ARTICLE IN PRESS

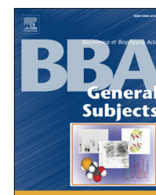
BBAGEN-27925; No. of pages: 10; 4C: 8, 9

Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen



Glycan specificity of a testis-specific lectin chaperone calmegin and effects of hydrophobic interactions

Masafumi Sakono [a], Akira Seko [a], Yoichi Takeda [a,*], Jun-ichi Aikawa [b], Masakazu Hachisu [a], Akihiko Koizumi [a], Kohki Fujikawa [a], Yukishige Ito [a,b,**]

[a] *Japan Science and Technology Agency (JST), ERATO, Ito Glycotrilogy Project, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*
[b] *Synthetic Cellular Chemistry Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*

ARTICLE INFO

Article history:
Received 9 October 2013
Received in revised form 19 March 2014
Accepted 14 April 2014
Available online xxxx

Keywords:
Lectin chaperone
Calnexin
Calmegin
Glycan
Hydrophobicity

ABSTRACT

Background: Testis-specific chaperone calmegin is required for the generation of normal spermatozoa. Calmegin is known to be a homologue of endoplasmic reticulum (ER) residing lectin chaperone calnexin. Although functional similarity between calnexin and calmegin has been predicted, detailed information concerned with substrate recognition by calmegin, such as glycan specificity, chaperone function and binding affinity, are obscure.
Methods: In this study, biochemical properties of calmegin and calnexin were compared using synthetic glycans and glycosylated or non-glycosylated proteins as substrates.
Results: Whereas their amino acid sequences are quite similar to each other, a certain difference in secondary structures was indicated by circular dichroism (CD) spectrum. While both of them inhibited protein heat-aggregation to a similar extent, calnexin exhibited a higher ability to facilitate protein folding. Similarly to calnexin, calmegin preferentially recognizes monoglucosylated glycans such as $Glc_1Man_9GlcNAc_2$ (G1M9). While the surface hydrophobicity of calmegin was higher than that of calnexin, calnexin showed stronger binding to substrate. We reasoned that lectin activity, in addition to hydrophobic interaction, contributes to this strong affinity between calnexin and substrate.
Conclusions: Although their similarity in carbohydrate binding specificities is high, there seems to be some differences in the mode of substrate recognition between calmegin and calnexin.
General significance: Calmegin exhibited higher hydrophobic interaction to aglycons than calnexin.
© 2014 Published by Elsevier B.V.

1. Introduction

Glycan processing plays an important role in glycoprotein quality control in the endoplasmic reticulum (ER) [1,2]. As nascent glycoproteins bearing triglucosylated high-mannose-type N-glycan ($Glc_3Man_9GlcNAc_2$; G3M9) are generally unfolded, various carbohydrate active proteins and chaperones in the ER assist them in achieving mature folding. Namely, in the beginning, nascent glycoproteins are trimmed by glucosidase-I and -II to the $Glc_1Man_9GlcNAc_2$ (G1M9) glycoform and then captured by ER-residing chaperones calnexin (CNX) or calreticulin (CRT). Both CNX and CRT are characterized by their property as lectins, which are specific to monoglucosylated glycans, typically G1M9. In addition, ERp57, a member of the protein disulfide isomerase family, is in association with CNX/CRT, and facilitates disulfide bond formation and isomerization [3]. Subsequently, upon removal of the remaining glucose residue by glucosidase II, client glycoproteins are liberated from CNX/CRT. At this stage, their folding states are surveyed by a folding sensor enzyme, UDP-glucose:glycoprotein glucosyltransferase (UGGT). When the folding is incomplete, liberated glycoproteins are reglucosylated by UGGT, regenerating the monoglucosylated glycoforms (*e.g.* G1M9), which are re-captured by CNX/CRT. After repeated interaction with glucosidase II, CNX/CRT, and UGGT (CNX/CRT cycle), glycoproteins that have achieved mature folding are exported to the Golgi apparatus.

