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Quantitative analysis of ascorbic acid permeability of aquaporin 0 in the lens

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ABSTRACT

Aquaporin 0 (AQP0) is a lens-specific protein comprising more than 30% of lens membrane protein content and is a member of the aquaporin family. Water permeates through AQP0 much more slowly than other aquaporin family members, and other compounds, such as glycerol, also permeate AQP0. In the lens, ascorbic acid (AA) is found at high concentrations, protecting the lens from photochemical events such as photo-oxidation. The aim of the present study was to clarify the function of AQP0. Mouse fibroblast L-cells stably expressing AQP0 were established and incubated in medium containing AA, and intracellular AA levels were measured by high-performance liquid chromatography (HPLC) and 2,6-dichlorophenol-indophenol (DCPIP) analysis. Intracellular AA levels in AQP0-expressing cells quickly rose and reached saturation 10 min after incubation in medium containing 1000 μM AA. In contrast, AA levels in cells slowly decreased when AA was washed out from the medium. Cells overexpressing AQP0 increased the cellular uptake of AA in a time- and concentration-dependent manner. These data suggest that AA as well as water permeates AQP0.

AQP0 expression on Xenopus oocyte membranes was achieved by the injection of *AQP0* cRNA into oocytes that were incubated in medium containing AA. Intracellular AA levels were then measured by HPLC. AA uptake was demonstrated in the AQP0-expressing oocytes and was shown to quickly reach saturation. Intracellular AA concentration in oocytes increased in a time- and concentration-dependent manner.

The data in the present study show that AA permeates AQP0, reveal the role of AQP0 in AA permeability *ex vivo*, and also indicate that there is a difference between the import and export of AA via AQP0. These findings suggest that AQP0 plays an important role in controlling lens AA content.

1. Introduction

The lens is a transparent tissue that allows visible light to be transmitted into the eye. The lens contains two major cell types, a single layer of epithelial cells on the anterior surface under the capsule, and numerous elongated fiber cells in the bulk of the lens. Lens fiber cells lose their cellular nuclei and organelles during differentiation, as these would scatter light and therefore hinder vision. The lens does not contain blood vessels and its nutrition is achieved though cell–cell communication. Aquaporin 0 (AQP0, also referred to as Major Intrinsic Polypeptide 26, MIP26) is a lens-specific membrane protein expressed in fiber cells; it plays a role in cell–cell adhesion and comprises more than 30% of the membrane protein content of the normal vertebrate lens [1,2]. Mutations in the *AQP0* gene produce autosomal dominant cataracts in mice and humans; therefore, this protein is believed to play an important role in the maintenance of lens transparency [3–7]. AQP0 is a member of the aquaporin family of water channels but has a very limited ability to transport water compared to other aquaporin family members [3,4]. AQP0 was reported to transport glycerol as well as water in AQP0-expressing Xenopus oocytes [4,8].

The lens contains high concentrations of antioxidants such as ascorbic acid (AA, also known as vitamin C) and glutathione, which protect it from photo-oxidation [9–12]. AA is transported into lens epithelial cells from the aqueous humor. The transport of ascorbate occurs though two general pathways: the sodium-dependent ascorbic acid transporter (SVCT) family pathway and the glucose transporter (GLUT) family pathway. The SVCT family transports ascorbic acid in a sodium-dependent manner and the GLUT family transports dehydroascorbic acid (DHA). The SVCT family comprises SVCT1 and SVCT2, and the AA transport capacity of SVCT1 is approximately 10-fold higher than that of SVCT2 [13,14]. In the lens, only SVCT2 is expressed. The GLUT family consists of GLUT1–GLUT5 [15]. GLUT1, GLUT3, and GLUT4 transport DHA, which is competitively inhibited by glucose, but GLUT2 and GLUT5 do not [16–18]. In the lens, SVCT2 and GLUT1 are expressed in epithelial cells. In fiber cells, GLUT3 is the only ascorbate transporter that transports DHA [19,20]. Thus, it is expected that fiber cells

Abbreviations: AQP0, aquaporin 0; AA, ascorbic acid.

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contain unknown membrane proteins that transport AA. AA levels in the lens are decreased in diabetic patients and in animal models of diabetes [21–23]. In diabetic rat lenses, in which the AA concentration is lowered, the transcription of GLUT1, GLUT3 and SVCT2 was shown to be normal, but the transcription of AQP0 was increased [24]. These data suggest that AA may be transported by AQP0 as well as by SVCT2. In this study, we show that AA permeates AQP0 *ex vivo* and discuss the function of AQP0.

