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Biophysical properties of UDP-glucose:glycoprotein glucosyltransferase, a folding sensor enzyme in the ER, delineated by synthetic probes

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ABSTRACT

UDP-glucose:glycoprotein glucosyltransferase plays a key role in glycoprotein quality control in the endoplasmic reticulum, by virtue of its ability to discriminate folding states. Although lines of evidence have clarified the ability of UGGT to recognize a partially unfolded protein, its mechanistic rationale has been obscure. In this study, the substrate recognition mechanism of UGGT was studied using synthetic substrate of UGGT. Although UGGT has high extent of surface hydrophobicity, it clearly lacks property of typical molecular chaperones. Furthermore, it was revealed that the addition of the substrate caused secondary structure change of UGGT in a dose-dependent manner, resulting that the K_d value of the UGGT–substrate interaction was estimated from theoretical formula based on 1:1 complexation between UGGT and the acceptor substrate. Moreover, the kinetic analysis of glucosyltransferase activity of UGGT elucidated Michaelis constant K_m correctly.

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1. Introduction

The endoplasmic reticulum (ER) is machinery that produces and modifies proteins, which also plays an important role in assisting in their folding [1]. A majority of newly generated polypeptides delivered into the ER are glycosylated on their asparagine (Asn) residues which are embedded in the tripeptide consensus sequence Asn-X-Ser/Thr. In the beginning, Asn-linked glycans are introduced as the tetradecasaccharide consisting of three D-glucose (Glc), nine D-mannose (Man), and two *N*-acetyl-D-glucosamine (GlcNAc) residues ($Glc_3Man_9GlcNAc_2$; G3M9). It is trimmed sequentially by glucosidase I (G-I), glucosidase II (G-II), and mannosidases, generating di- ($Glc_2Man_9GlcNAc_2$; G2M9), mono- ($Glc_1Man_9GlcNAc_2$; G1M9) and non-glucosylated ($Man_9GlcNAc_2$; M9) glycoforms, as well as their congeners lacking one or more Man residues.

Proteins carrying monoglucosylated glycans such as G1M9 enter the calnexin (CNX)/calreticulin (CRT) cycle, which plays the central role in glycoprotein quality control (GPQC) in the ER [2]. ER-residing chaperones CNX and CRT are characterized by their lectin-like property to specifically recognize glycoproteins that contain monoglucosylated high-mannose-type glycans. In the CNX/CRT cycle, G-II regulates both entry and exit of glycoproteins, due to its dual activity to convert G2 to G1 and G1 to G0 glycoforms. Another essential component of this cycle is UDP-glucose:glycoprotein glucosyltransferase (UGGT), an enzyme considered to play a role as the "folding sensor" in the ER. Intriguingly, this enzyme recognizes folding defective glycoproteins with nonglucosylated glycoforms, most typically Man9GlcNAc2 (M9). It catalyzes the glucose transfer reaction from UDP-glucose (UDP-Glc) to terminal mannose residue of their A-arm, resulting in regeneration of monoglucosylated glycoforms (e.g. G1M9) bound to mis(un)folded glycoprotein, which engage in the interaction with CNX/CRT (Fig. 1) [3]. Notwithstanding intense effort which has been devoted to understand the mechanistic foundation of its highly unique specificity [4], conclusion has been elusive. Although its specificity has been ascribed to hydrophobic interaction with mis(un)folded proteins, the fine picture is yet to be drawn. UGGT seems to discriminate subtle conformational differences of proteins. Curiously, it is poorly active toward folded as well as extensively misfolded glycoproteins [5], whereas it prefers partially misfolded proteins with molten-globule like structure as substrates [6,7].

Although the question of how the UGGT discriminate folding state of glycoprotein has remained to be answered [8], several studies have evidenced that the presence of both oligosaccharide and unfolded protein backbone is required for UGGT–substrate interaction [9–11]. Notably, study by Ritter et al., which employed

Abbreviations: ANS, 8-anilino-1-naphtalene sulfonic acid; CS, citrate synthase; ER, endoplasmic reticulum; Glc, glucose; GlcNac, *N*-acetylglucosamine; Man, mannose; UGGT, UDP-Glc:glycoprotein glucosyltransferase.

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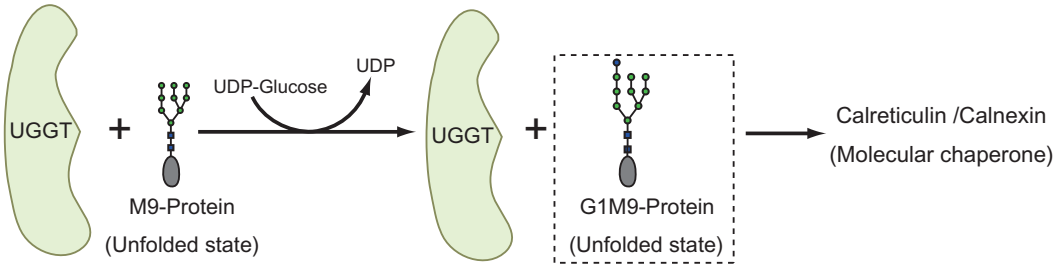

Fig. 1. Schematic illustration of UGGT function.

variously modified ribonuclease A and B, suggested that oligosaccharide fine structure is not of primary importance, while the presence of innermost GlcNAc is indispensable for binding [12]. In fact, our previous study showed that UGGT mainly recognizes core pentasaccharide ($Man_3GlcNAc_2$) region of the substrate, the affinity of which seemed to be higher than heptamannosyl homolog ($Man_7GlcNAc_2$) which retained B- and C-arms [13].

