ESRF	**Experiment title:** Structural Studies on Oestrogen Receptor Isoforms	**Experiment number:** ls-1070/ls-1071
Beamline: ID14-4	**Date of experiment:** from: 15th to: 17th Dec. 98	**Date of report:** 23/8/99
Shifts: BAG	**Local contact(s):** Sean McSweeney	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Ashley C.W. Pike* & A. Marek Brzozowski*

Structural Biology Laboratory, Chemistry Department, University of York, York YO10 5DD, United Kingdom

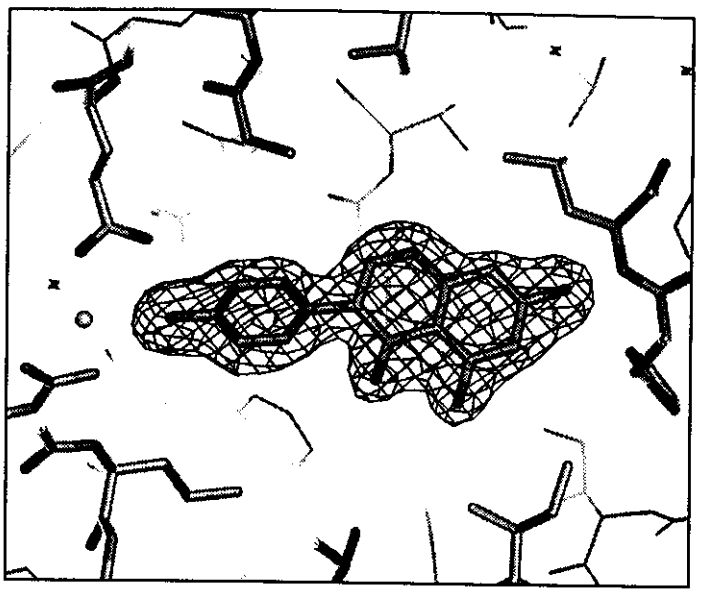
Report:

Oestrogens play a critical role in the growth, development and maintenance of a diverse range of tissues. They exert their physiological effects via the oestrogen receptor (ER), which functions as a ligand-activated transcriptional regulator [1]. Until recently, these effects were attributed to a single ER. The unexpected discovery of a second ubiquitous ER, termed ERβ [2], has added another layer of complexity to the action of oestrogens and prompted intense interest in the respective role of each isoform. The two ER isoforms exhibit overlapping but distinct tissue distribution patterns and differ in their ligand-binding ability and transactivational properties [3].

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.

As part of our on-going studies on ER, we have recently solved the structure of human ERβ-LBD in complex with the phyto-oestrogen genistein (GEN) [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 [3].

The hERβ-GEN complex was crystallised using the vapour diffusion technique at 18°C. Drops were composed of equal volumes of protein (8 mg ml^{-1}) and reservoir solution (6-9% (w/v) PEG 6000, 1.6-2.1M NaCl in 0.1M Tris *p*H8.1). The resultant hexagonal rods belong to space group $P6_122$ and have unit cell dimensions of $a=b=63.12$Å $c=250.23$Å with one LBD molecule per asymmetric unit. Data were collected to 1.8Å resolution from a single crystal on station ID14-EH4 using two sweeps. All data were integrated and reduced using DENZO and SCALEPACK. 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), using the coordinates of the ERα-LBD monomer (PDB entry: 1ERE; [5]) as a search model, and refined with REFMAC using all available data. The final model has a R_{cryst} of 21.5 and R_{free} of 25.2 for all data between 55 and 1.8Å.



Omit $|F_{obs}| - |F_{calc}|$ electron density map for GEN contoured at 3σ.

As expected, the overall structure of hERβ-LBD is very similar to that previously reported for ERα [5]. Twelve helices are arranged into a three-layered, anti-parallel α-helical sandwich motif. 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 (*see figure*). 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. We, and others, have shown that this precise alignment of H12, which occurs in the presence of agonists, allows ER to efficiently interact with coactivator proteins via the formation of a specific recruitment surface [6]. Instead, in the GEN complex, H12 lies in a similar orientation to that induced by ER 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.

References:

1. Tsai, M-J. & O'Malley, B.W. (1994) *Annu. Rev. Biochem.* **63**, 451-486.
2. Kuiper, G.G.J.M. *et al.* (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5925-5930.
3. Kuiper, G.G.J.M., *et al.* (1997) *Endocrinology* **138**, 863-870.
4. Pike, A.C.W. *et al.* (1999) *EMBO J.* **18**, 101-111.
5. Brzozowski, A.M. *et al.* (1997) *Nature* **389**, 753-758.
6. Moras, D. & Gronemeyer, H. (1998) *Curr. Opin. Cell Biol* **10**, 384-391.