2. Materials and methods

2.1. Animals

Three-week-old male Wistar rats were purchased from Sankyo Labo Service Corporation (Tokyo, Japan) and *Xenopus laevis* were purchased from Saitama Experimental Animals Supply Co., Ltd. (Saitama, Japan). The Keio University Animal Research Committee approved all animal procedures performed in this study.

2.2. Cell culture

Mouse fibroblast L-cells were obtained from the Health Science Research Resources Bank (Osaka, Japan). Cells were cultured under standard conditions of 5% CO_2 at 37 °C in Dulbecco's Modified Eagle minimum essential Medium (DMEM; Gibco, CA) and 10% fetal calf serum (FBS; Cansera International Inc., Pexdale, ON, Canada).

2.3. Expression plasmids for AQP0 and SVCT2 constructs

Total RNA from rat lens was extracted using TRIZOL® reagent (Invitrogen, CA) according to the manufacturer's instructions. For *AQP0* and *SVCT2* cloning, reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using the Takara RNA PCR™ Kit (AMV) v3.0 (Takara Biotech., Shiga, Japan). The oligonucleotide primer sets used in this study are shown in Table 1 (Hokkaido System Science Co., Ltd., Hokkaido, Japan). Amplified and purified *AQP0* and *SVCT2* cDNA was inserted into the pcDNA3.1 (+) Vector (Invitrogen). *In vitro* transcription of cRNA from the plasmids encoding AQP0 and SVCT2 using an *in vitro* transcription T7 kit (Takara Biotech., Tokyo, Japan).

2.4. Establishment of stably-expressing cell lines

Expression vectors were transfected into L-cells using Lipofectamine™ Reagent and Plus Reagent (Invitrogen). Stable L-cell line expressing AQP0 or SVCT2 was selected in DMEM containing 1 mg/mL G-418 (Gibco, CA) and was established L-AQP0 or L-SVCT2. A control cell line was prepared by the transfection of L-cells with pcDNA3.1 (+) control vector.

2.5. Preparation and injection of oocytes

Female *X. laevis* were anesthetized, and stage V and VI oocytes were removed and prepared as according to standard method [25]. Twenty-four hours after isolation, oocytes were injected with 25 ng of *AQP0* or *SVCT2* cRNA and then maintained in Modified Barth's Solution (MBS; 88 mM NaCl, 0.33 mM $Ca(NO_3)_2{\cdot}4H_2O$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, and 10 mM Hepes, pH 7.4) supplemented with 50 mg/mL gentamicin (Wako Pure Chemical Industries Ltd., Osaka, Japan) at 18 °C for 48 h. Control oocytes were injected with an equivalent volume of water.

Table 1
Primer sequences used for expression vectors.

Gene	Direction	Sequences
AQP0	Forward	5′-GAATTCATGGGAACTTCG-3′
	Reverse	5′-GCGGCCGCTTACAGGGC-3′
SVCT2	Forward	5′-ATGGCCATCTACACCACAGAG-3′
	Reverse	5′-CTATACTGTGGCCTGGGAGT-3′

2.6. Western blot analysis

Preparation of cell and oocyte lysates was performed as described previously with minor modifications [26]. Cells or oocytes were washed four times and homogenized in PBS, and then centrifuged at 20,000*g* for 20 min at 4 °C. The precipitates were suspended in 8 M urea and then centrifuged. The remainder (the urea-insoluble fraction) was subjected to SDS–PAGE on a 12.5% gel and transferred to a nitrocellulose membrane filter for Western blot analysis. Western blot analysis was performed using an anti-rat AQP0 carboxyl tail antibody (^{253}GEPVELKTQAL^{263}) and an anti-rat SVCT2 antibody (Alpha Diagnostic Intl. Inc., SA), and bands were visualized using the ECL Advance™ Western Blotting Detection Kit (GE Healthcare Bio-Sciences, NJ) [27].

2.7. AA uptake assay

The stable cell lines L-AQP0 or L-SVCT2 were plated at a density of 1.5×10^4 cells per 35 mm-diameter plate (Becton Dickinson, Franklin Lakes, NJ). Cells were incubated in DMEM for 12 h at 37 °C under 5% CO_2 in air, and then the medium was switched to DMEM containing 1–1000 μM AA. After incubation with AA, cells were washed four times in PBS and scraped from tissue culture plates with a rubber policeman in 100 μL PBS. Cells were homogenized in 100 μL PBS and then centrifuged at 20,000*g* for 20 min at 4 °C. AA concentrations in the supernatant were measured by HPLC and DCPIP analysis.