Our group previously developed fully synthetic non-proteinic substrates of UGGT (for e.g. see Fig. 2), which carry various chromophores as aglycon [14,15]. Given that corresponding glycans are, in their underivatized form, devoid of reactivity, introduction of one of these aglycons seems to convert quiescent glycans into glucosylation competent form. Although the entity which confers reactivity to UGGT substrates has not been entirely clear, the easiest interpretation would be that these synthetic substrates mimic common characteristics of unfolded proteins, such as surface exposed hydrophobicity [13]. Irrespective of the mechanistic justification, advantage of using synthetic substrates for precise analysis of UGGT is obvious, because their glycan structures are homogeneous and well-defined. In addition, any complexity associated with using denatured proteins can be circumvented. As a matter of fact, our previous study using synthetic substrates successfully provided quantitative estimate of glycan specificity of UGGT [16]. Since synthetic substrates carrying di- or monoglucosylated glycans have been used extensively in our analysis of G-II and CRT [17], they are expected to be highly powerful as tools for systematic study of glycoprotein quality control system in the ER.

In this study, we planned to explore UGGT's property responsible for its recognition of folding defective glycoproteins, using the *Drosophila* enzyme [18]. Our analysis showed that UGGT possesses high surface hydrophobicity, whereas it lacks the property common to typical chaperones which prevent aggregation of denatured proteins. Furthermore, we revealed that secondary structure change of UGGT was caused by complexation with an acceptor substrate, which was evidenced by CD experiments. Namely, addition of the acceptor substrate decreased the extent of α-helices in a concentration dependent manner. From these changes, biophysical constants of interaction between UGGT and substrate were acquired. Subsequently, kinetic analysis of glucose transfer reaction mediated by UGGT was carried out. These findings well support the assumption that substrate recognition mode of UGGT is bipartite, requiring both hydrophobic region and appropriately configured oligosaccharides in a single molecule.

2. Materials and methods

2.1. Reagents

Drosophila UGGT, which was tagged with a C-terminal His6 tag and expressed in Hi-5 insect cells (SDS–PAGE is shown in Fig. S1), was a kind from Dr. Karin Reinisch and Dr. Stephanie Hamill (Department of Cell Biology, Yale University School of Medicine). M9-G-E-BODIPY, M9-G-E-TAMRA and M9-Gly were synthesized following our previous report [13]. Deoxynojirimycin and deoxymannojirimycin were obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). UDP-glucose disodium salt, calcium chloride, Triton X-100, Tris hydroxymethyl aminomethane, hydrochloride and acetonitrile were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 8-Anilino-1-naphtalene sulfonic acid (ANS) was obtained from MP Biomedicals (Illkirch, France). Citrate synthase (CS) was purchased from Sigma Chemical Company (St. Louis, MO, USA). Triethylamine was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

2.2. Glucose transition reaction mediated by UGGT

The reaction mixture containing 10 μM acceptor substrate M9-G-E-BODIPY, 1 mM UDP-Glc, 0.6 μM UGGT, 250 μM deoxynojirimycin, 250 μM deoxymannojirimycin, 10 mM $CaCl_2$ and 4 mM Tris–HCl (pH 8.0), was prepared, and then the solution was incubated for 10 min at 37 °C. After incubation, 20 μL of the solution was removed by a micropipet and diluted with 20 μL of CH_3CN to stop the enzymatic reaction. The glucose transfer yield was analyzed by HPLC column (TSKGEL Amide-80 (4.6 mm i.d. × 25 cm)) with 3% AcOH-Et_3N (pH 7.3)/CH_3CN mixed solvent (35:65 to 50:50, linear gradient for 50 min) at 40 °C and a 1 mL/min flow rate. The glycan-G-E-BODIPY was detected by fluorescence intensity (fluorescence at 513 nm, excitation 504 nm). The initial velocity of each substrate was calculated from the value of glucose transfer after incubation.

2.3. Light scattering assay for thermal UGGT aggregation

Light scattering analysis was performed on fluorescent spectrophotometer (FP-6500, Jasco) equipped with temperature controller. Both monochromators were set at 400 nm, while slit-widths at 2 nm each. The solution containing 10 mM $CaCl_2$ and 4 mM Tris–HCl (pH 8.0) was preheated in cuvette holder at 40 °C or 50 °C for 5 min. After incubation, 0.6 μM UGGT was added into a 1 cm path-length cell, and then data acquisition was started.

2.4. Measurement of protein hydrophobicity by fluorescence probe ANS

UGGT (0.6 μM) dissolved in 10 mM $CaCl_2$ and 4 mM Tris–HCl (pH 8.0) was incubated with 50 μM ANS at 37 °C for 10 min. The ANS–binding fluorescence to UGGT was monitored with the excitation at 360 nm and the emission recorded from 400 to 600 nm, using fluorescent spectrophotometer (FP-6500, Jasco) at 37 °C in a 1 cm path-length cell.