While CNX is a transmembrane protein, previous studies have shown that its lectin and chaperone regions both localize in the luminal side of the ER [4]. As shown in Fig. 1, CNX is composed of N-, P-, and C-domains. The N-domain is proposed to have a carbohydrate binding activity, while the P- and C-domains are responsible for association with ERp57 and calcium storage, respectively [5]. In addition to the C-domain, a calcium binding site was also identified in the N-domain. X-ray diffraction study has revealed that the structure of the N-domain is globular and

Abbreviations: Glc, glucose; Man, mannose; GlcNAc, N-acetylglucosamine; CNX, calnexin; CRT, calreticulin; UGGT, UDP-glucose:glycoprotein glucosyltransferase; CMG, calmegin; CRT3, calsperin or calreticulin 3; ANS, 8-anilino-1-naphtalene sulfonic acid; CAB, bovine carbonic anhydrase B; Cbz, carboxybenzyl; CS, citrate synthase

* Corresponding author. Tel.: +81 48 485 8893; fax: +81 48 485 8892.
** Correspondence to: Y. Ito, Japan Science and Technology Agency (JST), ERATO, Ito Glycotrilogy Project, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. Tel.: +81 48 485 8893; fax: +81 48 485 8892.
E-mail addresses: yotakeda@riken.jp (Y. Takeda), yukito@riken.jp (Y. Ito).

http://dx.doi.org/10.1016/j.bbagen.2014.04.012
0304-4165/© 2014 Published by Elsevier B.V.

Please cite this article as: M. Sakono, et al., Glycan specificity of a testis-specific lectin chaperone calmegin and effects of hydrophobic interactions, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagen.2014.04.012

ARTICLE IN PRESS

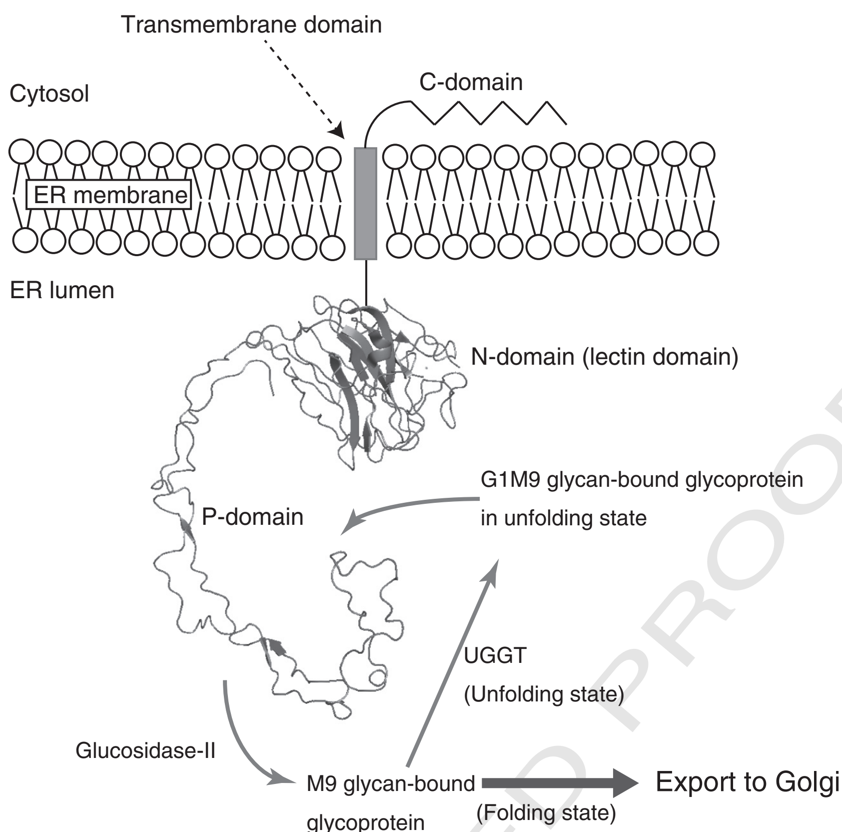


Fig. 1. Structure and schematic function of calnexin. The figure was drawn based on the structure of cCNX (PDB code 1JHN).

