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Expression, purification and preliminary characterization of glucagon receptor extracellular domain

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ABSTRACT

Glucagon is a pancreatic hormone that plays pivotal roles in regulating glucose homeostasis and metabolism. Glucagon exerts its action by binding to its receptor, glucagon receptor (GCGR), one of class B G-protein coupled receptors (GPCRs). Diabetes is a bihormonal disease in which excessive glucagon secretion is a major contributor in the pathogenesis of this disease; elucidation of how glucagon binds to GCGR will facilitate the rational design of the GCGR antagonist for treating diabetic hyperglycemia. Here we report the successful expression and purification of the GCGR extracellular domain (GCGR-ECD) and its fusion protein with the glucagon peptide at its C-terminus (GCGR-ECD-Gc). We utilized the maltose binding protein (MBP) fusion method and disulfide bond isomerase DsbC co-expression approach for the success of the soluble expression of both GCGR-ECD and GCGR-ECD-Gc in *Escherichia coli*. We also obtained a high yield production of secreted GCGR-ECD with the baculovirus expression system by optimizing its N-terminal secreting signal. We first utilized isothermal titration calorimetry approach to determine the *in vitro* binding affinities of glucagon to the GCGR-ECD. No significant differences were found between the prokaryotic expressed GCGR-ECD (7.6 μM) and the eukaryotic glycosylated one (6.6 μM). The observation of the intra ligand-receptor binding within the fusion protein GCGR-ECD-Gc suggests it as a good candidate for further structural study.

Introduction

Glucagon, a 29-amino-acid peptide produced by pancreatic alpha cells plays a crucial role in maintaining glucose homeostasis through activation of the glucagon receptor (GCGR)[1], a G-protein coupled receptor, expressed in a variety of tissues including liver and brain [1]. In conditions of hypoglycemia, the secretion of glucagon is increased to facilitate hepatic glucose production mainly through glycogenolysis and gluconeogenesis [2]. Glucagon plays an essential role in preventing hypoglycemia during fasting. However, a lack of glucagon suppression contributes to the postprandial hyperglycemia in type 2 diabetes patients [3]. Diabetes mellitus has been regarded as a bihormonal disorder, characterized by both insulin deficiency and glucagon excess [4]. Recent studies demonstrated that excessive glucagon secretion or action, rather than insulin deficiency, is predominant for the development of diabetic manifestations [5,6]. Therefore, pharmacological interventions that reduce glucagon secretion or block glucagon receptor signaling are promising approach for treating diabetes. Indeed, several glucagon receptor antagonist and antibodies that can improve glycemic control in animal models and humans have been reported recently [7–9].

Glucagon receptor belongs to the class B G-protein coupled receptors (GPCRs), all of which possess a large extracellular N terminal domain of 100–150 amino acids, which is the main determinant for the ligand binding specificity and plays a crucial role in receptor activation [10]. Although the structures of full-length class B GPCRs remain unknown [11], many structures of their extracellular domains (ECDs) have been determined by X-ray crystallography or nuclear magnetic resonance (NMR) spectrometry and most of them are the structures of the ligand-receptor complexes [12]. These structures revealed a detailed map of ligand-receptor interactions and gave insights into the ligand binding specificity and selectivity [13]. The bimolecular interface of the glucagon-GCGR complex has been extensively studied via a variety of molecular and biochemical methods [14,15]. The crystal structure of GCGR-ECD seized with a specific antibody was reported

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[1] *Abbreviations used:* GCGR, glucagon receptor; GPCRs, G-protein coupled receptors; NMR, nuclear magnetic resonance; ITC, isothermal titration calorimetry.

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recently to structurally map the glucagon binding surface [16]. However, without the structure of GCGR-ECD bound with glucagon, their detailed interactions and therefore the regulated conformation are still largely unknown, which has been becoming the obstacle of designing rational GCGR antagonists.

Here we report the successful expression and purification of GCGR-ECD by using both *Escherichia coli* expression and the insect cell expression system. We, for the first time, measured the *in vitro* binding affinities of glucagon to GCGR-ECDs by using isothermal titration calorimetry (ITC). We also designed the GCGR-ECD fusion protein with the glucagon peptide at its C-terminus (GCGR-ECD-Gc) and got it expressed in *E. coli*. ITC assays showed the intra ligand-receptor binding existed within the fusion protein, suggesting it a good candidate for future crystallographic study.