2.5. Citrate synthase aggregation assay

Thermal aggregation of CS was monitored by measuring light scattering at 400 nm with a fluorescent spectrophotometer for

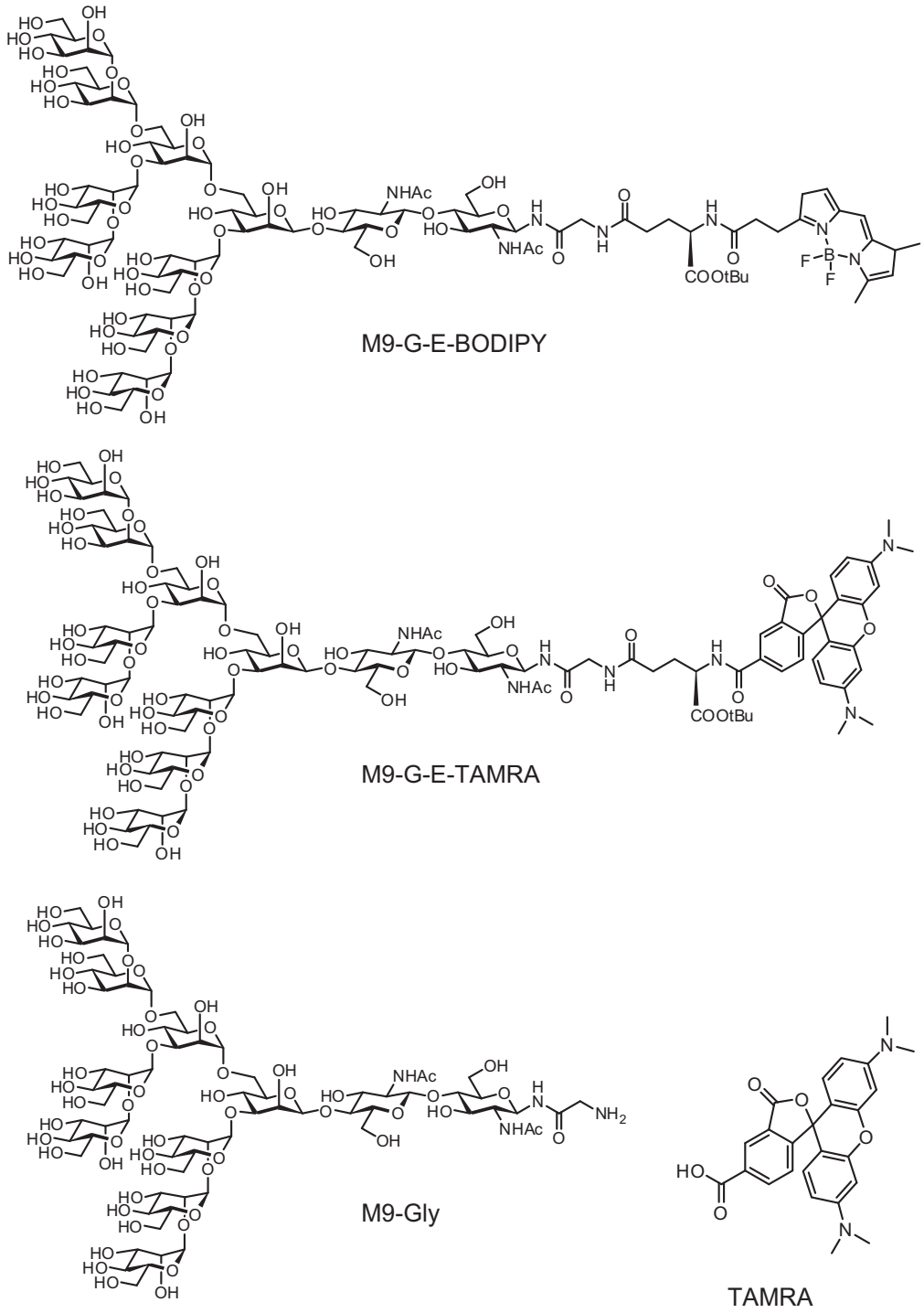

Fig. 2. Structure of acceptor substrates.

30 min at 45 °C. CS was diluted to a final concentration of 100 nM in 4 mM Tris–HCl buffer (pH 8.0) and 10 mM $CaCl_2$. The reaction solution was preincubated for 10 min at 45 °C and continuously stirred throughout the measurement. After incubation, 100 nM UGGT was added into the reaction solution, and then data acquisition was started.

2.6. Secondary structure analysis of UGGT by CD spectrum

CD spectra were recorded from 190 to 250 nm, with 100 nm min^{-1} scanning speed on a J-720 CD spectropolarimeter (JASCO, Japan). The spectra were collected and averaged over 10 scans. All experiments were performed at 37 °C, using quartz cells of 1.0 mm optical path length. The 4 mM Tris–HCl buffer solution (pH8.0) containing 0.3 μM UGGT and 10 mM $CaCl_2$ with M9-G-E-TAMRA ranging from 0 to 20 μM was used as samples of CD spectra assay.

