LETTER

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Structure of the glucagon receptor in complex with a glucagon analogue

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Class B G-protein-coupled receptors (GPCRs), which consist of an extracellular domain (ECD) and a transmembrane domain (TMD), respond to secretin peptides to play a key part in hormonal homeostasis, and are important therapeutic targets for a variety of diseases[1–8]. Previous work[9–11] has suggested that peptide ligands bind to class B GPCRs according to a two-domain binding model, in which the C-terminal region of the peptide targets the ECD and the N-terminal region of the peptide binds to the TMD binding pocket. Recently, three structures of class B GPCRs in complex with peptide ligands have been solved[12–14]. These structures provide essential insights into peptide ligand recognition by class B GPCRs. However, owing to resolution limitations, the specific molecular interactions for peptide binding to class B GPCRs remain ambiguous. Moreover, these previously solved structures have different ECD conformations relative to the TMD, which introduces questions regarding inter-domain conformational flexibility and the changes required for receptor activation. Here we report the 3.0 Å-resolution crystal structure of the full-length human glucagon receptor (GCGR) in complex with a glucagon analogue and partial agonist, NNC1702. This structure provides molecular details of the interactions between GCGR and the peptide ligand. It reveals a marked change in the relative orientation between the ECD and TMD of GCGR compared to the previously solved structure of the inactive GCGR–NNC0640–mAb1 complex. Notably, the stalk region and the first extracellular loop undergo major conformational changes in secondary structure during peptide binding, forming key interactions with the peptide. We further propose a dual-binding-site trigger model for GCGR activation—which requires conformational changes of the stalk, first extracellular loop and TMD—that extends our understanding of the previously established two-domain peptide-binding model of class B GPCRs.

Activation of GCGR by its endogenous ligand glucagon triggers the release of glucose from the liver during fasting, and thus has an important role in glucose homeostasis and is a potential drug target for type 2 diabetes[15]. We recently determined the crystal structure of the full-length GCGR in an inactive state in complex with the negative allosteric modulator (NAM) NNC0640 and the antigen-binding fragment of an inhibitory antibody mAb1 (ref. 16). To further understand the molecular mechanisms of peptide binding and receptor activation of GCGR, we solved the 3.0 Å-resolution crystal structure of the full-length GCGR bound to a glucagon analogue and low-potency partial agonist, des-H1-[E9, K24(4×γE), L27]glucagon (NNC1702) (Fig. 1a, Extended Data Fig. 1 and Extended Data Table 1; see Methods for design of this partial agonist).

In the structure of the GCGR–NNC1702 complex, the ECD and the bundle of seven transmembrane helices (I–VII) in the TMD adopt similar conformations to those of the corresponding domains in the previously determined structure of the GCGR–NNC0640–mAb1 complex, with C_α root-mean-square deviations of 1.2 Å and 1.5 Å, respectively. However, the relative orientation between the ECD and TMD in the peptide-bound GCGR structure differs markedly from that in the inactive GCGR–NNC0640–mAb1 structure (Fig. 1b, c). This was expected, considering that the ECD orientation in the GCGR–NNC0640–mAb1 structure is not compatible with the two-domain peptide-binding model for class B GPCRs[16]. Comparison between the GCGR–NNC1702 structure and the recently determined structures of peptide-bound glucagon-like peptide-1 receptor (GLP-1R) shows that the orientation of the ECD relative to the TMD is similar in the GCGR–NNC1702 structure and the structure of GLP-1R bound to glucagon-like peptide-1 (GLP-1) and Gs protein solved by cryo-electron microscopy[13] (Extended Data Fig. 2a, b), both of which contain the peptide ligands that interact with both the ECD and TMD. However, the ECD orientation in the crystal structure of the truncated peptide agonist (peptide 5)-bound GLP-1R[14] is substantially different from that in the other two structures (Extended Data Fig. 2c, d). This may be due to a lack of interactions between the ECD core and the truncated peptide ligand that either enables greater conformational flexibility or promotes a unique inter-domain conformation.

The GCGR–NNC1702 structure reveals secondary structure modifications of the stalk region (residues G125–K136) and the first extracellular loop (ECL1; residues S203–A220), compared to the inactive GCGR–NNC0640–mAb1 structure (Fig. 2a). These two regions of GCGR have previously been suggested[16,17] to be important modulators that regulate peptide ligand binding and receptor activation. In the GCGR–NNC0640–mAb1 structure, the N-terminal portion of the stalk (residues G125–Q131) and ECL1 (residues R201–S217) exhibit extended β-strand conformations and make close contacts with each other; they form a compact β-sheet structure, which is likely to stabilize the receptor in an inactive conformation[16]. By contrast, the stalk in the peptide-bound GCGR structure forms a 3-turn α-helical extension of

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