Biochimica et Biophysica Acta 1813 (2011) 1105–1110

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Cell surface targeting of myelin oligodendrocyte glycoprotein (MOG) in the absence of endoplasmic reticulum molecular chaperones ☆

Joanna Jung, Marek Michalak *

Department of Biochemistry, School of Molecular and Systems Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

ARTICLE INFO

Article history:
Received 22 November 2010
Received in revised form 7 December 2010
Accepted 8 December 2010
Available online 21 December 2010

Keywords:
Endoplasmic reticulum
Quality control
Chaperone
Calnexin
Calreticulin
ERp57

ABSTRACT

Myelin oligodendrocyte glycoprotein (MOG) is a type I integral membrane glycoprotein that localizes to myelin sheaths in the central nervous system. MOG has important implications in multiple sclerosis, as pathogenic anti-MOG antibodies have been detected in the sera of multiple sclerosis patients. As a membrane protein, MOG achieves its native structure in the endoplasmic reticulum where its folding is expected to be controlled by endoplasmic reticulum chaperones. Calnexin, calreticulin, and ERp57 are essential components of the endoplasmic reticulum quality control where they assist in the proper folding of newly synthesized glycoproteins. In this study, we show that expression of MOG is not affected by the absence of the endoplasmic reticulum quality control proteins calnexin, calreticulin, or ERp57. We also show that calnexin forms complexes with MOG and these interactions might be glycan-independent. Importantly, we show that cell surface targeting of MOG is not disrupted in the absence of the endoplasmic reticulum chaperones. This article is part of a special issue entitled: 11th European Symposium on Calcium.

1. Introduction

Myelin is the multilamellar sheath surrounding the large axons in the peripheral and central nervous systems and is essential for fast signal transmission. Myelin formation depends on myelin proteins. One of the minor (0.05%) components of the central nervous system (CNS) myelin is myelin oligodendrocyte glycoprotein (MOG).[1] This protein is expressed within the outermost lamellae of the myelin sheath, cell body, and processes of oligodendrocytes [1]. MOG is a 28-kDa cell surface glycoprotein with a single N-glycosylation site at N^{31} and one disulfide bond formed between C^{24} and C^{98} in the immunoglobulin (Ig)-like extracellular domain [2]. The amino acid sequence of MOG is highly conserved among animal species, and the crystal structure of its extracellular domain has demonstrated that it forms head-to-tail dimer in solution, which would suggest dimerization and potential homophilic (self-association) interactions [2]. MOG plays an important role in the pathology of multiple sclerosis, a chronic inflammatory disease of the CNS by influencing multiple sclerosis susceptibility as a primary target for autoimmune response [3]. Anti-MOG antibodies have been found in the sera of patients with multiple sclerosis, and their presence is directly associated with myelin damage. Previous studies suggest that encephalitogenic antibodies recognize conformation-dependent epitopes of MOG [4].

As an integral membrane protein, MOG is synthesized in the endoplasmic reticulum (ER) and subsequently targeted to the surface of oligodendrocytes. These events must be closely monitored by the ER quality control system that consists of molecular chaperones and folding enzymes. Calnexin and calreticulin, two ER lectin chaperones, play a crucial role in the quality control process. Calnexin and calreticulin are both calcium-binding chaperones in the ER [8,17,21]. Calreticulin plays especially important role in cellular calcium homeostasis as it is responsible for buffering of over 50% of the ER calcium and it affects the function of the store operated calcium influx [8]. The two ER lectin-like calcium-binding chaperones each consists of a globular domain, which contains a glycan-binding site and an extended, proline-rich domain (P-arm) that is known to associate with ERp57, an oxidoreductase that belongs to the protein disulfide isomerase (PDI) family [5,6]. ERp57 catalyzes formation and isomerization of disulfide bonds in newly synthesized glycoprotein (often calnexin and calreticulin substrates) within the ER. Despite MOG playing a critical role in the pathology of multiple sclerosis, the role of ER associated chaperones and folding enzymes in the biology of MOG and, consequently in the pathology of multiple sclerosis, is not known. Here we identified calnexin as an ER molecular chaperone for MOG and examined the role of ER quality control in the MOG expression, trafficking, and unfolded protein response (UPR). This work provides

☆ This article is part of a special issue entitled 11th European Symposium on Calcium.