The α-helicity of UGGT was calculated by Eq. (1) following Greenfield's method [19]:

$$\alpha\text{-helicity } [\%] = \frac{(\theta_{208} - 4000)}{(33000 - 4000)} \times 100 \quad (1)$$

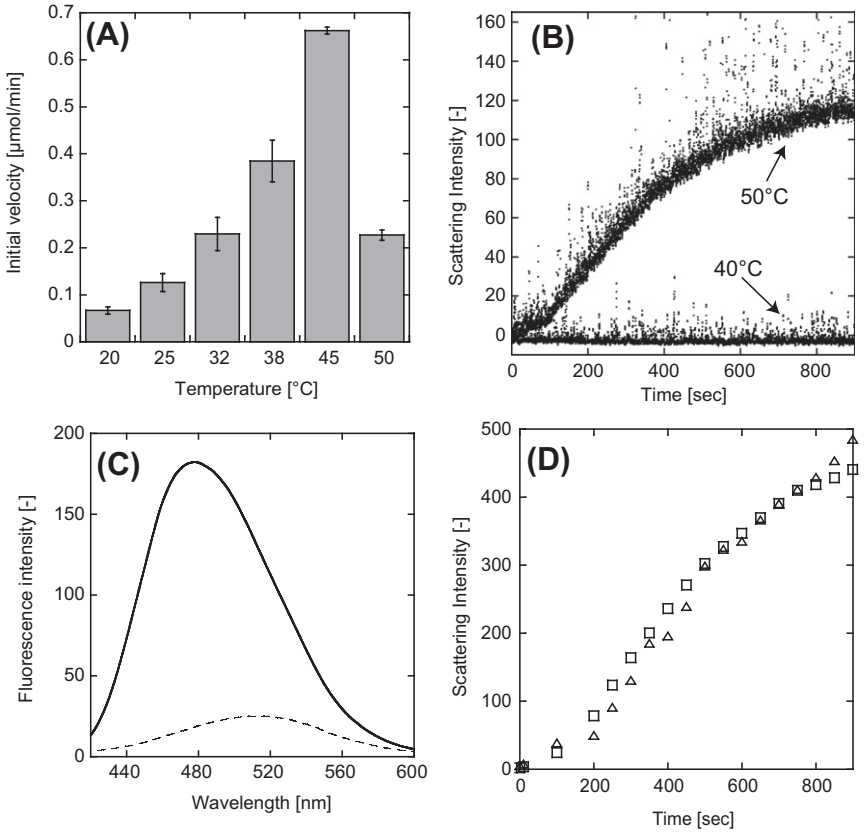

Fig. 3. (A) Effect of reaction temperature on glucosyltransferase activity of UGGT. The reaction mixture contained 10 μM M9-G-E-BODIPY, 1 mM UDP-Glc, 0.6 μM UGGT and 4 mM Tris–HCl (pH 8.0). The mix solution was incubated for 5 min at 37 °C. After incubation, the glucose transfer yield was analyzed by HPLC. The initial velocity of each substrate was calculated from the value of glucose transfer. (B) Observation of UGGT thermal stability by light scattering analysis. The buffer solution was preheated in cuvette holder at 40 or 50 °C for 5 min. After incubation, 0.6 μM UGGT was added into cuvette, and then scattering intensity was measured by fluorescent spectroscopy under stirring. (C) ANS binding assay to UGGT. Dotted and solid line indicates ANS alone and ANS with UGGT, respectively. UGGT (0.6 μM) was incubated with 50 μM ANS at 37 °C for 10 min. The ANS-binding fluorescence to UGGT was monitored with the excitation at 360 nm using fluorescent spectrophotometer at 37 °C. (D) Influence of UGGT on the thermal-aggregation of CS. Square (□) and triangle (△) symbols represent CS alone and CS with UGGT, respectively. The light scattering measurement was performed in 4 mM Tris–HCl (pH 8.0) containing 100 nM UGGT and 100 nM CS.

The dissociation constant (K_d) between UGGT and substrate was analyzed by nonlinear regression curve fitting (KaleidaGraph, Synergy Software, Inc.), using the equilibrium equation (2) [20].

$$\Delta\theta_{208} = \theta_{208,\max}\left(\frac{1 + K_a[\mathrm{UGGT}]_0 + K_a[\mathrm{subtrate}]_0}{2K_a[\mathrm{UGGT}]_0} - \sqrt{\left(\frac{1 + K_a[\mathrm{UGGT}]_0 + K_a[\mathrm{subtrate}]_0}{2K_a[\mathrm{UGGT}]_0}\right)^2 - \frac{[\mathrm{subtrate}]_0}{[\mathrm{UGGT}]_0}}\right) \quad (2)$$

$\theta_{208,\max}$ indicates maximum change in θ value at 208 nm. K_a (=1/K_d) means association constant. $[\mathrm{UGGT}]_0$ and $[\mathrm{substrate}]_0$ are the initial concentration of UGGT and substrate, respectively.

2.7. Determination of Michaelis constant

The Michaelis–Menten constant (K_m) for glucose transfer reaction by UGGT was determined by measuring the initial velocity of the reaction and analyzing the data according to bellow Eq. (3) (v: initial velocity, Vmax: maximum velocity, [S]: concentration of donor substrates, K_m: the Michaelis–Menten constant).

$$v = \frac{\mathrm{Vmax}[S]}{K_m + [S]} \quad (3)$$

Kinetic data were analyzed by nonlinear regression curve fitting, using the Michaelis–Menten equation (KaleidaGraph).

