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UGGT1 retains proinsulin in the endoplasmic reticulum in an arginine dependent manner

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Abstract

We sought to clarify a pathway by which *L*- and *D*-arginine simulate insulin secretion in mice and cell lines and obtained the following novel two findings. (1) Using affinity magnetic nanobeads technology, we identified that proinsulin is retained in the endoplasmic reticulum (ER) through UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1) when arginine availability is limited. (2) *L*- and *D*-arginine release proinsulin from UGGT1 through competition with proinsulin and promote exit of proinsulin from the ER to Golgi apparatus. The ability of arginine to release proinsulin from UGGT1 closely correlates with arginine-induced insulin secretion in several models of β cells indicating that UGGT1-proinsulin interaction regulates arginine-induced insulin secretion.

Graphical Abstract

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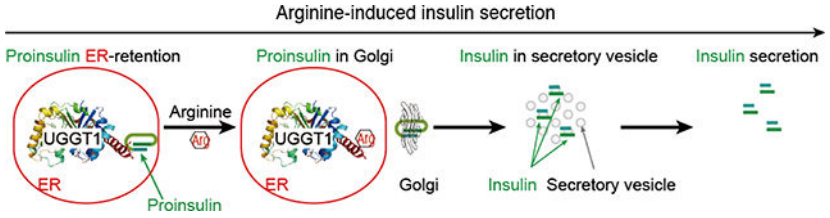
Author Contribution

JC, MH, YM, and SS performed the experiments, TI designed the experiments, analyzed the data, and wrote the manuscript. YoI, YuI and HH interpreted the data and wrote the manuscript.

Competing interest

The authors declare no competing financial interests.

Supplemental information is available in the online version of the article.



Keywords

Arginine; Insulin; Endoplasmic reticulum; UGGT1

Introduction

Insulin secreted from pancreatic β cells plays a central role in glucose homeostasis and the impairment in insulin secretion leads to diabetes[1–3]. Glucose is a principal stimulator of insulin secretion and glucose metabolism in pancreatic β cells is coupled with insulin secretion[4]. Glucose is taken up by β cells through glucose transporter 1 and 2 (GLUT1 and 2, solute carrier family 1 and 2; SLC2A1 and 2) is converted to glucose-6-phosphate by glucokinase (GCK) to initiate glucose metabolism that ultimately closes K_{ATP} channel and induces Ca^{2+} influx by opening the voltage-gated Ca^{2+} channels leading to exocytosis of insulin granules[4].

The semi-essential amino acid arginine that is mainly supplied from food augments insulin secretion when there is a permissive concentration of glucose[1,2,5,6], and isomer *D*-arginine also stimulates insulin secretion[5]. When arginine reaches the pancreatic β cells, arginine is transported into β cells by cationic amino acid transporter 1–2 (CAT1–2, SLCA1–2)[7–9]. It is proposed that uptake of arginine by the electrogenic transporter CAT1–2 generates a depolarizing current initiating Ca^{2+} influx and insulin secretion in the presence of a permissive concentration of glucose[4,6]. As arginine induced insulin secretion is considered to be independent from metabolism through the TCA cycle and oxidative phosphorylation, it has been widely used to evaluate metabolism independent secretory function of β cells *in vivo* and *in vitro*[6,10,11]. However, there appears to be additional pathways by which arginine promotes insulin secretion. Interestingly, both *L*- and *D*-isomers of arginine are capable of inducing insulin secretion[5]. As CAT1–2 are stereospecific and only mediate uptake of *L*-amino acid, a mechanism by which *D*-arginine stimulates insulin secretion is unanswered.

Other known membrane receptors for arginine include GPRC6A (G-protein-coupled receptors, GPCR[12,13]) and amino acid taste receptor (TAS1R5–24[14]). In addition to *L*-amino acids, ligands of GPRC6A include osteocalcin, steroids, and androgen, some of which are not strong insulin secretagogues making GPRC6A a less likely mediator of stimulation of insulin secretion. Moreover, *D*-amino acids do not serve as ligands of GPRC6A[5,13]. As for amino acid taste receptors, the expression of TAS1R5–24 is low in pancreas leaving its contribution to insulin secretion questionable. Thus, arginine transporters and membrane receptors are unlikely to mediate arginine's action to promote insulin secretion.

Alternatively, arginine may stimulate insulin secretion through intracellular targets. Arginine is known to bind to several proteins which serve as potential intracellular targets including nitric oxide synthase (NOS), arginase, Cellular arginine sensor for mTORC1 (CASTOR1), phosphofructokinase 1 and 2 (PFK1 and 2), RuvB-like 1, and RuvB-like 2[15–18]. However, we previously reported that an arginine analogue, arginine methyl ester (AME), stimulates insulin secretion to similar extent as arginine, despite AME inhibition of NOS activity, indicating that arginine-induced insulin secretion cannot be explained by arginine-NOS-NO signals[15]. In addition, a non-selective inhibitor of NOS, N^G-Nitro-*L*-arginine methyl ester (*L*-NAME), also stimulates insulin secretion as much as *L*- and *D*-arginine do[15]. These data indicate that NOS is a less likely target of arginine to increase insulin secretion. Arginine is also reported to activate mTORC1 through CASTOR1 in eukaryotes[17,18], but its expression in human and mouse β cells appears to be low[19]. Thus, the pathway by which *L*- and *D*-arginine stimulate insulin secretion remains unclear.

