Biochemical and Biophysical Research Communications **257,** 139–144 (1999)
Article ID bbrc.1999.0425, available online at http://www.idealibrary.com on IDEAL®

Molecular and Functional Study of *AQY1* from *Saccharomyces cerevisiae:* Role of the C-Terminal Domain

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Received February 4, 1999

The yeast *YPR192w* gene, which encodes a protein (Aqy1p) with strong homology to aquaporins (AQPs), was cloned from nine *S. cerevisiae* strains. The osmotic water permeability coefficient (P_f) of *X. laevis* oocytes expressing the gene cloned from the Σ1278b strain (*AQY1-1*) was 5.7 times higher than the P_f of oocytes expressing the gene cloned from other strains (*AQY1-2*). Aqy1-1p, initially cloned without its C-terminus (Aqy1-1ΔCp), mediated an ~3 times higher water permeability than the full-length protein. This corresponds to a 3-fold higher protein density in the oocyte plasma membrane, as shown by freeze-fracture electron microscopy. P_f measurements in yeast spheroplasts confirmed the presence of functional water channels in Σ1278b and a pharmacological study indicated that this strain contains at least a second functional aquaporin. © 1999 Academic Press

Key Words: **aquaporin; *AQY1; Saccharomyces cerevisiae;* water transport; genetic heterogeneity.**

Since the discovery and functional characterisation of human AQP1 in 1992 [1], many aquaporin water channels from mammals, plants, insects, amphibians or bacteria have been cloned and characterised [2]. Functional studies of most of these proteins were essentially performed after heterologous expression in *Xenopus laevis* oocytes. Data from reconstitution studies are rare due to difficulties to purify large quantities of proteins from their native membranes. Therefore, to obtain amounts of proteins of interest compatible with biophysical and structural analyses, several other expression systems have been investigated.

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We have previously reported on a yeast system for both the functional characterisation and the production of mammalian aquaporins [3,4]. The use of *Saccharomyces cerevisiae* was convenient due to the apparent lack of endogenous aquaporin activity in the *sec6-4* mutant strain we used. However, the expression level of exogenous aquaporin was relatively low in this mutant, and we started to consider other, better performing, strains. At the same period, the screening of databases after the release of the complete genome of *S. cerevisiae*, indicated the presence of four ORFs encoding proteins of the Major Intrinsic Protein (MIP) family [5]. Fps1p-Yll043p has been described as the yeast glycerol facilitator required for export, and was shown to be involved in the yeast osmoregulation [6,7] but also in cell fusion during mating [8]. Amino acid sequence analysis shows that Yfl054p is highly homologous to Fps1p and other glycerol facilitators, but its physiological role is unknown. *YPR192w* and the two overlapping ORFs *YLL052c-YLL053c* are more closely related to aquaporin genes. Ypr192p, renamed Aqy1p, is highly homologous to *Arabidopsis thaliana* plasma membrane aquaporins. Yll052p and Yll053p are strongly homologous to the second and the first half of Aqy1p, respectively, and may correspond to fragments of a second aquaporin, named Aqy2p.

In order to investigate the presence of endogenous water channels in various yeast strains, we cloned the *AQY1* gene from nine laboratory and wild-type *S. cerevisiae* strains. The functional characterisation we performed in oocytes indicated that, among the nine cloned cDNAs, only the cDNA of the Σ1278b strain significantly increased the P_f of oocytes. This result is in agreement with the recent work of Bonhivers et al. [9]. Aqy1-1p exhibited a three times lower P_f than its C-terminal truncated variant (Aqy1-1ΔCp), for which we showed by freeze-fracture electron microscopy that it was three times as much expressed at the oocyte

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plasma membrane. Finally, stopped flow experiments, performed on whole spheroplasts, confirmed the presence of a water channel activity in the Σ1278b strain.