Oocytes injected with *AQP0* or *SVCT2* cRNA were washed four times with ND96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes, pH 7.4), and then the buffer was switched to ND96 containing 1–30 μM AA, followed by incubation at 18 °C. After incubation, oocytes were washed four times with PBS, homogenized in 50 μL PBS and then centrifuged at 20,000*g* for 20 min at 4 °C. AA concentrations in the supernatant were measured by HPLC.

2.8. Measurement of AA concentrations

AA concentrations were determined using HPLC or DCPIP analysis (Merck, Darmstadt, Germany) [28]. For HPLC, the supernatant was mixed with 5% trichloroacetic acid solution (Wako) for deproteinization and then centrifuged. After centrifugation at 20,000*g* for 20 min at 4 °C, the supernatant was subjected to HPLC analysis. A liquid chromatograph (LC-10AD, Shimadzu, Kyoto, Japan) was equipped with a UV detector (SPD-10A, Shimadzu) and a chromatopac data analyzer (C-R8A, Shimadzu). Separation was achieved by isocratic elution in an L-column ODS C18 column (4.6 × 100 mm; Chemicals Evaluation and Research Institute, Saitama, Japan) with a mobile phase consisting of 60% 0.1 M Phosphate buffer (pH 6.0)/40% acetonitrile/0.1% triethylamine (Wako) at a flow rate of 0.1 mL/min. Twenty microliters of sample was injected into the HPLC system and AA was detected at 10 min as the peak retention time.

For DCPIP analysis, samples were homogenized in four volumes of PBS relative to the supernatant volume. Samples were mixed with 2.5% metaphosphoric acid solution and centrifuged at 20,000*g* for 20 min at 4 °C for deproteinization. After centrifugation, the supernatant was titrated with 0.15 mg/mL DCPIP. The changing absorbance of the solution was measured with an infinite M1000 microplate reader at 540 nm (TECAN Ltd., Männedorf,

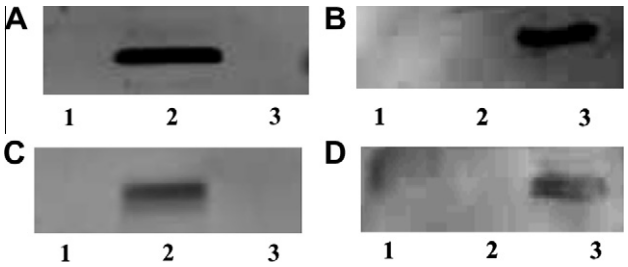

Fig. 1. Expression of AQP0 or SVCT2 in stable L-cell or Xenopus oocyte. Expression of AQP0 or SVCT2 in L-cells or oocytes was detected by Western blot analysis. AQP0 was detected using an anti-AQP0 antibody (A, C), and SVCT2 was detected using an anti-SVCT2 antibody (B, D). Lane 1: L-cells transfected with control vector, Lane 2: L-cells stably expressing AQP0, Lane 3: L-cells stably expressing SVCT2 (A, B). Lane 1: Xenopus oocytes injected with water, Lane 2: oocytes injected with *AQP0* cRNA, Lane 3: oocytes injected with *SVCT2* cRNA (C, D).

Switzerland). Intracellular AA concentration was calculated by subtracting the control cell or oocyte concentration from the experimental cell concentration.

3. Results

3.1. Detection of AQP0 or SVCT2 expression

AQP0 or *SVCT2* expression vector was transfected into L-cells, and cell stably expressing the corresponding protein were selected. The cells were then subjected to Western blot analysis for AQP0 or SVCT2. AQP0 or SVCT2 was detected in the urea-insoluble fractions of L-AQP0 or L-SVCT2 cell, respectively (Fig. 1A and B).

cRNA for *AQP0* or *SVCT2* was injected into Xenopus oocytes and expressed the corresponding protein, and then oocyte extracts were subjected to Western blot analysis. The urea-insoluble fractions of extracts from oocytes injected with *AQP0* or *SVCT2* cRNA contained AQP0 or SVCT2 protein, respectively, but control oocytes injected with water did not express either protein (Fig. 1C and D).

3.2. Uptake of AA in stable L-cell lines

To study AA transport, L-AQP0 or L-SVCT2 cell was incubated in culture medium containing various concentrations of AA, and intracellular AA concentration was subsequently measured by HPLC or DCPIP analysis. HPLC analysis showed that both L-AQP0 and L-SVCT2 cells incorporated intracellular AA, and that the level of incorporation was AA dose-dependent (Fig. 2A and B). DCPIP analysis also showed that L-AQP0 and L-SVCT2 cells incorporated AA in a dose-dependent manner (Fig. 2C and D).