3. Results

Since UGGT specifically recognizes folding defective glycoproteins, we speculated that it may also be functional as a chaperone which facilitates protein folding by preventing aggregation. Before addressing this notion, thermal stability of UGGT was first examined by testing its activity at elevated temperatures. As a substrate, fluorescently labeled M9-G-E-BODIPY (Fig. 2) was employed, since it was previously shown to have an excellent activity toward UGGT (Fig. 2). As shown in Fig. 3A, UGGT retained its activity at elevated temperatures and the glucose transfer was rather accelerated as the temperature increased. Maximum rate enhancement was observed at 45 °C, which was 5 times higher than at 25 °C, while abrupt fall-off of the activity occurred at 50 °C.

To identify the cause of the declination of the activity, light scattering experiments were conducted. As shown in Fig. 3B, aggregation of UGGT was observed at 50 °C. The scattering became more intense as the incubation time was elongated. In the event, this assay revealed that UGGT denatured at temperatures over 50 °C, while it was fully stable at 45 °C or lower temperatures. It deserves being emphasized that the temperature profile of UGGT activity is accurately deduced only from analysis with synthetic substrate, because temperature change would perturb glycoprotein's folding state and, in consequence, reactivity to UGGT.

In order to reveal elements crucial for the substrate recognition of UGGT, its surface hydrophobicity was evaluated by ANS binding