MATERIAL AND METHODS

Yeast strains and growth conditions. Nine *S. cerevisiae* strains, FL100 (*MAT*a) [10], Σ1278b (*MAT*α) [11], NY17 (*MAT*a, *ura3-52 sec6-4*) [12], X2180-1A (*MAT*a, *gal2 CUP1 mal0, SUC2*), W303-1A (*MAT*a, *ura3-1 leu2-3/112 trp1-1 his3-11/15 ade2-1 can1-100*) [13], CMY375 (*MAT*a, *ura3-52*), a derivative of S288C kindly provided by Dr C. Mann, as well as SIHA3, SIHA7, SIHA varioferm, 3 wine strains marketed by E. Begerow Co, Langenlonsheim, Germany, were used for cloning studies and water transport assays. Cells were grown in YPD (1% yeast extract, 2% bacto-peptone (Difco) and 2% dextrose) and plated on YPD supplemented with 2% bacto-agar (Difco).

PCR amplifications. Oligonucleotides were designed according to sequences from the Yeast Genome Database. Genomic DNA was prepared according to [14]. *YPR192w* (*AQY1*) ORF was amplified from genomic DNA of all strains, except Σ1278b, using the primers AQY1-UP (5′-GAGATCTTTAACTATAACATGTCTTCGAACG-3′ with a *Bgl*II restriction site (underlined) at the 5′ end) and AQY1-DO1 (5′-TACTAGTATTAGACTTCAGCCACAGCAGAGG-3′ with a *Spe*I restriction site (underlined) at the 5′ end). *AQY1* amplification in the Σ1278b strain was performed using the AQY1-UP and the AQY1-DO2 (5′-CACTAGTAAAAACACTAATTACCTCAGTAG-3′ with a *Spe*I restriction site (underlined) at the 5′ end) primers. The truncated Σ1278b *AQY1* (*AQY1-1ΔC*) was amplified with AQY1-UP and AQY1-DO1 primers. PCR amplifications were performed with 1 unit of $Vent_R$ DNA polymerase (New England Biolabs) in the presence of 1 × buffer, 4 mM $MgSO_4$, 0.2 mM dNTPs, 0.5 μM primers and 1 μg of genomic DNA. PCR reactions were submitted to the following conditions: 3 min at 95°C, 30 cycles [30 s at 95°C, 30 s at 55°C, 1 min at 72°C] and 10 min at 72°C.

Plasmid constructions. All PCR fragments were cloned in the pT7Ts expression vector (kindly provided by P. Krieg), downstream of the T7 promoter between the 5′ and the 3′ non-coding regions of *X. laevis* β-globin gene. Constructions were created by ligating either the 943 bp *Bgl*II-*Spe*I PCR fragments amplified from all strains with AQY1-UP and AQY1-DO1 primers, or the 1075 bp *Bgl*II-*Spe*I PCR fragment amplified from Σ1278b with AQY1-UP and AQY1-DO2 primers, in the *Bgl*II-*Spe*I pT7Ts fragment. Standard cloning methods were used [15] and all inserts were sequenced after cloning.

Expression of Aqy1p in X. laevis oocytes—Determination of water and solute permeabilities. Constructions were linearized with *Xba*I and transcribed *in vitro* using T7 RNA polymerase. The integrity of the cRNAs was checked by agarose gel electrophoresis and the concentration was determined at 260 nm. Defolliculated stage V and VI oocytes from *X. laevis* were prepared as described by Abrami et al. [16] and injected with either 50 nl of water (controls) or 50 nl of a solution containing 0.4 μg/μl of cRNAs (0.2 μg/μl for AQP3 cRNA). Oocytes were then incubated for 48-72 h at 18°C in a 200 mosm/kg H_2O Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 10 mM HEPES, 50 μg/ml geneticin (Life Technologies), pH 7.4). To determine the osmotic water permeability coefficient (P_f, in cm/s), oocytes were incubated at 21°C in a diluted Barth's solution at 40 mosm/kg H_2O, to create a water influx. Oocyte swelling was monitored by video microscopy and P_f was calculated from initial rates of swelling as previously described [16]. In some experiments, oocytes were preincubated 15 min in the presence of 1 mM $HgCl_2$ (diluted Barth also contained 1 mM $HgCl_2$). To determine glycerol and urea permeabilities (P_{gly} and P_{urea}), oocytes were incubated at 18°C for 5 min in the presence of either 1 mM ^{14}C-glycerol or 1 mM ^{14}C-urea. The solute permeability was calculated according to the equation: P_{solute} = solute apparent volume (cm^3)/[t (s) × S (cm^2)]. All data are expressed as means ± SE.

