previously in the presence of AF2-antagonists is consistent with these ligands' distinctive antagonistic characters and provides some clues as to the origins of full antagonism.

References:

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