While Ca^{2+} influx is primarily responsible for initiating exocytosis of insulin granules, continuous production of insulin granules is critically important for insulin secretion as well[4]. Insulin undergoes highly regulated processing after translation of its precursor protein preproinsulin to form mature insulin granules[20,21]. After translation, preproinsulin is immediately translocated to the endoplasmic reticulum (ER) where a signal peptide is removed to produce proinsulin. At the ER, proinsulin undergoes disulfide bond formations and then moves to the Golgi network where proinsulin is cleaved into mature insulin in the secretory granules ready for secretion[1,2]. Animal models of insulin gene mutations (Akita and Munich mice) and humans with insulin gene mutations caused a dominant negative form of diabetes (maturity-onset diabetes of the young type 10, MODY10) and revealed that a proper folding of insulin in the ER is critically important for transit of proinsulin out of ER[22]. However, it is unknown whether there is an additional factor that regulates retention and release of proinsulin at the ER in β cells. Interestingly, we found that arginine depletion increases the retention of proinsulin and arginine acutely promotes exits of proinsulin from the ER in insulin secreting cells[23]. Here, we identified UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1), a protein known as gatekeeper of N-glycoprotein quality control (NGPQC)[24], serves as a molecular scaffold to retain proinsulin in the ER when arginine availability is low. Arginine disrupts the binding of proinsulin to UGGT1 and promotes transport of proinsulin out of the ER. UGGT1 is known to selectively re-glucosylate unfolded or mutated N-glycoproteins, thus providing quality control for protein transport out of the ER. The current study has revealed a new role UGGT1 plays as a regulator of arginine dependent transport of proinsulin out of the ER.

Materials and Methods

Antibodies and cell culture

The following antibodies were purchased: β-actin (sc-47778, Santa Cruz Biotechnology, Santa Cruz, CA, USA), glyceraldehyde 3-phosphate dehydrogenase (ab9485, Abcam, Cambridge, UK), insulin (L6B10, Cell Signaling Technology, Danvers, MA, USA, and sc-9168, Santa Cruz Biotechnology), UGGT1 (14170–1-AP, Proteintech, Rosemont, IL, USA; sc-374565, Santa Cruz Biotechnology; and ab13520–50, Abcam), KDEL (ER

staining, SPA-827, Stressgen, Victoria, BC, Canada), gp96 (2104S, Cell Signaling Technology), FLAG (F-1804, Sigma, St. Louis, MO, USA), GM130 (*cis*-Golgi network staining, 610822, BD Transduction Laboratories, New York, NY, USA), and Alexa Fluor-conjugated secondary antibodies (Thermo Fisher, Rockford, IL, USA). Mouse pancreas-derived NIT-1 cells (CRL-2055™ purchased from ATCC^{R}, Manassas, VA, USA) were maintained as described previously[15,23]. HEK293FT cells were maintained with DMEM +10% FCS[15,23].

Analysis of insulin secretion from cells and mice

Insulin secretion was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi, Gunma, Japan)[15,23] as described previously. We did not use phosphate-buffered saline (PBS) or Krebs–Ringer HEPES buffer because slight cell damage was detected with PBS/KRH without FCS even in 30 min. For mouse studies, plasma was collected from the mice at specific time points (0, 5, 30, and 120 min) after *L*-arginine or glucose administration via IP injection. Insulin concentration was measured using an ELISA kit (Shibayagi, Gunma, Japan). Mutated insulin secretion (i. e. R46Q-INS variant) secretion is analyzed using anti-Myc antibody.

Immunofluorescence study of β cells

Proinsulin (defined as in the ER and the Golgi network) or insulin (defined as in the secretory vesicles) was visualized using anti-insulin as green, and the ER (anti-KDEL antibody) and cis-Golgi network as red with Alexa Fluor-conjugated secondary antibody. Scale bars, 20 μm. For experiments using mice, pancreas was obtained from the animals fasted 24 h to decrease circulating *L*-arginine concentration for processing pancreas for histology. For Movie S1, HEK293FT cells transfected with pCDNA-insulin-mCherry and pCDNA-signal-GFP-KDEL* expression vectors 1 day prior to the experiment were arginine depleted for 30 min followed by arginine administration and confocal time-lapse microscopy was performed using a BX51W1 microscope (Olympus) equipped with a CSU-X1 laser scanning confocal unit (Yokogawa, Tokyo, Japan) with a 100× objective lens (LUMPLFL100XW, Olympus) and an iXon+ EMCCD camera (Andor) equipped with an on-stage culture chamber (Tokai Hit) filled with 45% N_2, 40% O_2, and 5% CO_2. For the excitation of eGFP and mCherry, 488-nm and 561-nm laser lines were used, respectively. Images were acquired every 1 s for 10 s, with an exposure time of 30–50 ms under control of METAMORPH software (Universal Imaging, Downingtown, PA, USA).

Affinity purification of UGGT1.

Affinity purification using magnetic nanobeads and isolation of arginine-binding proteins were performed as described previously[15].

Analysis of trypsin and chymotrypsin partial digestion.

Partial digestion with trypsin and chymotrypsin was performed as described previously[15].

Mice studies.