UNCORRECTED PROOF

composed of β sandwich folds, while the P-domain holds a β strand hairpin form due to the presence of a proline-rich amino acid sequence [6].

Testis-specific chaperones calmegin (CMG) and calsperin (CRT3) are homologues of CNX and CRT, respectively [7–9]. Although CNX gene deletion was shown to cause early-postnatal death in mouse [10], CMG knockout mice were fully viable, while the generation of normal spermatozoa was affected [11]. In particular, expression of spermatozoal proteins such as ADAM1, ADAM2 and ADAM3, which are involved in zona pellucida binding or migration from the uterus into the oviduct, was reduced by CMG deletion, which caused male infertility [12–14]. Whereas CMG is required for the formation of a heterodimeric complex consisting of ADAM1 and ADAM2, CNX is not able to support this function [11]. Moreover, it has been reported that CMG is specifically expressed in germ cells of pachytene spermatocyte to spermatid stage, while CNX is expressed throughout all stages of spermatogenesis [15]. Therefore, the physiological role of CMG is assumed to be distinct from CNX. However, unlike CNX, the mode of substrate–CMG interaction, such as glycan specificity, chaperone activity and binding affinity, has been obscure.

Our previous studies have employed synthetic oligosaccharides modified by various aglycons as versatile substrates to study carbohydrate active proteins involved in the glycoprotein quality control system [16,17]. For instance, we have previously clarified that the reactivity of synthetic M9 derivatives as substrates of UGGT was affected by the nature of aglycons, particularly the magnitude of their hydrophobicity [18]. Synthetic N-glycan derivatives have proven valuable also as substrates of glucosidase-II and CRT [18–20]. Kinetic constants of enzyme reactions were found to be quite similar between glycoproteins and synthetic N-glycans [21], indicating that our approaches based on non-proteinic substrates are reasonable for the analyses of the glycoprotein quality control system mediated by carbohydrate–protein interactions.

In this study, to clarify its substrate recognition mechanism, we examined the biochemical properties of CMG, in comparison with CNX, using synthetic N-glycan derivatives and glycosylated or non-glycosylated proteins. Although their amino acid sequences are quite similar to each other, some differences in the secondary structure were observed by CD analysis. Both of them were able to inhibit protein aggregation to a similar extent, whereas differences were seen in their ability to assist refolding of a non-glycosylated protein. Although, similarly to CNX, CMG was able to interact specifically with monoglucosylated high-mannose-type glycans such as G1M9, subtle differences between them in terms of fine specificity of substrate recognition were observed.

2. Materials and methods

2.1. Materials

M7-BODIPY, M8-BODIPY, M9-BODIPY, G1M9-BODIPY, G2M9-BODIPY, G3M9-BODIPY, G1M8B-BODIPY, G1M8C-BODIPY, G1M7BC-BODIPY, G1M5B2C2-BODIPY, G1M6BC2-BODIPY, and G1M9-Cbz were prepared as previously reported [20,22]. ClogP values were calculated using ChemDraw Ultra version 6.0, Cambridge Soft (Cambridge, MA). Calcium chloride, calcium carbonate, guanidine hydrochloride, 10–20% Tris–glycine gels, tris–hydroxymethyl aminomethane hydrochloride, β-mercaptoethanol, dithiothreitol and acetonitrile were purchased from Wako Pure Chemical Industries (Osaka, Japan). 8-Anilino-1-naphtalene sulfonic acid (ANS) was obtained from MP Biomedicals (Illkirch, France). Recombinant firefly luciferase, citrate synthase, bovine carbonic anhydrase (CAB) and *p*-nitrophenylacetate were purchased from Sigma-Aldrich (St. Louis, MO). IgY was isolated from chicken egg yolk as previously reported [23]. VIVACON500 was obtained from Sartorius Stedim Biotech S.A. (Goettingen, Germany).

Please cite this article as: M. Sakono, et al., Glycan specificity of a testis-specific lectin chaperone calmegin and effects of hydrophobic interactions, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagen.2014.04.012

Download English Version:

https://daneshyari.com/en/article/1947579

Download Persian Version:

https://daneshyari.com/article/1947579

Daneshyari.com