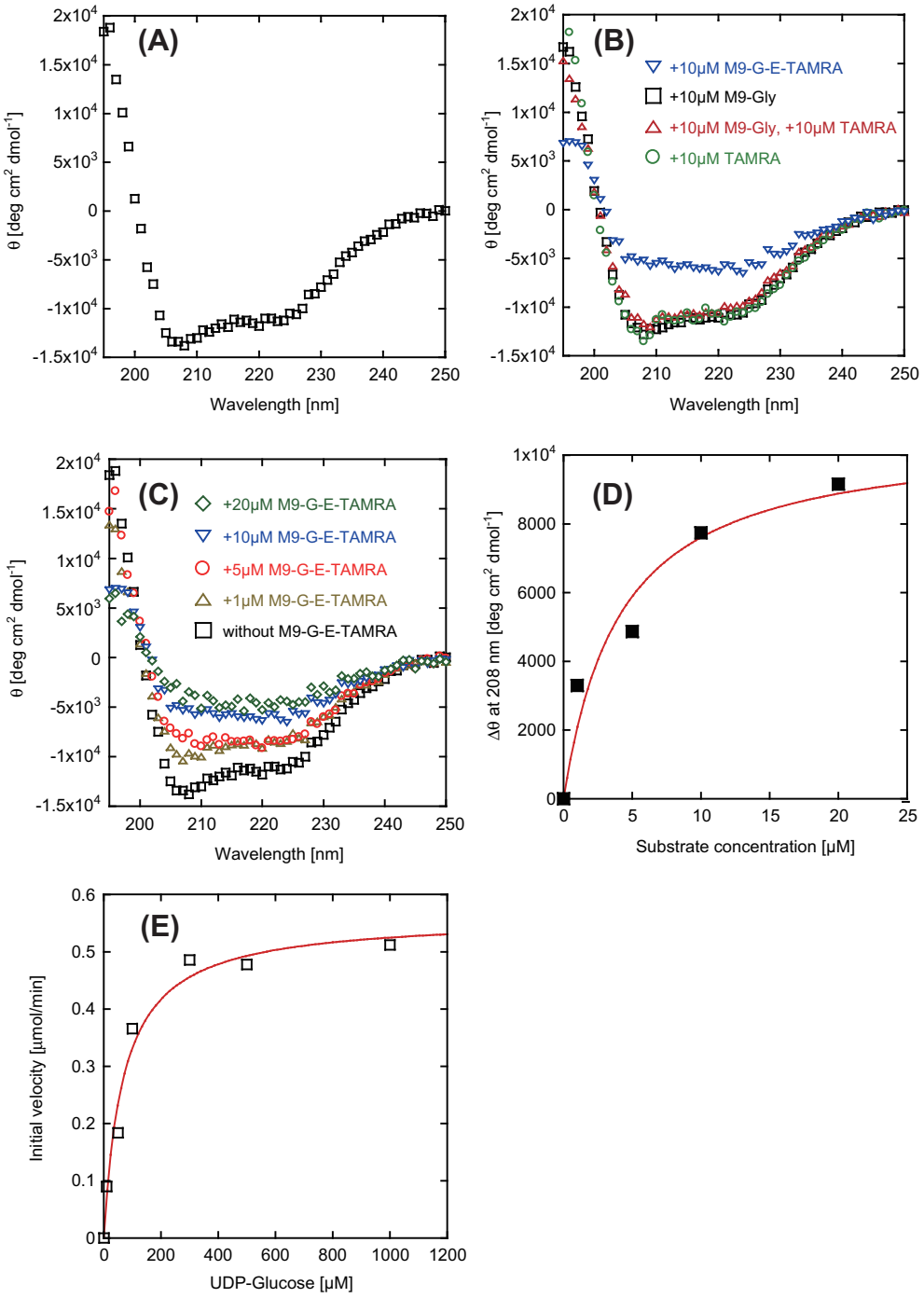

Fig. 4. CD spectrum measurement of UGGT. All CD spectra were analyzed by 0.3 μM UGGT solution dissolved with 4 mM Tris–HCl (pH 8.0) by CD spectropolarimeter. (A) CD spectrum of UGGT in the absence of acceptor substrate. (B) CD spectra of UGGT in the presence of acceptor substrate or substrate analog. (C) CD spectra of UGGT with various concentration of acceptor substrate. The concentration of substrate was adjusted from 0 to 20 μM. (D) Change of θ value at 208 nm of UGGT by acceptor substrate addition. Line shows theoretical formula based on 1:1 complex formation between UGGT and acceptor substrate. (E) Effect of concentration of glucose donor substrate on glucosyltransferase activity. The reaction mixture contained 10 μM M9-G-E-BODIPY, appropriate concentration of UDP-Glc, 0.6 μM UGGT and 4 mM Tris–HCl (pH 8.0). The mix solution was incubated for 5 min at 37 °C. After incubation, the glucose transfer yield was analyzed by HPLC. The initial velocity of each substrate was calculated from the value of glucose transfer.

assay. ANS has been widely used as a fluorescent probe that quantifies exposed hydrophobicity of proteins [21]. In the presence of UGGT, fluorescence intensity of ANS elevated dramatically and maximum emission wavelength was shifted from 515 to 480 nm (Fig. 3C). This behavior clearly indicates that UGGT possesses high surface hydrophobicity. Recently, Izumi et al. employed fully synthetic glycoprotein that was isolated in folded as well as intentionally misfolded states, as substrates of UGGT. Their study clearly showed that high surface hydrophobicity exhibited by unfolded proteins is critical in order for the UGGT catalyzed glucose transfer to proceed in a maximum velocity [22]. Accordingly, hydrophobic interaction between UGGT and substrate is likely to be crucial for the productive substrate recognition. In accordance with this surmise, previous study revealed that glucose transfer reaction was

highly sluggish when oligosaccharide was either unmodified or derivatized with an aglycon of low hydrophobicity [13].

With an aim of elucidating the function of UGGT's surface hydrophobicity, protein denature suppression capacity was then examined, which was estimated by its aggregation inhibition activity toward citrate synthase (CS). CS has been used as a benchmark probe for evaluating the activity of molecular chaperones, because it readily aggregates at temperatures over 40 °C [23]. Potency of UGGT to suppress aggregation of CS was assessed by light scattering at 45 °C. As shown in Fig. 3D, the scattering intensity increased in a time-dependent manner when CS was incubated alone. Somewhat unexpectedly, however, no inhibition of the aggregation was observed when the incubation was conducted in the presence of UGGT, although UGGT itself was shown to retain its activity at this temperature (*vide supra*). This result indicates that UGGT does not have an activity as a bona fide chaperone, in spite of the presence of surface hydrophobicity which must be functional in interacting with unfolded proteins. In our synthetic substrates of UGGT, hydrophobic aglycons were shown to play critical role in order to be recognized by UGGT, most likely through their interaction with the enzyme's hydrophobic region. However, although interference of glucose transfer may be expected, addition of excess aglycon did not exhibit any effect on the activity of UGGT (Fig. S2). Consequently, whereas the interaction of UGGT with aglycon is an important factor, the affinity seems to be rather weak as such.

We then planned to see if any structural change of UGGT arises upon acceptor binding, by means of CD experiments. For this purpose, M9-G-E-TAMRA (Fig. 2) was employed as the probe, which was shown to be an excellent acceptor substrate of UGGT [13]. As shown in Fig. 4A, CD spectrum of UGGT revealed substantial α-helicity, which exhibited negative bands at 208 and 222 nm. From the intensity of the band at 208 nm, proportion of α-helix was estimated to be ca. 33% [19]. Subsequently, influence of the acceptor substrate M9-G-E-TAMRA on UGGT secondary structure was examined (Fig. 4B), in comparison with TAMRA and a poorly active substrate M9-Gly [13]. The analysis showed that the addition of the active acceptor substrate caused a significant change of the secondary structure, which diminished the α-helicity of UGGT in a concentration dependent manner (Fig. 4C). Content of the α-helix decreased from 33% to 21%, 16%, 6.1%, and 1.2%, at substrate concentrations 1, 5, 10, and 20 μM, respectively. In stark contrast, neither M9-Gly nor TAMRA gave any effect on the CD spectrum. Taking together, these results indicate that the structural change of UGGT was caused by simultaneous binding of the glycan portion and a hydrophobic aglycon, both of which are also required for the glucose transfer reaction.

Clear CD spectral changes allowed us to estimate the affinity of the substrate to UGGT. Change of the θ value at 208 nm is exhibited in Fig. 4D. From the result, $\Delta\theta_{208}$ was converged on constant value accompanied with concentration increasing of acceptor substrate M9-G-E-TAMRA, indicating that the interaction between UGGT and acceptor substrate consists of quantitative relationship. A comparison of theoretical formula, which is based on 1:1 complex formation between UGGT and acceptor substrate, with experimental plot represents strong correlation. From the theoretical formula, the dissociation constant K_d between UGGT and acceptor substrate was estimated to be 4 μM. It may deserve being stressed that as the acceptor substrate used in this study lacks any Cotton effect at the wavelength from 190 to 250 nm, the measurement of secondary structure change of UGGT was successfully carried out by CD spectrum.