The animals overexpressing UGGT1 specifically in β cells under rat insulin promoter were generated. Two-month old male transgenic mice were used for experiments.

Immunoprecipitation and Western blot

Immunoprecipitation and Western blot analysis was performed as described previously[15,23].

Structure analysis

Homology models of human proinsulin were generated using the Swiss-Model server. The template *Thermomyces dupontii* UGGT1 (PDB ID: 5Y7O)[28] was selected for model building. Computer modeling of mouse UGGT1 was performed using the PyMOL. Only the rotamer with the highest frequency of appearance in proteins is shown. Global and per-residue model quality was evaluated using the QMEAN4 scoring function. The obtained scores were −5.83 for mouse UGGT1.

Statistical analysis

The values are reported as the means ± standard error (SE). Statistical significances (the Student's *t*-test and one-way/two-way analysis of variance=ANOVA) are indicated in figure legends as follows: $*p < 0.05$; $**p < 0.005$; $***p < 0.0001$. The insignificant differences ($p > 0.05$) are indicated as NS. The experiments were repeated five times.

Ethical approval

All mice experiments were performed in accordance with the ethical guidelines for the animal care of NCGG, and the experimental protocols were approved by the Animal Care Committee of NCGG[23].

Results

Arginine releases proinsulin from the endoplasmic reticulum (ER).

To analyze intracellular distribution of insulin, mCherry-tagged insulin expression vector was established and transfected to HEK293 FT cells with higher efficiency[15]. At first, mCherry-tagged insulin secretion is induced by arginine-administration after arginine-depletion for 30 min (Fig 1a). In these time period, time lapse microscopy was taken HEK293 FT cells transfected with insulin-mCherry and signal-GFP-KDEL vectors (Fig. 1b and Movie S1). After arginine-depletion for 30 min (time 0), insulin-mCherry located in the ER. Arginine administration induced the translocation of proinsulin from the ER to secret. Collectively, arginine depletion appears to cause proinsulin retention in the ER and arginine addition allows the release of proinsulin from the ER, suggesting the presence of an arginine sensitive proinsulin binding protein in the ER.

Identification of UGGT1 binding to arginine and proinsulin

To isolate and identify a protein that is capable to bind both arginine and proinsulin, arginine- and proinsulin-immobilized magnetic nanobeads[15] were mixed with cell extracts. Among proteins that showed binding to arginine- and proinsulin-immobilized nanobeads

(Fig. 1c), a protein of approximately 170 kDa was found to be absolutely identical and was identified as UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1, GenBank accession no. NP_942602/NM_198899) based on liquid chromatography-mass spectrometry (LC/MS) that revealed four common peptides (A-D, Fig. 1d). Importantly, UGGT1 contains the ERRS in its C-terminal region (Fig. 1e) to allow retention in the ER. Recombinant UGGT1 prepared by *in vitro* transcription and translation (*ivt*) was used to confirm that UGGT1 binds to both arginine and proinsulin (Fig. 1f and Supplementary Fig. S1a). Arginine binds to UGGT1 by competing with proinsulin and *vice versa* (Fig. 1f). Next, HEK293FT cells transfected with insulin-Myc and UGGT1F expression vectors were pretreated in arginine-depleted medium for 30 min followed by arginine administration as above to analyze the intensity and distribution of proinsulin/insulin-Myc and UGGT1F immunofluorescence staining (Fig. 1g, 1h and Supplementary Fig. S1b). While UGGT1F and proinsulin-Myc co-localized inside of the ER after arginine-depletion (Fig. 1b, 1g, Supplementary Fig. S1b, and Movie S1), arginine-administration dissociated UGGT1F and proinsulin-Myc signals in HEK293FT cells (Fig. 1b, 1h, Supplementary Fig. S1b and Movie S1). These data also support that UGGT1 binds to proinsulin at a low arginine concentration, and that arginine administration breaks the interaction and dissociates proinsulin from UGGT1.

UGGT1 binds to both *L*-arginine and *D*-arginine.

As isomer *D*-arginine is also known to stimulate insulin secretion[5], we tested binding of both *L*- and *D*-arginine to UGGT1. Trypsin and chymotrypsin partial digestion analyses showed that both *L*- and *D*-arginine partially protect UGGT1 from digestion (Fig. 2a and Supplementary S2). In *L*-arginine-immobilized beads analysis (Fig. 2b), both *L*- and *D*-arginine eluted UGGT1 bound to *L*-arginine-immobilized beads. Since arginine is a cationic *L*-amino acid, we tested whether other cationic amino acids bind UGGT1 and promote insulin secretion. Interestingly, there appears to be a positive correlation between pKa of amino acids, affinity to UGGT1, and potency to stimulate insulin secretion among different amino acids.

UGGT1 regulates arginine-induced insulin secretion in β cell line and mice.

Next, we analyzed the contribution of UGGT1 to arginine-induced insulin secretion using NIT-1 cells and mouse pancreatic β cells (Fig. 3, and Supplementary Fig. S3). In NIT-1 cells overexpressing FLAG-tagged UGGT1 (Fig. 3a and Supplementary Fig. S3a), insulin secretion was significantly blunted at low doses of arginine (0.2 mM and 0.6 mM) but increased at high concentrations of arginine compared with control cells (Fig. 3a). Of note, the concentration of *L*-arginine in the blood is approximately 50–200 μM in the blood of mice and 2 mM in F-12k medium. These results indicated that UGGT1 is indispensable for NIT-1 cells to increase insulin secretion in response to arginine.