AA uptake at various time intervals was assessed by incubation in DMEM culture medium containing 1000 μM AA at 37 °C. L-AQP0 and L-SVCT2 cells incorporated AA in a time-dependent manner, as determined by HPLC (Fig. 3A) and DCPIP analysis (Fig. 3B). Incorporation of AA increased over time for the first 10 min in L-AQP0 cells, reaching a plateau of 100 μmol/10^6 cells. In L-SVCT2 cells, AA uptake increased over time for the first 30 min and reached a plateau of 450 μmol/10^6 cells.

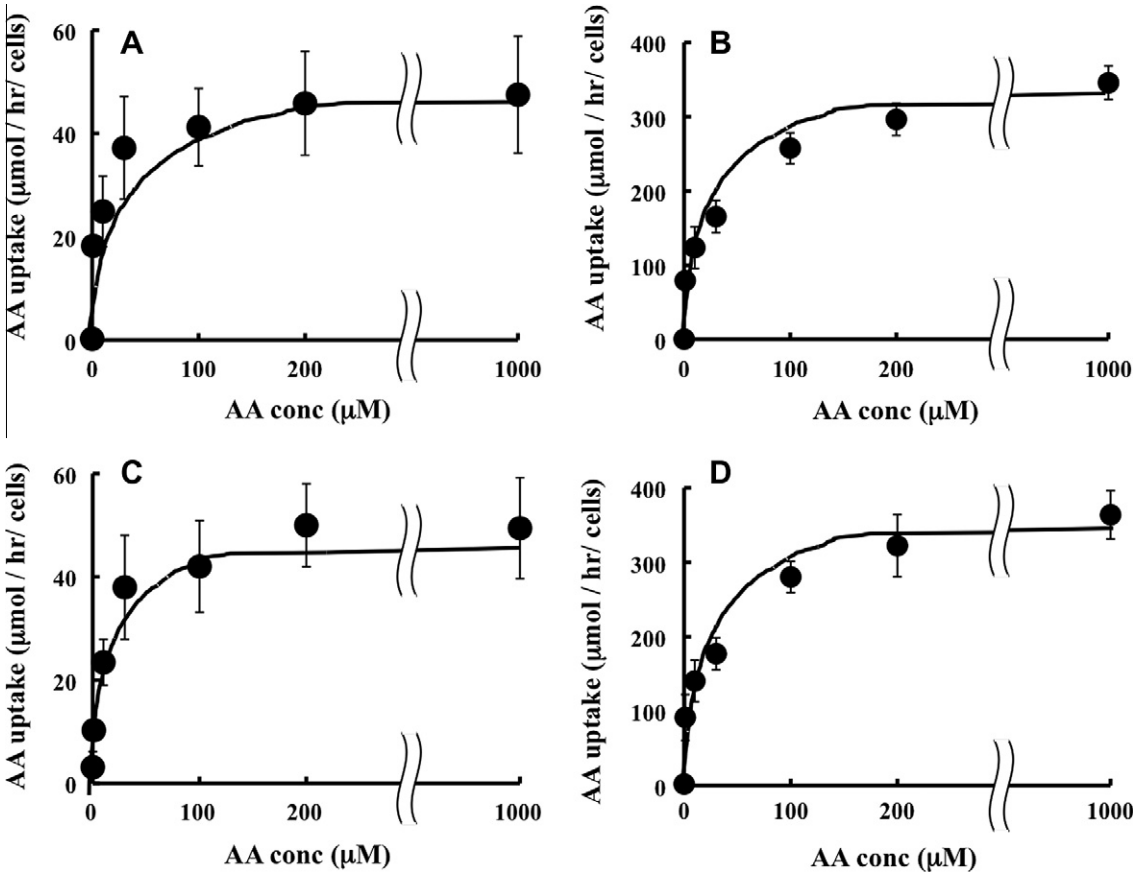

Fig. 2. Ascorbic acid uptake in L-cells stably expressing AQP0 or SVCT2. L-cells stably expressing AQP0 (L-AQP0) or SVCT2 (L-SVCT2) were incubated in DMEM containing various concentrations of AA for 60 min at 37 °C, and intracellular AA concentrations were then quantitated by HPLC and DCPIP analysis. (A) L-AQP0 cells were incubated in medium containing AA, and then AA was quantitated by HPLC. (B) L-SVCT2 cells were incubated in medium containing AA, and then AA was quantitated by HPLC. (C) L-AQP0 cells were incubated in medium containing AA, and then AA was quantitated by DCPIP analysis. (D) L-SVCT2 cells were incubated in medium containing AA, and then AA was quantitated by DCPIP analysis. All results are expressed as means ± SE. Each experiment was independently performed three times (n = 12 per group).