Taking account of the estimated K_d value (4 μM), all UGGT in solutions was regarded being complexed with the acceptor substrate, when the concentration of the latter adjusted to higher than 10 μM. Assuming that the interaction between UGGT and M9-G-E-TAMRA was rigid enough to keep the complex from dissociation, the mixture was subjected to the glucose transfer reaction by adding UDP-Glc, and initial velocity of glucose transfer reaction to M9-G-E-TAMRA was measured. Michaelis constant K_m was estimated by assuming the following equilibrium:

$$[\text{UGGT} - \text{M9 substrate}] + [\text{UDP-Glc}] \Longleftrightarrow [\text{UGGT} - \text{M9 substrate} - \text{UDP-Glc}] \rightarrow [\text{UGGT}] + [\text{G1M9 substrate}] + [\text{UDP}]$$

Correlation of initial velocity with concentration of the donor substrate is depicted in Fig. 4E. The K_m value of UGGT estimated from fitting curve of Michaelis–Menten kinetics are given as 69 μM.

4. Discussion

Although UGGT lack property as a molecular chaperone, the ANS-binding experiment clarified its high surface hydrophobicity. As M9-G-E-BODIPY, but not M9-Gly, exhibits high reactivity, the hydrophobic aglycon would seem to play a decisive role in UGGT-acceptor recognition. However, inclusion of excess BODIPY did not cause any retardation of glucose transfer to M9-G-E-BODIPY, indicating that the hydrophobic interaction between UGGT and the aglycon is insignificant as such. In line with this notion, CD spectrum of UGGT was not influenced by the addition of the hydrophobic aglycon (Fig. 4B). Since inclusion of M9- was equally ineffective in affecting the secondary structure of UGGT, our results suggest that substrate recognizing mechanism of UGGT is distinct both from molecular chaperones and lectins [24].

Drosophila UGGT, which was used in this study, is known to be highly homologous to human UGGT in its sequence [25]. Sequence analysis of the human enzyme suggested that UGGT possesses a single catalytic domain for glucose transfer reaction and a recognition site for the donor substrate [26]. Secondary structure change of UGGT caused by the addition of an acceptor substrate was clearly seen by the CD spectrum, extent of which increased in a dose dependent manner. Theoretical formula based on 1:1 complexation between UGGT and the acceptor substrate leads to the K_d value of the latter which was estimated to be ca. 4 μM. The estimated K_d value between UGGT and the acceptor substrate indicates that interaction of UGGT with M9 substrate is comparable in its magnitude with that of G1M9 with CNX/CRT [27]. Several studies clarified that certain glycosyltransferases exhibit secondary structure change upon interaction with donor substrates or analogs [28,29], suggesting that glycosyltransferases cause significant conformational change in order to exhibit its catalytic activity. The complex formed by mixing UGGT and M9-G-E-TAMRA smoothly underwent glucosylation upon addition of UDP-Glc, indicating that the observed conformational change reflects productive enzyme-acceptor interaction. This experiment also gave us the estimate of kinetic parameters of UDP-Glc. Thus obtained K_m value (69 μM) was quite similar to that reported for the rat enzyme (44 μM) [30], indicating reliability of our system.

In conclusion, our study revealed characteristic properties of UGGT, surface hydrophobicity, lack of chaperone activity, and bipartite mode of substrate recognition, which may hint UGGT's unique specificity toward of folding defective glycoproteins. In addition, significant change of its secondary structure was observed upon complexation with a synthetic acceptor substrate, allowing us to estimate its affinity to acceptor substrate.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.08.112.