* Corresponding author. Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2S7. Tel.: +1 780 492 2256; fax: +1 780 492 0886.
E-mail addresses: joanna1@ualberta.ca (J. Jung), marek.michalak@ualberta.ca (M. Michalak).

[1] Abbreviations used: CNS, central nervous system; ER, endoplasmic reticulum; GAPDH, glyceraldehyde phosphate dehydrogenase; GFP, green fluorescent protein; Ig, immunoglobulin; MOG, myelin oligodendrocyte glycoprotein; PDI, protein disulfide isomerase; UPR, unfolded protein response

0167-4889/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2010.12.014

new important insights into the biology of MOG. We have identified new potential players, which may contribute to folding and conformation of MOG and therefore to its encephalitogenic properties.

2. Experimental procedures

2.1. Vector DNA

The full-length cDNAs encoding mouse MOG were cloned into pEGFP-N1 vector to create vectors encoding MOG with green fluorescent protein (GFP) at its carboxyl termini MOG–GFP. The following DNA primers containing an *attB* recombination site (Gateway Cloning, Invitrogen) were used: forward primer 5′-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA TAC CAT GAT GGC CTG TTT GTG GAG CTT CTC-3′ reverse primer 5′-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTT TTA CTT GTA CAG CTC GTC CAT GCC-3′. First the PCR products were cloned into the pDONR vector (Invitrogen) using LR Clonase Enzyme (Invitrogen). Next, the recombination reaction was carried out using BP Clonase Enzyme Mix (Invitrogen) with the gene of interest, the ubiquitously expressed promoter, EF1α (cellular polypeptide chain elongation factor 1 alpha), and a destination vector $2K7_{bsd}$ containing a blasticidin resistance gene that allows the easy selection of transfected cells [7]. Virus isolation and transduction were carried out as described previously [7].

2.2. Cell culture, cell lines, and immunofluorescence

Wild-type (K41) and calreticulin-deficient (K42) mouse embryonic fibroblasts were described previously [8]. ERp57-deficient mouse embryonic fibroblasts were isolated from *ERp57*$^{-/-}$ embryos and immortalized [8,9]. Wild-type and calnexin-deficient mouse fibroblasts were isolated from newborn mice and immortalized [10]. Lentiviral expression constructs encoding MOG–GFP were used to create wild-type, calnexin-deficient (*cnx*$^{-/-}$), calreticulin-deficient (*crt*$^{-/-}$) and *ERp57*$^{-/-}$-deficient cell lines stably expressing recombinant MOG–GFP. Cells were cultured in the presence of 7 μg/ml blasticidine for 14 days. Expression of MOG–GFP protein was monitored by Western blot analysis using anti-GFP antibodies or by immunofluorescence, co-staining with ER marker TexasRed Concanavalin A conjugate using confocal microscopy. For immunofluorescence, cells expressing MOG–GFP were grown for 24 h after seeding in culture media and fixed in 4% paraformaldehyde for 12 min [10]. Images were collected by spinning-disk microscopy (Guelph, Canada) set up on an Olympus IX-81 inverted stand (Markham, Canada). Images were acquired through a 60× objective (N.A. 1.42) with an Electron Multiplying Charge Coupled Device (EMCCD) camera (Hamamatsu, Japan).

2.3. Western blot analysis and immunoprecipitation

Brains and cerebellum from mice were isolated, crushed in liquid nitrogen, and lysed in RIPA buffer containing 150 mM NaCl, 50 mM Tris–HCl, pH 7.4, 0.5% Nonidet P-40, 1% deoxycholate, 0.1% sodium dodecyl sulphate, and protease inhibitors for 15 min on ice [11]. For Western blot analysis, cells expressing MOG–GFP were lysed on ice in RIPA buffer for 15 min [10]. The following antibodies were used: goat anti-GFP 1:10,000, rabbit anti-MOG 1:300, rabbit anti-calnexin (Stressgene), rabbit anti-calreticulin 1:300, rabbit anti-ERp57 1:1000, and rabbit anti-glyceraldehyde dehyrodenase (GAPDH) 1:1000.