Electrophoresis and immunoblotting. Whole membranes from 10 water- or cRNA-injected oocytes were prepared as described by Preston [17]. Membrane pellets were resuspended in Laemmli buffer (2 μl/oocyte), heated for 10 min at 65°C, and were loaded on 12.5% polyacrylamide gels with the equivalent of 1 oocyte/lane. Proteins were transferred onto PVDF membranes and probed with polyclonal anti-Aqy1p antibodies raised in rabbits against a synthetic peptide located in the N-terminus sequence (TDKQHTR) (NeoSystem, France). A cysteine residue was added to the peptide C-terminus, to allow binding for further serum purification by affinity chromatography (immunobilization Kit2, Pierce). Immunoreactive proteins were revealed by the ECL Western blotting technique (Enhanced ChemiLuminescence, Amersham Pharmacia Biotech).

Freeze-fracture electron microscopy. Control and cRNA-injected oocytes were fixed in 2.5% glutaraldehyde in Barth for 2 h at 18°C, and washed in Barth. Oocytes were then incubated in Barth supplemented with 30% glycerol for 1 h at room temperature. They were first cut in halves and, after partial cytoplasm removal, into smaller pieces. These membrane fragments were sandwiched between two copper specimen holders and frozen in melting freon. The samples were fractured in a Balzers 300 at −150°C under 10^{-7} Torr vacuum. Fractured surfaces were coated with platinum at 45° and carbon at 90°. The replicas were cleaned in a solution of bleach, washed in distilled water and observed in a Philips EM 400 microscope at 80 kV. A representative series of images (15 photos/cRNA from 3 independent experiments) of the protoplasmic fracture faces (P face) were enlarged at 94600× final magnification. Quantification of particle density was performed by counting P face particles from known areas of the membrane, as described by Zampighi et al. [18].

Spheroplast preparation and stopped flow experiments. Σ1278b and FL100 yeasts, grown at 30°C to midexponential phase (A_{600nm}∼1), were washed two times with 10 mM sodium azide, and digested by zymolyase to remove the cell wall [19]. The pelleted spheroplasts (5 min centrifugation at 2200×g) were resuspended in: 10 mM Tris/MES pH 7.0, 1 mM EDTA, 0.5 M sorbitol, at a concentration of $\sim 1 \times 10^7$ cells/ml. Cells were conserved at 4°C until use, and gently resuspended prior to the stopped flow experiment. Kinetics of spheroplast volume changes were followed at 10°C, by 90° light scattering (λ_{ex} = 475 nm) using a previously described stopped-flow spectrophotometer (SFM3, Biologic, Claix, France) [20]. Cell osmotic water permeability was measured by mixing 100 μl of spheroplasts with an equal volume of a hyperosmotic solution of sorbitol, to produce a 125 mosm/kg H_2O inwardly-directed osmotic gradient. Spheroplasts submitted to hyperosmotic shocks of varying amplitude developed a time-dependent increase in scattered light, linearly correlated to the ratio of initial extra- to intracellular osmolalities (data not shown). Data from 8-10 time courses were averaged and fitted to single exponential functions (k is the rate constant, in s^{-1}) by using the Simplex procedure of the BIOKINE software (Biologic, France). The osmotic water permeability coefficient, P_f, in cm/s, was determined using the following equation,

$$dV(t)/dt = (P_f) \times (S) \times (MVW) \times [(C_{in} \times (1 - b)/V(t) - V_0 \times b) - C_{out}]$$

where V(t) is the spheroplast volume as a function of time, S and V_0 are the initial cell surface area and volume, MVW is the molar volume of water (18 cm^3/mol), C_{in} and C_{out} (mol/cm^3) are the initial concentrations of intra- and extracellular solute, and b is the fraction of cell volume that does not participate in the osmotic shrinkage [21] and is equal to 0.24 [22]. Theoretical kinetics were simulated for various arbitrary values of P_f using inverse relationship between scattered light intensity and vesicle volume, and fitted by the BIOKINE software as single exponentials. The obtained calibration curve (P_f versus k) was used to determine P_f from the experimental k values. In some experiments, spheroplasts were preincubated 10 min at room temperature in the presence of 0.5 mM $HgCl_2$.