References

[1] J.L. Brodsky, W.R. Skach, Protein folding and quality control in the endoplasmic reticulum: recent lessons from yeast and mammalian cell systems, Curr. Opin. Cell Biol. 23 (2011) 464–475.
[2] L.A. Rutkevich, D.B. Williams, Participation of lectin chaperones and thiol oxidoreductases in protein folding within the endoplasmic reticulum, Curr. Opin. Cell Biol. 23 (2011) 157–166.
[3] L.W. Ruddock, M. Molinari, *N*-Glycan processing in ER quality control, J. Cell Sci. 119 (2006) 4373–4380.
[4] C. D'Alessio, J.J. Caramelo, A.J. Parodi, UDP-GlC:glycoprotein glucosyltransferase-glucosidase II, the ying-yang of the ER quality control, Semin. Cell Dev. Biol. 21 (2010) 491–499.
[5] C. Ritter, A. Helenius, Recognition of local glycoprotein misfolding by the ER folding sensor UDP-glucose:glycoprotein glucosyltransferase, Nat. Struct. Biol. 7 (2000) 278–280.
[6] J.J. Caramelo, O.A. Castro, L.G. Alonso, G. De Prat-Gay, A.J. Parodi, UDP-Glc:glycoprotein glucosyltransferase recognizes structured and solvent accessible hydrophobic patches in molten globule-like folding intermediates, Proc. Natl. Acad. Sci. USA 100 (2003) 86–91.
[7] J.J. Caramelo, O.A. Castro, G. de Prat-Gay, A.J. Parodi, The endoplasmic reticulum glucosyltransferase recognizes nearly native glycoprotein folding intermediates, J. Biol. Chem. 279 (2004) 46280–46285.
[8] M. Molinari, *N*-Glycan structure dictates extension of protein folding or onset of disposal, Nat. Chem. Biol. 3 (2007) 313–320.
[9] M. Sousa, A.J. Parodi, The molecular basis for the recognition of misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase, EMBO J. 14 (1995) 4196–4203.
[10] M.C. Sousa, M.A. Ferrero-Garcia, A.J. Parodi, Recognition of the oligosaccharide and protein moieties of glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase, Biochemistry 31 (1992) 97–105.
[11] E.S. Trombetta, A. Helenius, Conformational requirements for glycoprotein reglucosylation in the endoplasmic reticulum, J. Cell Biol. 148 (2000) 1123–1129.
[12] C. Ritter, K. Quirin, M. Kowarik, A. Helenius, Minor folding defects trigger local modification of glycoproteins by the ER folding sensor GT, EMBO J. 24 (2005) 1730–1738.
[13] K. Totani, Y. Ihara, T. Tsujimoto, I. Matsuo, Y. Ito, The recognition motif of the glycoprotein-folding sensor enzyme UDP-Glc:glycoprotein glucosyltransferase, Biochemistry 48 (2009) 2933–2940.
[14] Y. Ito, Y. Takeda, Analysis of glycoprotein processing in the endoplasmic reticulum using synthetic oligosaccharides, Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 88 (2012) 31–40.
[15] Y. Takeda, K. Totani, I. Matsuo, Y. Ito, Chemical approaches toward understanding glycan-mediated protein quality control, Curr. Opin. Chem. Biol. 13 (2009) 582–591.
[16] K. Totani, Y. Ihara, I. Matsuo, H. Koshino, Y. Ito, Synthetic substrates for an endoplasmic reticulum protein-folding sensor, UDP-glucose: glycoprotein glucosyltransferase, Angew. Chem. Int. Ed. Engl. 44 (2005) 7950–7954.
[17] K. Totani, Y. Ihara, I. Matsuo, Y. Ito, Substrate specificity analysis of endoplasmic reticulum glucosidase II using synthetic high mannose-type glycans, J. Biol. Chem. 281 (2006) 31502–31508.
[18] C.G. Parker, L.I. Fessler, R.E. Nelson, J.H. Fessler, *Drosophila* UDP-glucose:glycoprotein glucosyltransferase: sequence and characterization of an enzyme that distinguishes between denatured and native proteins, EMBO J. 14 (1995) 1294–1303.
[19] N. Greenfield, G.D. Fasman, Computed circular dichroism spectra for the evaluation of protein conformation, Biochemistry 8 (1969) 4108–4116.
[20] N.J. Greenfield, V.M. Fowler, Tropomyosin requires an intact N-terminal coiled coil to interact with tropomodulin, Biophys. J. 82 (2002) 2580–2591.
[21] I. Sirangelo, E. Bismuto, S. Tavassi, G. Irace, Apomyoglobin folding intermediates characterized by the hydrophobic fluorescent probe 8-anilino-1-naphthalene sulfonate, Biochim. Biophys. Acta 1385 (1998) 69–77.
[22] M. Izumi, Y. Makimura, S. Dedola, A. Seko, A. Kanamori, M. Sakono, Y. Ito, Y. Kajihara, Chemical synthesis of intentionally misfolded homogeneous glycoprotein: a unique approach for the study of glycoprotein quality control, J. Am. Chem. Soc. 134 (2012) 7238–7241.
[23] J. Buchner, H. Grallert, U. Jakob, Analysis of chaperone function using citrate synthase as nonnative substrate protein, Meth. Enzymol. 290 (1998) 323–338.
[24] J.P. Hendrick, F.U. Hartl, The role of molecular chaperones in protein folding, FASEB J. 9 (1995) 1559–1569.
[25] S.M. Arnold, L.I. Fessler, J.H. Fessler, R.J. Kaufman, Two homologues encoding human UDP-glucose:glycoprotein glucosyltransferase differ in mRNA expression and enzymatic activity, Biochemistry 39 (2000) 2149–2163.
[26] S.M. Arnold, R.J. Kaufman, The noncatalytic portion of human UDP-glucose: glycoprotein glucosyltransferase I confers UDP-glucose binding and transferase function to the catalytic domain, J. Biol. Chem. 278 (2003) 43320–43328.
[27] M. Kapoor, H. Srinivas, E. Kandiah, E. Gemma, L. Ellgaard, S. Oscarson, A. Helenius, A. Surolia, Interactions of substrate with calreticulin, an endoplasmic reticulum chaperone, J. Biol. Chem. 278 (2003) 6194–6200.
[28] H. Jamaluddin, P. Tumbale, S.G. Withers, K.R. Acharya, K. Brew, Conformational changes induced by binding UDP-2F-galactose to alpha-1,3 galactosyltransferase-implications for catalysis, J. Mol. Biol. 369 (2007) 1270–1281.
[29] J.A. Alfaro, R.B. Zheng, M. Persson, J.A. Letts, R. Polakowski, Y. Bai, S.N. Borisova, N.O. Seto, T.L. Lowary, M.M. Palcic, S.V. Evans, ABO (H) blood group A and B glycosyltransferases recognize substrate via specific conformational changes, J. Biol. Chem. 283 (2008) 10097–10108.
[30] D.C. Tessier, D. Dignard, A. Zapun, A. Radominska-Pandya, A.J. Parodi, J.J. Bergeron, D.Y. Thomas, Cloning and characterization of mammalian UDP-glucose glycoprotein: glucosyltransferase and the development of a specific substrate for this enzyme, Glycobiology 10 (2000) 403–412.