For immunoprecipitation, cells were grown to 80–90% confluency followed by the addition of 500 μl/10 cm dish of A lysis buffer containing 50 mM Hepes, pH 7.4, 200 mM NaCl, 2% CHAPS and protease inhibitors [12]. Samples were incubated on ice for 30 min and then spun at 11,600*g* for 15 min to remove insoluble material. Supernatant was pre-cleared with a 1/15th of 10% protein A/G Sepharose bead suspension in an HBS buffer containing 50 mM HEPES, pH 7.4, and 200 mM NaCl. Beads were centrifuged followed by addition of 2 μl of an appropriate antibody. The samples were incubated overnight at 4 °C with rotation followed by addition of 100 μl of 10% protein A/G Sepharose in HBS buffer and incubated for an additional 4 h. Beads were centrifuged, washed 3 times with HBS containing 1% CHAPS, one time with HBS, followed by addition of 30 μl of SDS-PAGE sample buffer [13]. Proteins were separated by SDS-PAGE (10% acrylamide). Cells were also culture with 1 mM castanospermine for 16 h.

2.4. Endoglycosidase digestions

Cellular proteins were extracted with RIPA buffer and 20 μg of total protein was incubated with EndoH or PNGaseF at 37 °C for 3 h according to the manufacturer's protocols. Digested and undigested samples were separated by SDS-PAGE (10% acrylamide) followed by Western blot analysis with rabbit anti-GFP antibodies at 1:10,000.

2.5. Analysis of unfolded protein response (UPR)

The UPR was measured using the luciferase reporter gene assay [10]. In brief, wild-type, *cnx*$^{-/-}$, *ERp57*$^{-/-}$ and *crt*$^{-/-}$cells stably expressing MOG–GFP were transfected with pRL-XFL vector encoding *Renilla* luciferase and *Firefly* luciferase reporter genes [10,14]. Briefly, 1 μg of DNA was transfected using Effectene (Qiagen). Cells were treated with 1 μM thapsigargin for 16 h, lysed, diluted, and assayed with the Dual-Luciferase Assay Kit (Promega) using a luminometer (Berthold-Lumat LB 9501). Relative light units (RLUs) were normalized to internal control. Average ± standard deviation (SD) ($n = 3$).

3. Results and discussion

3.1. Calnexin interacts with MOG

In eukaryotic cells, membrane and secreted proteins fold and achieve their native structures in the ER. Calnexin is one of the ER associated molecular chaperones and, together with oxidoreductase ERp57, assists many glycoproteins to achieve their final confirmation [15]. MOG is a glycoprotein with a single N-glycosylation site and a single disulfide bond, which makes it a potential candidate for calnexin and ERp57 substrate. We asked whether calnexin and ERp57 form complexes with the newly synthesized MOG. Stable expression of MOG–GFP in wild-type cells was followed by immunoprecipitation with anti-GFP antibodies and Western blot analysis with either anti-ERp57 or anti-calnexin antibodies. Fig. 1 shows that calnexin formed complexes with MOG indicating that calnexin may play a role in MOG folding. Attempts to immunoprecipitate MOG with ERp57 failed indicating that ERp57 may not interact directly with MOG. To examine

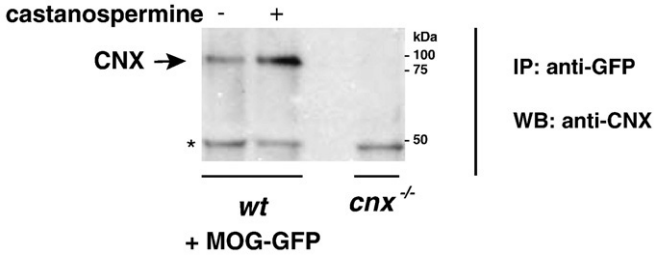

Fig. 1. Interaction between calnexin and MOG. Cell lysates were collected from wild-type (*wt*) cells stably expressing MOG green fluorescent protein (GFP) followed by addition of the goat anti-GFP antibodies as indicated in the figure and as described under "Experimental procedures". Proteins were separated by SDS-PAGE followed by Western blot analysis with anti-calnexin (*anti-CNX*) antibodies. A representative Western blot of 3 independent experiments is shown. Cells were cultured in the presence of 1 mM castanospermine for 16 h followed by immunoprecipitation and Western blot analysis to test for a glycan-dependent interaction between calnexin and MOG–GFP. Calnexin-deficient cells (*cnx*$^{-/-}$) stably expressing MOG–GFP were used as a negative control. The arrow indicates the location of calnexin (CNX) and the asterisk mark unspecific bands.