doi:10.1017/S1431927617006596
Microsc. Microanal. 23 (Suppl 1), 2017

Structure of the Insulin Receptor in Complex with Insulin using Single Particle CryoEM Analysis

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Insulin Receptor (IR) mediated signaling is crucial in controlling glucose homeostasis, regulating lipid, protein and carbohydrate metabolism, and modulating brain neurotransmitter levels [1, 2]. Aberrations in Insulin signaling have been associated with a variety of disease states, including diabetes, cancer and Alzheimer's [1, 3, 4]. IR is composed of two heterodimers (α and β chains), each containing an extracellular portion (ectodomain), a single transmembrane helix (TM), and a cytoplasmic tyrosine kinase domain (TK) (Figure 1). One single disulfide bond links the α and β chains in the monomer, while the dimer is stabilized by two interchain disulfide bonds (Figure 1). Insulin is thought to bind to two distinct sites (per monomer), in a complex process that exhibits negative cooperativity [5]. Insulin binding site 1 was mapped by alanine scanning to portion of the L1 domain (Asp12-Asn15, Leu37, Phe39, Phe64 and Arg65) and to the αCT helix (Gln692-Pro718) located at the C-terminal end of the ID domain. Site 2 was mapped to loop regions near the junction between the FNIII-1 and FNIII-2 domains [5 and references therein].

Although insulin and IR have been studied for over 40 years, the structure of the IR ectodomain and the atomic details of the interactions with insulin are poorly understood. Only a medium resolution (3.3 Å) structure for the IR ectodomain, in complex with two FABs, has been reported to date (PDB id 4ZXB) [6], and it represents the un-ligated form. This structure contains one monomer per asymmetric unit; a V-shaped dimer is generated by crystallographic symmetry (Figure 2A). More detailed information on the interaction between insulin and its receptor were illustrated when low resolution (3.9-4.4 Å) crystal structures of Insulin bound to an "Insulin microreceptor" (L1 and CR domains, plus exogenous αCT helix) were published [7, 8]. These structures show that insulin is mostly bound to the αCT helix, and barely interacts with L1. Overlay of the insulin-microreceptor structures onto the dimeric receptor (using L1 as reference, Figure 2B) suggests that insulin binding site 1 involves L1 from one monomer and αCT from the other. In addition, given the large steric clashes between the insulin and the FNIII-1 and FNIII-2 domains, and the αCT helix, some large conformational change must happen upon insulin binding.

Crystallization of the IR ectodomain is impaired by several factors, including its intrinsic flexibility. The use of alternative structure determination techniques may bypass these issues and still provide high resolution structures. Over the past 5 years, single particle cryoEM has proven to be a powerful alternative to the more traditional structure determination methods [9], and it may potentially provide insights into the dynamics of complex systems. We report here a 6.8 Å preliminary map (Figure 2C) of the IR ectodomain in the presence of a 10x excess of insulin obtained using single particle cryoEM analysis of vitrified samples. The individual domains can be assigned, although positioning of the insulin has not yet been achieved. The map suggests that the receptor is dimeric and it has a mushroom-like shape, with the FNIII-2 and FNIII-3 domains forming the stem. Although the IR is mostly β-

strands and loops, there appears to be clear density for an alpha helix between L1 and FNIII-1; if this density corresponds to the αCT, it appears to be about 40 Å away from the position observed in the crystal structure. While L2 and FNIII1-3 superimpose well to the corresponding domains in 4ZXB, the L1 and CR domains move as much as 60 Å from the position in the crystal structure. Further work to improve the resolution of the structure is in progress but even at this stage the map allows for visualization of the conformational changes that occur upon insulin binding and provide an improved understanding of the system.

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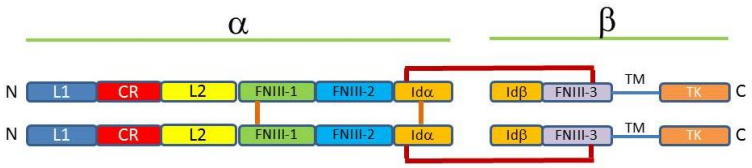

Figure 1. Schematic representation of the IR dimer. The disulfide bonds are indicated by orange lines. The ectodomain contains two leucine-rich domains (L1 and L2), a cysteine-rich domain (CR) and three fibronectin type III domains (FNIII-1, 2, and 3). An Insert Domain (ID) connects FNIII-2 and FNIII-3 and contains the αβ proteolytic site.

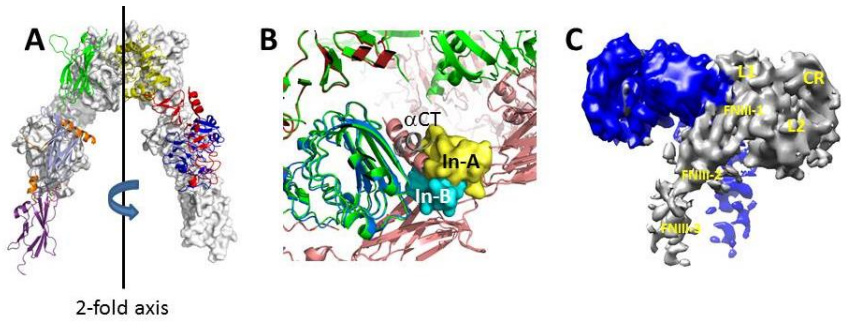

Figure 2. A) The proposed IR dimer from PDB 4ZXB. One monomer is shown as ribbon, with the domains color-coded as in figure 1. The crystallographic mate is shown as a grey surface. B) Overlay of the microreceptor (blue and red ribbon for L1 and CR, and grey αCT)- insulin (yellow and cyan) complex onto the chain A (green) of the proposed IR dimer. The symmetry mate is shown in pink. C) 6.8 Å map of the IR in the presence of insulin. The two monomers are colored in blue and grey. The individual domains are labelled.

https://doi.org/10.1017/S1431927617006596