We generated transgenic mice that overexpress FLAG-tagged UGGT1 (UGGT1F, Fig. 1d) under rat insulin promoter. The expression of UGGT1F was selectively increased at the mRNA level in the pancreas and UGGT1F protein was preferentially seen in the ER of pancreatic β cells (Supplementary Fig. S3b). Histological analyses of pancreas from transgenic mice revealed that β cells are slightly but significantly larger in transgenic mice compared with WT littermates (Supplementary Fig S3c), although other parameters

including number of β cells per islets or islet area per pancreas were not significantly different (Supplementary Fig. S3d–i). Since arginine is mainly obtained from food, transgenic mice and control littermates were fasted to assess the impact of arginine deprivation for glucose homeostasis. Plasma arginine concentrations decreased rapidly over 24 h of fasting (gray rectangle in Fig. 3b). The transgenic mice showed significantly lower plasma insulin levels and higher plasma glucose concentrations only up to 2 h of fasting (Fig. 3b), indicating that UGGT1 overexpression blunted insulin secretion *in vivo* when blood arginine concentration is relatively high. To further test the impact of arginine load on insulin secretion, the serum insulin level 5 min after several doses of arginine injection was obtained. Arginine tolerance testing (3.0 g/kg arginine) showed that transgenic mice have increased insulin at 5 min from baseline but they secreted significantly less insulin at all tested time points compared with WT control (Fig. 3c). Combined with data from NIT-1 cells (Fig. 3a), the levels of UGGT1 expression regulates arginine-induced insulin secretion in mice and cultured β cells.

The C-terminal of UGGT1 is responsible for competitive interaction with arginine and proinsulin.

To determine the region of UGGT1 that interacts with *L*-arginine and proinsulin, we tested binding of *L*-arginine and proinsulin to various UGGT1 fragments (Fig. 4a) and found that C40 was sufficient to bind both *L*-arginine and proinsulin in nanobeads assay (Supplementary Fig. S4a). UGGT1 C40 showed binding to *L*- and *D*-arginine in trypsin partial digestion analysis (Supplementary Fig. S4c) as well. Furthermore, radiolabeled *L*-arginine attached to his-tagged UGGT1 C40 immobilized to Ni-NTA-agarose was eluted by non-radiolabeled *L*-arginine or proinsulin in a dose dependent manner (Fig. 4b). There also was dose dependent elution of C40 and proinsulin when arginine was added to C40 mixed with proinsulin and Ni-NTA-agarose indicating that C40 has competitive binding to arginine and proinsulin (Supplementary Fig. S4b).

UGGT1 is responsible for arginine- and proinsulin-binding, but UGGT2 is responsible for proinsulin-binding.

UGGT is known as an enzyme that re-glucosylates N-glycoproteins. There are two isoforms of UGGT in mammals (or vertebrates), while plants and insects have only one UGGT[25–28]. Since insulin is exceptional among secreted proteins and is not glycosylated[29], UGGT1 is not able to function as a glucosyltransferase for proinsulin in β cells. Instead, our study suggests a new function of UGGT1 as an ER receptor protein that binds and retains proinsulin in the ER. Arginine competes with the interaction of UGGT1 with proinsulin, but fails to compete against the interaction of UGGT2 with proinsulin (Fig. 4c and 4d). This unique interaction of UGGT1 and proinsulin is mediated through the C-terminal region of UGGT1 that serves as a binding site for arginine and proinsulin, the region that does not exist in (plants) UGGT and non-homologues in (rodents and primates) UGGT2 (Fig. 4e)[25–28,30,31]. On the other hand, in rodents and primates, all two UGGTs (UGGT1 and UGGT2) function as UGGT by re-glucosylating N-glycoproteins[25–28,30,31]. Thus, the retention of proinsulin in the ER is a previously unknown, unique function of UGGT1 not shared with UGGT2. We designated arginine/proinsulin binding domain (Fig. 4e).

Glutamate residues in the C-terminal of UGGT1 is responsible for competitive interaction with arginine and proinsulin.

As *L*-arginine is the most cationic amino acid (Fig. 2d) and likely has strong interactions with anionic amino acids, we focused on EEkE and EE and generated expression vectors of 3E>3A and 2E>2A mutations (Supplementary Fig. S4e) for analysis (Fig. 4f, 4g and Supplementary Fig. S4e–g). When IP of proinsulin was blotted for UGGT1, proinsulin was associated with $UGGT1^{WT}$ only in the absence of arginine as expected (Fig. 4f, 4g and Supplementary Fig. S4e, and 4f). However, both 3E>3A and 2E>2A UGGT1 mutations disrupted the association of UGGT1 with proinsulin in both the presence and absence of arginine when proinsulin was appropriately immunoprecipitated. As expected, both 3E>3A and 2E>2A mutations of UGGT1 impaired arginine-induced insulin secretion (Fig. 4f). Thus, these five glutamate residues in the C-terminal region of UGGT1 are critical for ER-retention of proinsulin and arginine-induced insulin secretion (Supplementary Fig. S4g). Using pdb-5Y7O of crystal structure of folding sensor region of UGGT from *Thermomyces dupontii*[28], we analyzed crystal structure of mouse UGGT1 *in silico* using the PyMOL (Fig. 4h). As expected, arginine/proinsulin binding domain is apart from UGGT1 main protein structure and very flexible. It suggests that arginine/proinsulin binding activity is different function to UDP-glucose:glycoprotein glucosyltransferase activity.