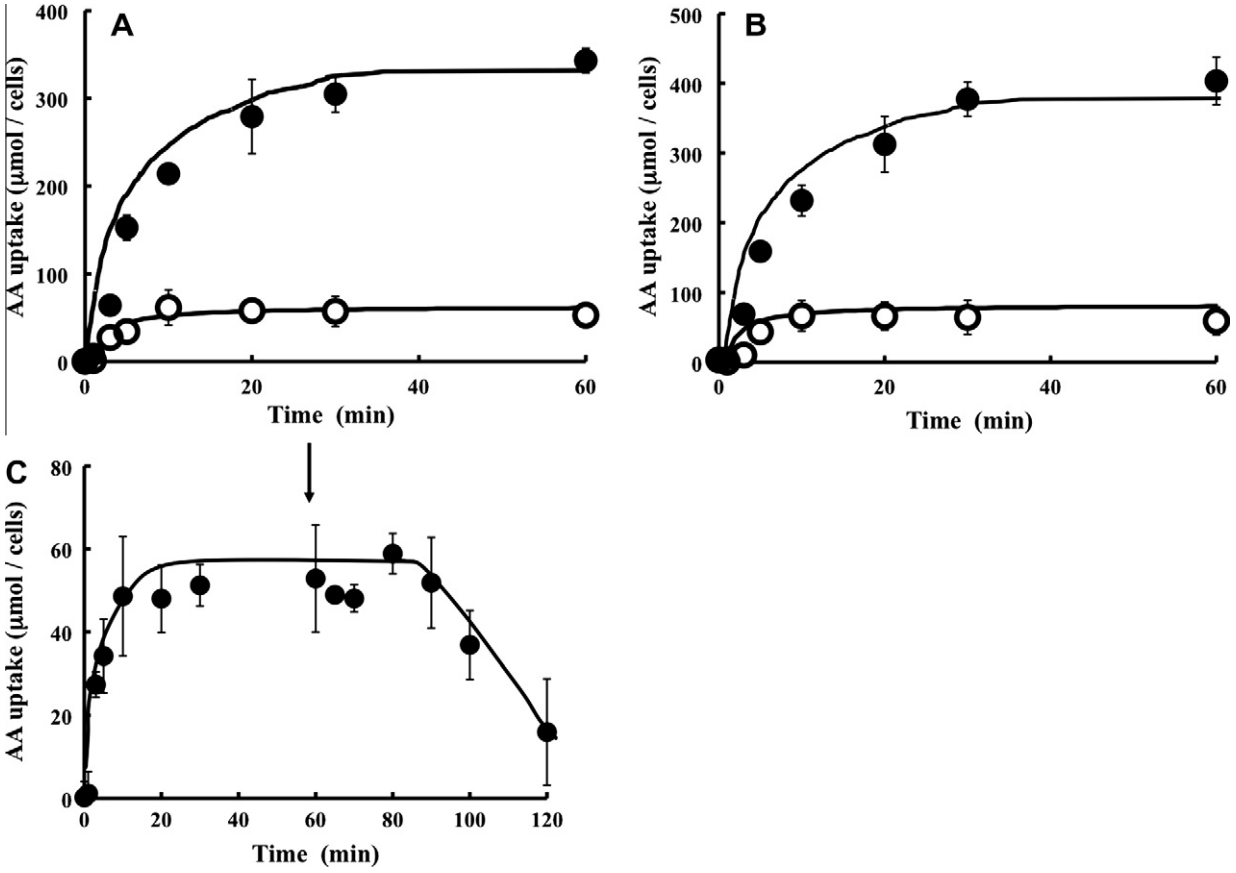

Fig. 3. Time course of AA uptake in L-cells stably expressing AQP0 or SVCT2. The time course of AA uptake in L cells was measured by HPLC (A) and DCPIP (B). L-AQP0 cells (open circles; ○) and L-SVCT2 cells (closed circles; ●) were incubated in DMEM containing 1000 μM AA at 37 °C. (C) L-AQP0 cells were first incubated in culture medium containing AA and then switched to AA-free medium at the timepoints indicated. Intracellular AA concentrations were measured by HPLC. All results are expressed as means ± SE. This experiment was independently performed three times (n = 12 per group).

