FEBS Open Bio 4 (2014) 315–320

FEBS openbio

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

The role of Cysteine 227 in subcellular localization, water permeability, and multimerization of aquaporin-11

Saki Takahashi, Kanako Muta, Hiroko Sonoda, Ayaka Kato, Ahmed Abdeen, Masahiro Ikeda *

Department of Veterinary Pharmacology, University of Miyazaki, Miyazaki 889-2192, Japan

ARTICLE INFO

Article history:
Received 6 January 2014
Revised 12 March 2014
Accepted 12 March 2014

Keywords:
Aqauaporin-11
Water channel
Water permeability

ABSTRACT

Aquaporin-11 (AQP11) is the latest member of the mammalian water channel protein family to be described. Recent in vivo studies have shown that mutation at Cys^{227} causes renal failure. However the importance of Cys^{227} for the molecular function of AQP11 is largely unknown. In this study, we examined the subcellular localization, water permeability, and multimerization of AQP11 with a mutation at Cys^{227}. Interestingly, cells expressing the mutants had significantly higher osmotic water permeability. In contrast, the mutation lowered the cell surface expression and multimerization levels. Our observations suggest that Cys^{227} is crucial for the proper molecular function of AQP11.

1. Introduction

The aquaporins (AQPs) are a family of integral membrane proteins, which transport water and small solutes including glycerol, CO_2, ammonia, urea, and hydrogen peroxide across the membranes of biological cells [1]. So far, sequence analyses have revealed thirteen functionally and phylogenetically distinct members of the AQP family in mammals, of which AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8 are orthodox AQPs that primarily transport water molecules, AQP3, AQP7, AQP9, and AQP10 are aquaglyceroporins that are permeated by water and neutral solutes, and AQP11 and AQP12 are a recently proposed third group of AQPs whose functions are largely unknown [1–4].

It has been demonstrated that the orthodox AQPs and aquaglyceroporins share the same molecular architecture [3,5,6]. These proteins exist as multi-subunit oligomers and each subunit has the channel pore. Two highly conserved Asn-Pro-Ala (NPA) motifs in each subunit are present in each member, and these are known to be the signature motifs of the AQPs. Structural analyses have shown that these motifs reside on opposite sides of each monomer and are important for pore formation.

Compared with the orthodox AQPs and aquaglyceroporins, AQP11 and AQP12 are the most distantly related paralogs with low amino acid sequence identity (AQP11 is approximately 10% identical and AQP12 is approximately 25% identical to the sequences of the orthodox AQPs and aquaglyceroporins) [2–4,6–8]. With regard to the signature motifs, the N-terminal NPA motif for AQP11 is Asn-Pro-Cys (NPC) and that for AQP12 is Asn-Pro-Thr (NPT), the C-terminal NPA motif being conserved.

In comparison with AQP12, AQP11 has been better characterized [4,7–10,12]. AQP11 was first described by our group and studies with AQP11-deficient ($Aqp11^{-/-}$) mice revealed the importance of AQP11 during post-natal kidney development in mammals [7]. Immunohistochemical studies showed that AQP11 was localized mainly in the endoplasmic reticulum (ER) in certain tissues such as kidney, liver, testis, intestine, and brain. An osmotic water permeability assay with Sf9 membrane vesicles and mammalian cells expressing AQP11 indicated that AQP11 was a water-permeable channel [8,9].

Recently, Tchekneva and colleagues have characterized sudden juvenile death syndrome (sjds) mutation ($Aqp11^{sjds/sjds}$) mice that are characterized by cell injury in the renal cortex, resembling the phenotype of $Aqp11^{-/-}$ [10]. They also identified the Cys^{227} to Ser mutation in $Aqp11^{sjds/sjds}$ mice as the causative mutation and found that the phenotype of heterozygous $Aqp11^{-/sjds}$ compound mice was similar to that of $Aqp11^{-/-}$ mice, indicating that the Cys^{227} to Ser mutation was a loss-of-function one. However, the importance of Cys^{227} for the molecular function of AQP11 has yet to be elucidated.

In order to gain insight into the nature of AQP11, we examined the role of Cys^{227} in AQP11 in terms of subcellular localization, water permeability, and multimerization in transfected mammalian cells.

Abbreviations: AQP, aquaporin; ER, endoplasmic reticulum; GFP, green fluorescent protein; DsRM, DsRed-Monomer

* Corresponding author. Address: Department of Veterinary Pharmacology, Faculty of Agriculture, University of Miyazaki, Gakuenkibanadai-Nishi 1-1, Miyazaki 889-2192, Japan. Tel./fax: +81 985 58 7268.

E-mail address: a0d302u@cc.miyazaki-u.ac.jp (M. Ikeda).

http://dx.doi.org/10.1016/j.fob.2014.03.005

Unexpectedly, the mutation at Cys^{227} was found to confer a gain-of-function in terms of water permeability, suggesting that Cys^{227} is crucial for the proper molecular function of AQP11.

2. Materials and methods

2.1. Plasmid construction, cell culture, transfection and confocal microscopy

Constructs encoding Myc-, green fluorescent protein- (GFP-), or DsRed-Monomer- (DsRM-) -tagged proteins were made by in-frame subcloning of human AQP11 into pCMV-Myc (BD Biosciences Clontech, CA), pEGFP-C1 (BD Biosciences Clontech), or pDsRed-Monomer (BD Biosciences Clontech). C-terminal GFP-tagged human AQP1 (AQP1-GFP) was also made by in-frame subcloning of human AQP1 into pcDNA-DEST47 (Invitrogen, CA). For fluorescence labeling of the ER, the pDsRed2-ER vector (BD Biosciences Clontech) was employed. A site directed mutagenesis kit (Stratagene, CA) was used to introduce the mutations of the constructs.