Conclusion

In summary, we combined chemical, cell-based, and a transgenic mouse model to demonstrate that arginine sensitive interaction between proinsulin and UGGT1 contributes to the release of insulin in response to both *L*- and *D*-arginine.

In mice, we observed that arginine stimulates the mobilization of proinsulin from the ER after fasting. Combining biochemical and NIT-1 cell models, we demonstrated that proinsulin binds to UGGT1 when arginine availability is low in the ER, leading to the retention of proinsulin in the ER of pancreatic β cells. When arginine availability increases in the ER of pancreatic β cells, arginine disrupts binding between proinsulin and the C-terminal domain of UGGT1 competitively resulting in the release of proinsulin from UGGT1 and the promotion of further processing of proinsulin at the Golgi network and secretory vesicles for insulin secretion. We propose that this newly identified interaction of proinsulin with UGGT1 in the ER plays a role in arginine-induced insulin secretion and fine tunes insulin secretion.

Discussion

It has been known that the isomer *D*-arginine stimulates insulin secretion like *L*-arginine. However, the stimulation of insulin secretion by *D*-arginine occurs without Ca^{2+} influx and the opening of voltage-gated channels[11,12] leaving a mechanism responsible for the stimulation of insulin secretion by *D*-arginine unanswered. In the present study, we have shown that both *L*- and *D*-arginine stimulate insulin secretion by promoting release of proinsulin bound to UGGT1 (Fig. 1c, 1f, 4c, 4f, 4g, and Supplementary Fig. S1a, S4a, S4b, and S4f). Therefore, two independent pathways are utilized by arginine to induce insulin secretion: the release of proinsulin from UGGT1 in the ER by *L*- and *D*-arginine and the

stimulation of insulin secretion from secretory granules by *L*-, but not *D*-arginine (manuscript in preparation). The release of proinsulin from UGGT1 by *L*- and *D*-arginine is a consequence of competitive binding of proinsulin and arginine to UGGT1 (Fig. 2a, 2b, and Supplementary Fig. S2). Thus, UGGT1 serves as an intracellular amino acid receptor for *L*-amino acid and *D*-amino acid, which is a novel concept for amino acid biology. In addition to arginine, other cationic amino acids such as lysine and ornithine bind to UGGT1 and stimulate insulin secretion (Fig. 2c and 2d), indicating that anionic amino acids in UGGT1 interact with cationic amino acids. Indeed, we demonstrated that five (acidic) glutamate residues in the arginine/proinsulin binding domain (Supplementary Fig. S4d) are involved in arginine competition.

Arginine is one of the semi-essential amino acids mainly obtained from food and its concentration in the circulation reflects a nutritional state like concentrations of glucose and insulin[2]. Our study of UGGT1 transgenic mice showed that the change in arginine levels in the circulation affects the ER retention of proinsulin by UGGT1, indicating that UGGT1 mediated retention and release of proinsulin regulates insulin secretion *in vivo*. Although type 2 diabetes mellitus is characterized by the impairment in glucose-induced insulin secretion, reduced response to arginine is also reported[6]. A future study is required to address whether the retention of proinsulin by UGGT1 is increased in β cells affected by type 2 diabetes and serves as a target to improve insulin secretion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data and materials availability

Requests for data and materials should be addressed to TI (timai@ncgg.go.jp).

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Highlights

- UGGT1 binds to proinsulin in the absence of arginine.
- Arginine competes with proinsulin and binds to UGGT1 in the ER.
- Released proinsulin moves to Golgi apparatus and secretory vesicles to secrete.