Materials and methods

Materials and reagents

The Prime Star HS DNA polymerase used for PCR and the T4 DNA ligase used for ligation were from Takara (Dalian, China). Restriction enzymes and pMalC2x plasmid were from NEB (MA, USA). The competent *E. coli* cell (one Shot®Mach1™-T1R, MT), insect cell line Sf21, culture medium SF-900 II SFM and the transfection reagent Cellfectin II were from Invitrogen (CA, USA). The BacMagic™ DNA kit, pET40b, pETDuet-1, pIEx/Bac™-4 vectors and the *E. coli* strains Origami B (DE3) were from Novagen. The chromatographic resins and columns (Superdex 75, Superdex 200 and Mono Q) were from GE Healthcare (MA, USA). The ultra-filtration apparatus were from Millipore (MA, USA). The

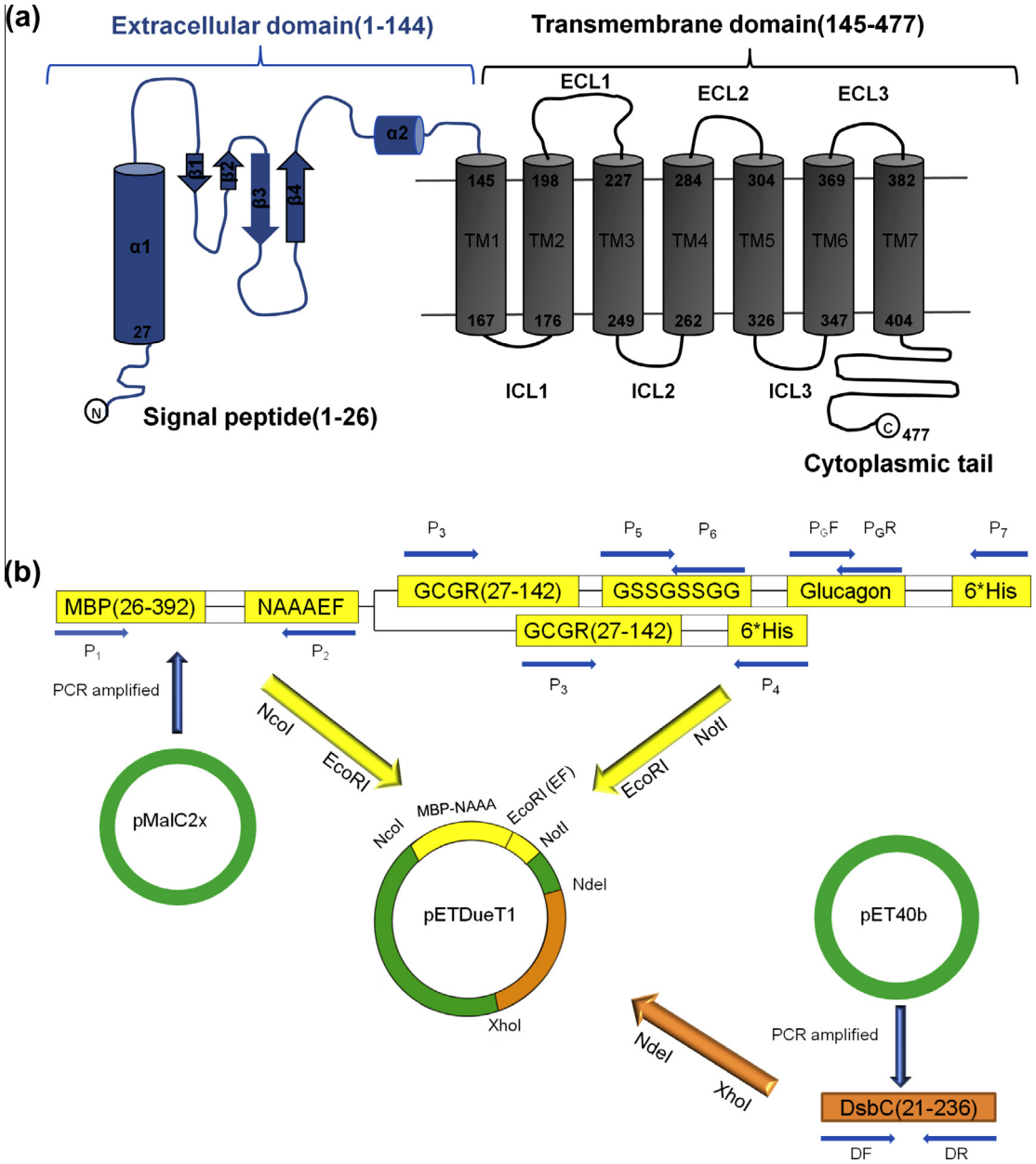

Fig. 1. Constructions of GCGR-ECDs for prokaryotic expression. (a) A cartoon representation of the predicted topology of GCGR with the extracellular domain highlighted in blue. The numbers denote the amino acid position in the sequence of GCGR. (b) Schematic representation of the construction of the MBP fusion GCGR-ECD and GCGR-ECD-Gc, which were co-expressed with DsbC by using pETDuet-1 vector. The sequences of all primers are listed in Table 1. MBP, maltose binding protein; GCGR, glucagon receptor; DsbC, protein disulfide bond isomerase. The vectors pET40b, pMalC2x and pETDuet-1 are labeled accordingly. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)