CHO-K1 cells (obtained from ATCC, #CCL-61) were transfected at 50–60% confluency using Lipofectamine 2000, Lipofectamine LTX (Invitrogen), or a Neon® Transfection System in accordance with the manufacturer's instructions. A Neon® Transfection System was employed only to examine the total expression level of DsRM-hAQP11-C227S. When fluorescence microscopy was conducted, cells on glass coverslips were observed 24 h after transfection using a laser scanning confocal microscope (Olympus FV300, Tokyo, Japan).

2.2. Surface biotinylation

A cell surface biotinylation assay was performed as described previously [8]. Briefly, transfected cells were biotinylated in 1 mg/ml sulfo-NHS-SS-biotin (Pierce, IL) at 4 °C for 10–15 min, washed, and then lysed in lysis buffer (25 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 2% Nonidet P-40, Complete protease inhibitor mixture) for 20 min at 4 °C. The cell lysate was centrifuged at 12,000*g* for 10 min, and the supernatant was precipitated with immobilized NeutrAvidin beads (Pierce).

2.3. Cross-linking experiments

Cross-linking with paraformaldehyde was performed as described previously [8]. Briefly, cells were incubated with PBS containing 4% paraformaldehyde at room temperature for 15 min. After washing, the cells were lysed in lysis buffer (25 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1.5% Nonidet P-40, Complete protease inhibitor mixture) and the lysate was then mixed with 2× Laemmli sample buffer supplemented with 0.1 M DTT at 37 °C for 20 min.

2.4. Measurement of osmotic water permeability (Pf)

One day after transfection, cells were treated with trypsin–EDTA for a short time (around 15 s) to create a round cell shape, allowing easy calculation of cell volume [8]. The rounded cells were then washed and bathed in an extracellular solution (ES) containing (in mM) 150 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, and 10 Hepes (pH 7.4 with Tris). The cells expressing DsRM were identified under a laser scanning confocal microscope. Osmotic swelling was caused by puff-application of a hypotonic solution made by 1:1 dilution of ES in MilliQ water at room temperature (around 25 °C), as described previously [8,11]. An image frame of each cell was recorded every 1.12 s using a time-lapse image-capture system attached to the laser scanning confocal microscope. *Pf* was calculated from osmotic swelling data using the formula:

$$Pf = [V_0 \times d(V/V_0)/dt]/[S \times V_w \times (\mathrm{osm}_{in} - \mathrm{osm}_{out})]$$

where V_0 and S are the initial cell volume and surface area, respectively, $d(V/V_0)/dt$ is the slope of linear fit for the plot of cell volume vs. time between 1.12 and 17.92 s during osmotic swelling, and V_w - $\times (\mathrm{osm}_{in} - \mathrm{osm}_{out})$ is the osmolality gradient.

2.5. Western blot analysis and chemicals

After separation by SDS–PAGE, the protein was transferred to a polyvinylidene difluoride membrane and the protein on the membrane associated with antibodies was detected by a Super Signal® chemiluminescence detection system (Pierce). The anti-Myc antibody was from BD Biosciences Clontech. All other chemicals and reagents were from Sigma or Wako Pure Chemicals (Japan).

3. Results

3.1. Subcellular localization of the AQP11 mutants at Cys^{227}

AQP11 has been shown to be localized at the ER membrane [7,8]. We investigated whether the introduction of a mutation at Cys^{227} replacing it with Ser (C227S) or Ala (C227A) affected the subcellular localization of AQP11. In this experiment, we used C-terminal GFP-tagged human AQP1 (hAQP1-GFP) as a control protein and N-terminal GFP-tagged human AQP11 (GFP-hAQP11), because it has been reported that the N-terminus of AQP1 functions as a signal for the proper localization, and tagging of GFP to the N- and C-terminus of AQP11 resulted in virtually the same subcellular localization [8,12]. When CHO cells were transfected with hAQP1-GFP expression plasmid together with a plasmid encoding an ER marker protein, hAQP1-GFP was clearly localized at the plasma membrane as well as at the ER (Fig. 1A–C), in line with a previous observation [8]. In contrast, the localization of GFP-hAQP11 virtually overlapped that of the ER marker (Fig. 1D–F). Similarly to GFP-hAQP11, GFP-hAQP11-C227S, and -C227A were clearly localized at the ER.

It has been shown that although the level of AQP11 expression is less than that at the ER, a significant amount of the protein is expressed at the plasma membrane [8]. Therefore, we checked the cell surface expression levels of Myc-hAQP11, Myc-hAQP11-C227S, and Myc-hAQP11-C227A using a cell surface biotinylation assay. The levels of both total and biotinylated protein for Myc-hAQP11-C227S and Myc-hAQP11-C227A were significantly lower than those of the wild type (Fig. 2A and B). On the other hand, the ratio of the level of biotinylated protein to that of total protein was not significantly altered between the wild type, Myc-hAQP11-C227S, and Myc-hAQP11-C227A (Fig. 2C).

It is possible that when using a chemically based lipofection technique to introduce a plasmid into cells, transfection efficiency may differ according to the plasmid employed, which might result in a reduced level of mutant expression being observed. Therefore, we also compared the total expression level of DsRM-hAQP11-C227S with that of DsRM-hAQP11 using electroporation, a mechanical transfection technique that is considered to introduce a fairly constant amount of plasmid into cells, even if different plasmids are used. The results showed that the protein expression level of DsRM-hAQP11-C227S was reduced by about 25% ($n = 3$) relative to that of its wild-type, thus corroborating the results obtained using Myc-tagged proteins.

Taken together, these data indicated that introduction of the mutation at Cys^{227} of AQP11 decreased the total level of expression, but did not affect trafficking to the plasma membrane.