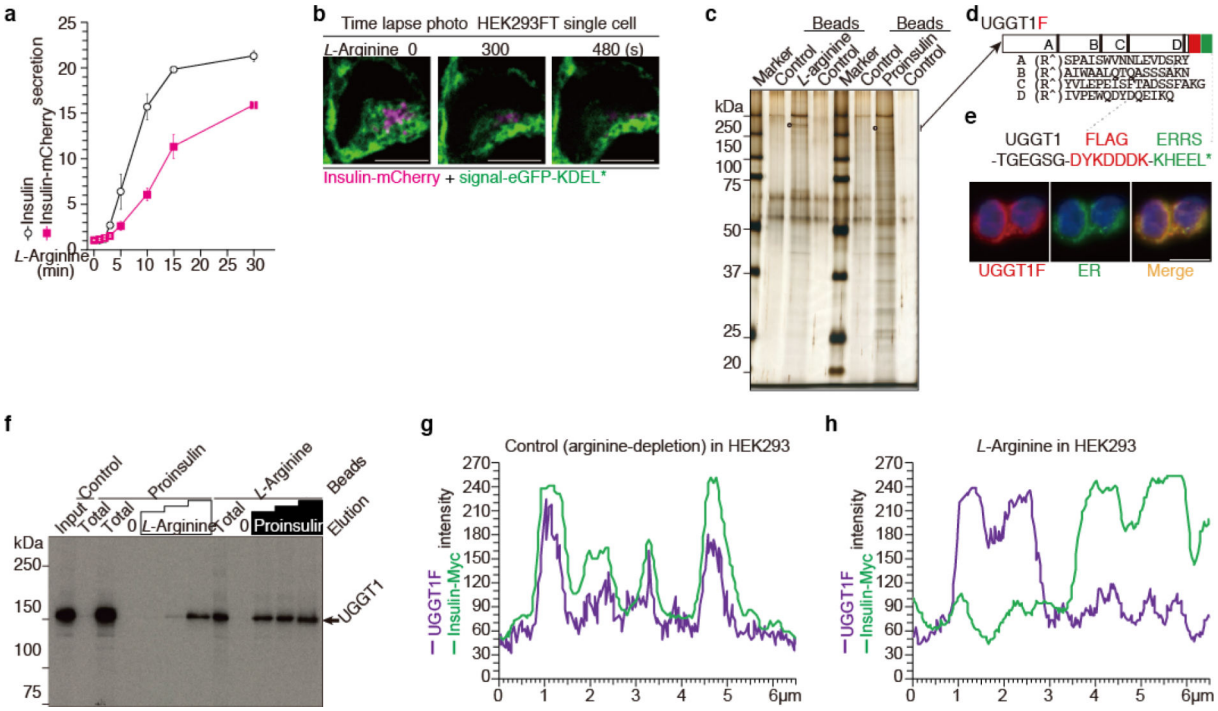

Figure 1. UGGT1 retains proinsulin in the endoplasmic reticulum (ER) in an arginine dependent manner.

(a) Insulin-mCherry-Myc secretion following arginine administration. Two expression vectors of insulin-Myc and insulin-mCherry-Myc were transfected, and arginine-dependent insulin-Myc secretion in the medium was analyzed using Myc-enzyme-linked immunosorbent assay (ELISA). Data are mean ± S.E. n=5.

(b) Arginine depletion retained proinsulin in the ER, and arginine released proinsulin from the ER in HEK293FT cells that express insulin-mCherry-Myc and signal-GFP-KDEL*. Cells were arginine starved in arginine-free medium followed by addition of arginine at a final concentration of 1 mM for indicated durations. The mCherry fused proinsulin is shown in magenta, and signal-GFP-KDEL* is shown the ER in green. Time-lapse photos are shown in (**e**), and the original movie is shown as Movie S1. Scale bars, 20 μm.

(**c**-**e**) Isolation of UGGT1 as a protein bound to both arginine and proinsulin using magnetic nanobeads technology. The two silver-stained protein bands (open circles) eluted from the *L*-arginine and proinsulin-immobilized beads were identified as UGGT1 (**c**). The purified protein bands were analyzed using LC/MS and four amino acid sequences (A–D) were obtained from two protein bands. To construct UGGT1F, the FLAG peptide was inserted immediately before the ER-retention signal (ERRS) of UGGT1 (**d**). The exogenous UGGT1F protein was localized in the ER of NIT-1 cells. The UGGT1F protein was stained red (anti-FLAG) and the ER was stained green (anti-KDEL). The orange region in the right panel indicates the localization of UGGT1F in the ER (merged image, **e**).

(**f**) Radiolabeled recombinant UGGT1 proteins were mixed and bound to the proinsulin-immobilized and *L*-arginine-immobilized nanobeads (Supplementary Fig. S1a). Then, these beads were eluted with *L*-arginine and proinsulin respectively. Data showed that arginine and proinsulin compete for binding to UGGT1 proteins.

(**g** and **h**) UGGT1F and proinsulin-Myc co-localized after arginine-depletion (**e**) and arginine administration dissociates co-localization (**f**). The HEK293FT cells transfected UGGT1F and insulin-Myc vectors were control (arginine-depleted for 30 min) and arginine was administered. Fluorescence intensity of intracellular UGGT1F and insulin analyzed by confocal microscope was plotted. The original image is listed in Supplementary Fig. S1b.