To compare AA import and export, cells were switched to an AA-free culture medium and AA concentration was measured in L-AQP0 and L-SVCT2 cells incubation in the absence of AA at 60 min (Fig. 3C). Intracellular AA content in L-AQP0 cells began to decrease after 20 min and was equal to that of control cells 60 min after the change to AA-free medium (Fig. 3C). Intracellular AA content in L-SVCT2 cells did not change after incubation in AA-free medium (data not shown). These results show that AQP0 channels enable both the import and export of AA.

3.3. AA uptake in Xenopus oocytes

To validate the above L-cell data, AA transport was studied in oocytes injected with *AQP0* or *SVCT2* cRNA. The oocytes were incubated in ND96 containing 1–30 μM AA, and intracellular AA was measured by HPLC. Oocytes expressing AQP0 or SVCT2 showed increased intracellular AA levels, with uptake occurring in a dose-dependent manner (Fig. 4A). To assess AA uptake over time in oocytes expressing AQP0 or SVCT2, the oocytes were incubated in ND96 buffer containing 30 μM AA for various intervals at 18 °C, and intracellular AA content was measured by HPLC. Oocytes injected with *AQP0* or *SVCT2* cRNA imported AA in a time-dependent manner (Fig. 4B).

4. Discussion

AA is a potent antioxidant known to protect tissues from oxidative stress [29,30]. The high concentration of AA in the lens protects this organ against oxidative damage; therefore, it is believed that the lens has the mechanism to import large amounts of AA from the aqueous humor. In this report, we show that AQP0 can transport AA through the lens fiber cell membrane.

There are several reports on transport by AQP0 using an *in vitro* incorporation assay with lens AQP0 in reconstituted lipid vesicles [8,31–33]. In these reports, it was shown that AQP0 transported not only water but also glycerol, sucrose and ions. In another report, lens AQP0 was shown using an *in vitro* incorporation assay to function as a nonspecific channel that transported molecules as large as MW 1500 in a gap junction-like manner [8,31,33]. In addition, lens AQP0 in reconstituted liposomes was shown to transport AA [32]. However, in these experiments, it was suggested that lens AQP0 may have been denatured and/or degraded, or contaminated with other lens membrane proteins such as Gja8 and Gja3, which are also known as connexins during extraction [34]. It may change the original properties of AQP0 from denaturation and/or contamination. In this paper, we used L-cell and Xenopus oocyte expressing AQP0, and confirmed that AQP0 permeated AA *in vivo*.

AQP0 expressed in mouse fibroblast L-cells and Xenopus oocytes was shown to localize to the cell membrane and have the same function in these cells as it does *in vivo* [35]. AQP0 expressed in L-cells and Xenopus oocytes does not have the possibility of being denatured, degraded or contaminated and should undergo the same post-translational modifications as it does *in vivo*, representing a valid model for the study of AQP0 physiological function. AQP0 expressed in Xenopus oocytes transported water and glycerol but not sucrose [4].

In this report, we also clarified the nature of AA transport by AQP0. Intracellular AA levels in L-cells expressing AQP0 increased

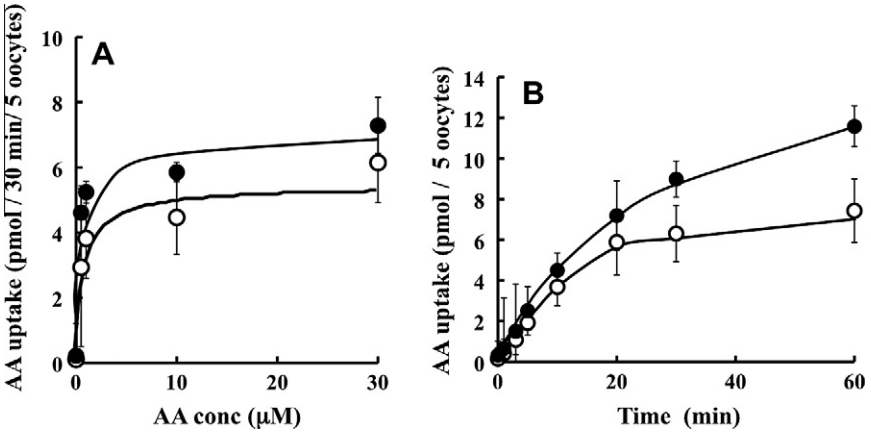

Fig. 4. Ascorbic acid uptake in oocytes expressing AQP0 or SVCT2. Xenopus oocytes injected with *AQP0* or *SVCT2* cRNA were incubated in ND96 containing AA for 30 min at 18 °C, and then intracellular AA was quantitated by HPLC (A). The time course of AA uptake in oocytes was assayed over a 60 min period (B). Open circles (○) indicate oocytes injected with *AQP0* cRNA, and closed circles (●) indicate oocytes injected with *SVCT2* cRNA. All results are expressed as means ± SE. This experiment was independently performed three times (n = 10 per group).

in an AA dose-dependent manner when incubated in AA-containing medium for 60 min (Fig. 2) and quickly increased and reached saturation after 10 min in DMEM containing 1000 μM AA (Fig. 3A and B). Similar results were obtained in oocytes expressing AQP0 (Fig. 4). When cells were switched to AA-free medium, intracellular AA levels in L-AQP0 cells began to decrease after 20 min and reached the levels of control L-cells after 60 min (Fig. 3C). To the contrast, L-SVCT2 did not decrease AA level at least 20 min when cells were switched to AA-free medium from AA containing medium. These results also showed that AQP0 forms bidirectional channels and imports AA more quickly than it exports it.

We previously reported that AA concentration is decreased in lenses of diabetic rats, that AQP0 expression is increased 7- to 9-fold, and that the expression of SVCT2, GLUT1 and GLUT3 is normal in diabetic rat lens [24]. Furthermore, AA concentrations in the lenses of control rats were not increased, but those of diabetic rats were normalized by AA supplementation [24]. These results suggest that increased AA concentration in the lenses of diabetic rats induced by AA supplementation contributes to an increase in AQP0 expression. Lenses need to maintain a high concentration of AA, and based on the present findings, AQP0 may responsible for maintaining AA content in the lens.

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References

[1] M.B. Gorin, S.B. Yancey, J. Cline, et al., The major intrinsic protein (MIP) of the bovine lens fiber membrane: characterization and structure based on cDNA cloning, Cell 39 (1984) 49–89.
[2] S.B. Yancey, K. Koh, J. Chung, et al., Expression of the gene for main intrinsic polypeptide (MIP): separate spatial distributions of MIP and beta-crystallin gene transcripts in rat lens development, J. Cell Biol. 106 (1988) 705–714.
[3] S.M. Mulders, G.M. Preston, P.M. Deen, et al., Water channel properties of major intrinsic protein of lens, J. Biol. Chem. 270 (1995) 9010–9016.
[4] C. Kushmerick, S.J. Rice, G.J. Baldo, et al., Ion, water and neutral solute transport in Xenopus oocytes expressing frog lens MIP, Exp. Eye Res. 61 (1995) 351–362.
[5] A. Shiels, S. Bassnett, Mutations in the founder of the MIP gene family underlie cataract development in the mouse, Nat. Genet. 12 (1996) 212–215.
[6] V. Berry, P. Francis, S. Kaushal, et al., Missense mutations in MIP underlie autosomal dominant 'polymorphic' and lamellar cataracts linked to 12q, Nat. Genet. 25 (2000) 15–17.
[7] D.J. Sidjanin, D.M. Parker-Wilson, A. Neuhäuser-Klaus, et al., A 76-bp deletion in the Mip gene causes autosomal dominant cataract in Hfi mice, Genomics 74 (2001) 313–319.
[8] S.J. Girsch, C. Peracchia, Lens cell-to-cell channel protein: I. Self-assembly into liposomes and permeability regulation by calmodulin, J. Membr. Biol. 83 (1985) 217–225.
[9] S.P. Wolff, G.M. Wang, A. Spector, Pro-oxidant activation of ocular reductants. 1: Copper and riboflavin stimulate ascorbate oxidation causing lens epithelial cytotoxicity in vitro, Exp. Eye Res. 45 (1987) 777–789.
[10] M. Bando, H. Obazawa, Ascorbate free radical reductase and ascorbate redox cycle in the human lens, Jpn. J. Ophthalmol. 32 (1988) 176–186.
[11] J.J. Trout, C.Y. Lu, A.D. Goldstone, et al., Polyamines mediate coronary transcapillary macromolecular transport in the calcium paradox, J. Mol. Cell Cardiol. 26 (1994) 369–377.
[12] T. Ishikawa, A.F. Casini, M. Nishikimi, Molecular cloning and functional expression of rat liver glutathione-dependent dehydroascorbate reductase, J. Biol. Chem. 273 (1998) 28708–28712.
[13] H. Tsukaguchi, T. Tokui, B. Mackenzie, et al., A family of mammalian Na^+-dependent L-ascorbic acid transporters, Nature 399 (1999) 70–75.