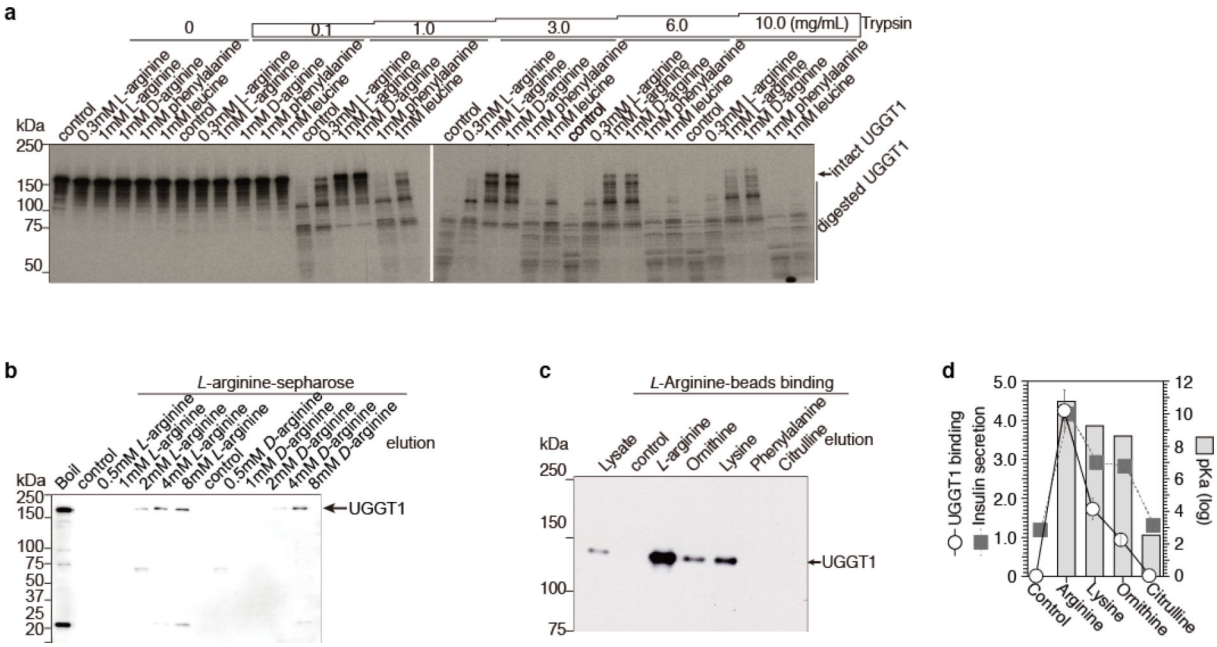

Figure 2. UGGT1 binds to *L*-arginine, *D*-arginine, and proinsulin regulating arginine-dependent insulin secretion.

(a) Both *L*- and *D*-arginine bound to UGGT1 and protected UGGT1 from trypsin (**a**) and chymotrypsin (Supplementary Fig. 2) partial digestion. A similar result was obtained using C40 mutant protein (Supplementary Fig. S3c).

(b) Both of *L*- and *D*-arginine bound to UGGT1 analyzed using *L*-arginine-immobilized nanobeads analysis. First, recombinant UGGT1 proteins were mixed with *L*-arginine-immobilized beads and the beads was washed several times. Then, UGGT1 proteins bound to beads were eluted with buffer containing with *L*- or *D*-arginine.

(c) UGGT1 binding to arginine was competed by cationic amino acids, but not by non-cationic amino acids.

(d) Correlation of amino acid basicity (pKa), UGGT1 binding, and insulin secretion. The UGGT1 binding activity of amino acids is correlated with its pKa and insulin secretion.

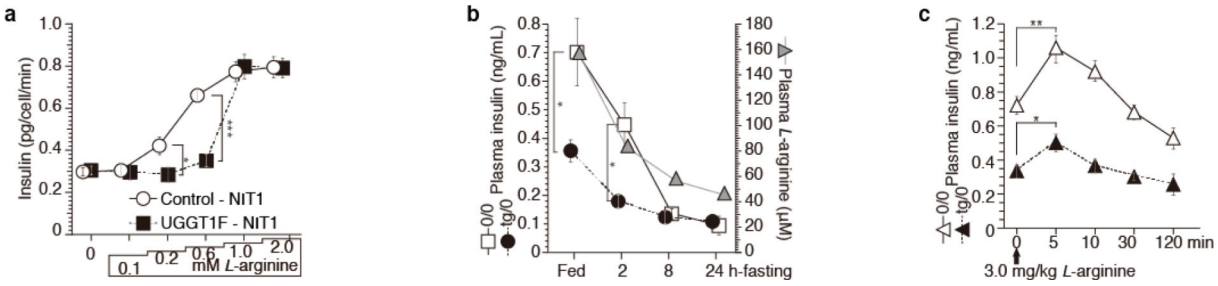

Figure 3. UGGT1 regulates *L*-arginine-induced insulin secretion in β cell line and mice.

(**a**) *L*-arginine-induced insulin secretion in the NIT-1 cells with UGGT1 overexpression (**a**). The UGGT1 expression vector was introduced into NIT-1 cells, and the two-fold higher transgene expression was confirmed using RT–PCR (Supplementary Fig. S3a). The analysis of the secreted insulin indicated that UGGT1 retained proinsulin in cells and *L*-arginine released insulin from the retention.

(**b** and **c**) UGGT1 retained proinsulin in the pancreas and *L*-arginine released insulin from intracellular retention in β cell-specific UGGT1-overexpressing transgenic mice. Using the UGGT1 expression vector driven by the rat insulin promoter, β cell-specific UGGT1-transgenic mice were generated. The expression of UGGT1 RNA in pancreas were confirmed (Supplementary Fig. S3b). The transgenic mice (tg/0) and their littermates (0/0) were subjected to fasting (**b**) and *L*-arginine tolerance test (**c**). Several doses of *L*-arginine (0.75, 1.5, and 3.0 mg/g body weight) were IP-injected into these mice, and plasma insulin was measured (**c**). The UGGT1-transgenic mice (tg/0) showed impaired *L*-arginine-induced insulin secretion. Data are presented as mean ± S.E. n=7 *p < 0.05; **p < 0.005; ***p < 0.0001. Nonsignificant differences (p > 0.05) are indicated as NS.