[14] R. Daruwala, J. Song, W.S. Koh, et al., Cloning and functional characterization of the human sodium-dependent vitamin C transporters hSVCT1 and hSVCT2, FEBS Lett. 460 (1999) 480–484.
[15] H.S. Hundal, A. Ahmed, A. Gumà, et al., Biochemical and immunocytochemical localization of the 'GLUT5 glucose transporter' in human skeletal muscle, Biochem. J. 286 (1992) 339–343.
[16] S.C. Rumsey, O. Kwon, G.W. Xu, et al., Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid, J. Biol. Chem. 272 (1997) 18982–18989.
[17] S.C. Rumsey, R. Daruwala, H. Al-Hasani, et al., Dehydroascorbic acid transport by GLUT4 in Xenopus oocytes and isolated rat adipocytes, J. Biol. Chem. 275 (2000) 28246–28253.
[18] B.R. Merriman-Smith, A. Krushinsky, J. Kistler, et al., Expression patterns for glucose transporters GLUT1 and GLUT3 in the normal rat lens and in models of diabetic cataract, Invest. Ophthalmol. Vis. Sci. 44 (2003) 2458–2466.
[19] R. Kannan, A. Stolz, Q. Ji, et al., Vitamin C transport in human lens epithelial cells: evidence for the presence of SVCT2, Exp. Eye Res. 73 (2001) 159–165.
[20] R. Merriman-Smith, P. Donaldson, J. Kistler, Differential expression of facilitative glucose transporters GLUT1 and GLUT3 in the lens, Invest. Ophthalmol. Vis. Sci. 40 (1999) 3224–3230.
[21] D.K. Yue, S. McLennan, E. Fisher, et al., Ascorbic acid metabolism and polyol pathway in diabetes, Diabetes 38 (1989) 257–261.
[22] J. DiMattio, Decreased ascorbic acid entry into cornea of streptozotocin-diabetic rats and guinea-pigs, Exp. Eye Res. 55 (1992) 337–344.
[23] J. DiMattio, Alterations in ascorbic acid transport into the lens of streptozotocin-induced diabetic rats and guinea pigs, Invest. Ophthalmol. Vis. Sci. 33 (1992) 2926–2935.
[24] Y. Nakazawa, M. Oka, M. Bando, et al. The role of ascorbic acid transporter in the lens of streptozotocin-induced diabetic rat, Biomed. Pharmacother. [Epub ahead of print].
[25] K. Sato, Y. Sai, T. Nishimura, et al., Influx mechanism of 2′,3′-dideoxyinosine and uridine at the blood-placenta barrier, Placenta 30 (2009) 263–269.
[26] Y. Matsubara, M. Oka, S. Shumiya, et al., Changes of MIP26K linked with cataract formation and maturation in the lenses of the Shumiya Cataract Rat, J. Jap. Soc. Cat. Res. 15 (2003) 47–49.
[27] M. Oka, K. Shimizu, K. Nakamura, et al., Lentoid body formation and expression of major intrinsic polypeptide (MIP) 26 on a PTFE membrane, J. Jap. Soc. Cat. Res. 16 (2004) 63–68.

[28] L.J. Harris, L.W. Mapson, Y.L. Wang, Vitamin methods: a simple potentiometric method for determining ascorbic acid, suitable for use with coloured extracts, Biochem. J. 36 (1942) 183–195.

[29] B. Frei, Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage, Am. J. Clin. Nutr. 54 (1991) 1113–1118.

[30] B. Frei, On the role of vitamin C and other antioxidants in atherogenesis and vascular dysfunction, Proc. Soc. Exp. Biol. Med. 222 (1999) 196–204.

[31] C. Peracchia, S.J. Girsch, Permeability and gating of lens gap junction channels incorporated into liposomes, Curr. Eye Res. 4 (1985) 431–439.

[32] M. Gooden, D. Rintoul, M. Takehana, et al., Major intrinsic polypeptide (MIP26K) from lens membrane: reconstitution into vesicles and inhibition of channel forming activity by peptide antiserum, Biochem. Biophys. Res. Commun. 30 (1985) 993–999.

[33] K. Varadaraj, C. Kushmerick, G.J. Baldo, et al., The role of MIP in lens fiber cell membrane transport, J. Membr. Biol. 170 (1999) 191–203.

[34] S. Bassnett, P.A. Wilmarth, L.L. David, The membrane proteome of the mouse lens fiber cell, Mol. Vis. 15 (2009) 2448–2463.

[35] S.S. Kumari, K. Varadaraj, Intact AQP0 performs cell-to-cell adhesion, Biochem. Biophys. Res. Commun. 390 (2009) 1034–1039.