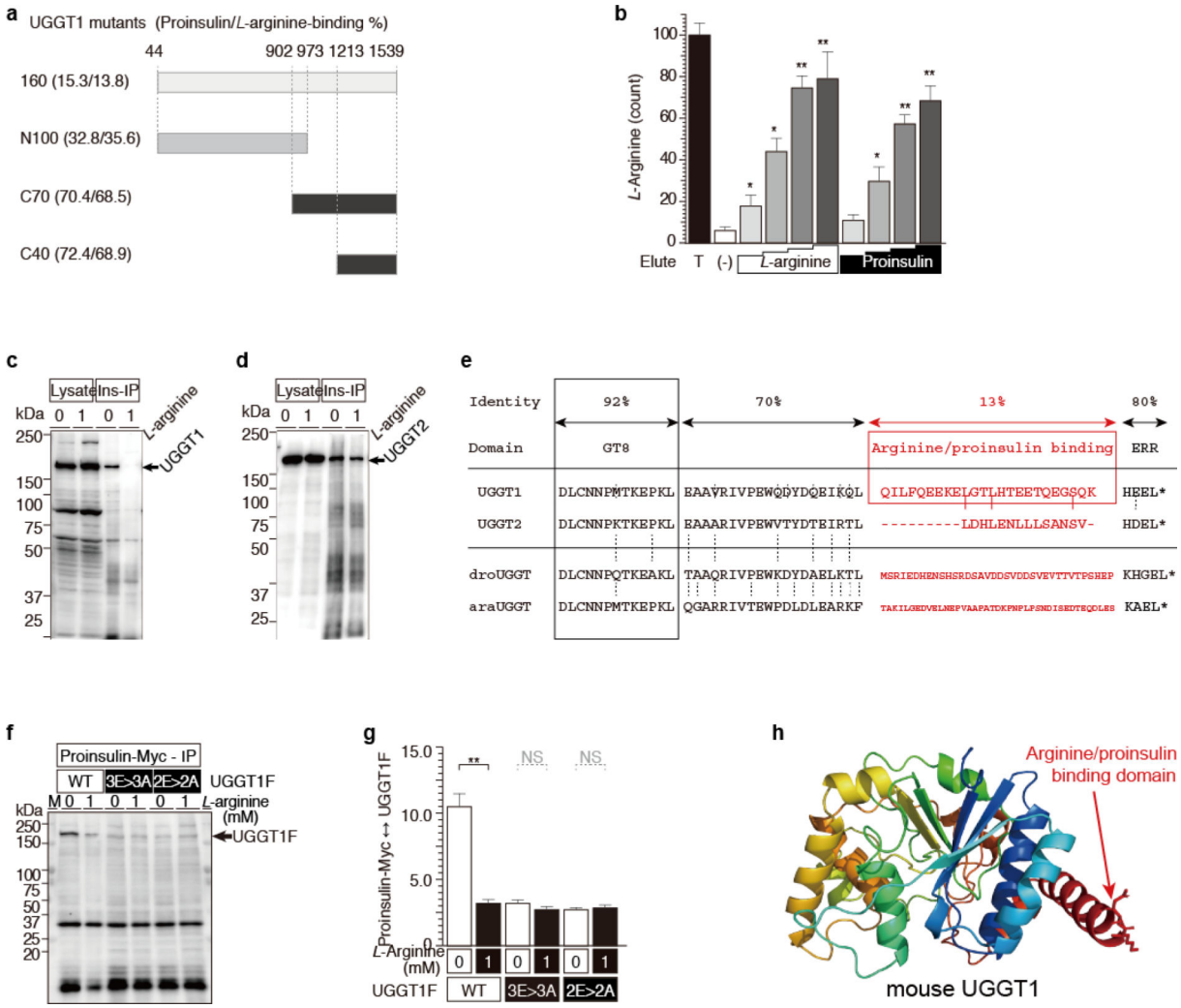

Figure 4. Glutamate residues in the C-terminal of UGGT1 is responsible for competitive interaction with arginine and proinsulin.

(a) UGGT1 C-terminal protein C40 minimal protein binding to proinsulin and *L*-arginine using magnetic nanobeads (Supplementary Fig S4a).

(b) UGGT1 C40-bound radiolabeled *L*-arginine was released from the UGGT1 C40 protein by both cold *L*-arginine and the proinsulin protein in a dose-dependent manner. The C40 proteins with His-tag were first mixed with radiolabeled *L*-arginine and subsequently with Ni-agarose. After the agarose was washed with binding buffer, radiolabeled *L*-arginine + C40-agarose was eluted with non-radiolabeled *L*-arginine or non-radiolabeled proinsulin. The eluted fractions were collected, and radiolabeled *L*-arginine was estimated. Stoichiometry of C40:*L*-arginine:proinsulin is 1.0:0.36:0.32. Data are presented as mean ± SE. n=5 *p < 0.05; **p < 0.005.

(**c** and **d**) Arginine releases proinsulin from endogenous UGGT1 (**c**) but does not compete for proinsulin-UGGT2 interaction (**d**).

(**e**) Highly diversity in C-terminal region of UGGTs.

(**f** and **g**) The 3E>3A and 2E>2A mutations of UGGT1F (Supplementary Fig. S4d) impaired arginine dependent interaction with proinsulin demonstrated by IPWB.

(**h**) Structure model of arginine/proinsulin binding domain of mouse UGGT1. Original data is from PDB- 5Y7O crystal structure of folding sensor region of UGGT from *Thermomyces dupontii* (PDB ID: 5Y7O)[28].