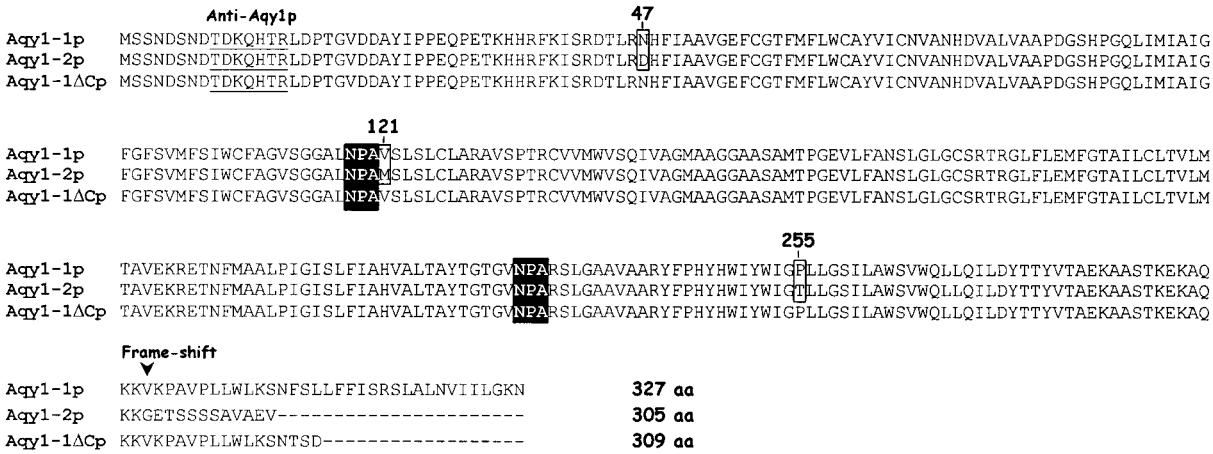

FIG. 1. Sequence alignments of Aqy1p. Aqy1-1p and Aqy1-1ΔCp were from Σ1278b, and Aqy1-2p was from all other tested strains. Protein length is indicated at the end of each sequence, the highly conserved NPA repeats are boxed in black, single amino acid substitutions (D47N, M121V and T255P) are indicated, as well as the frame-shift leading to a longer C-terminal extension in Aqy1-1p. N-terminus peptide used for polyclonal antibody production is underlined.

RESULTS

Cloning

YPR192w-AQY1 was initially amplified by PCR from 9 *S. cerevisiae* strains using the AQY1-UP/AQY1-DO1 primer set. PCR fragments were cloned in the pT7Ts expression vector and sequenced. All sequences except that obtained from strain Σ1278b were identical to that determined by systematic sequencing of the genome of strain S288C. The *AQY1* gene cloned from the Σ1278b strain exhibited 7 nucleotide changes when compared with that from the other strains, leading to 4 differences in the deduced amino acid sequence: 3 single amino acid substitutions (D47N, M121V and T255P) and a longer C-terminal extension due to a frame-shift at residue 294 (Fig. 1). *AQY1* from the Σ1278b strain will now be referred to as *AQY1-1* and *AQY1* from the other strains as *AQY1-2* (note that the sequence present in the databases corresponds to *AQY1-2*). As the AQY1-UP/AQY1-DO1 primer set initially led to the amplification of a C-terminal truncated form of *AQY1-1* (*AQY1-1ΔC*), a second set of primers (AQY1-UP/AQY1-DO2) was used to amplify the full length gene.

Expression in Oocytes

Osmotic water permeability. The P_f of oocytes injected with the cRNAs synthesized from *AQY1-1*, *AQY1-1ΔC*, *AQY1-2* and AQP3 as a positive control was measured at 21°C. In oocytes expressing Aqy1-1p, P_f was increased 5.7-fold as compared to control oocytes ($(0.68 \pm 0.08) \times 10^{-2}$ cm/s *versus* $(0.12 \pm 0.01) \times 10^{-2}$ cm/s, n = 14) (Fig. 2A). In contrast, oocytes ex-

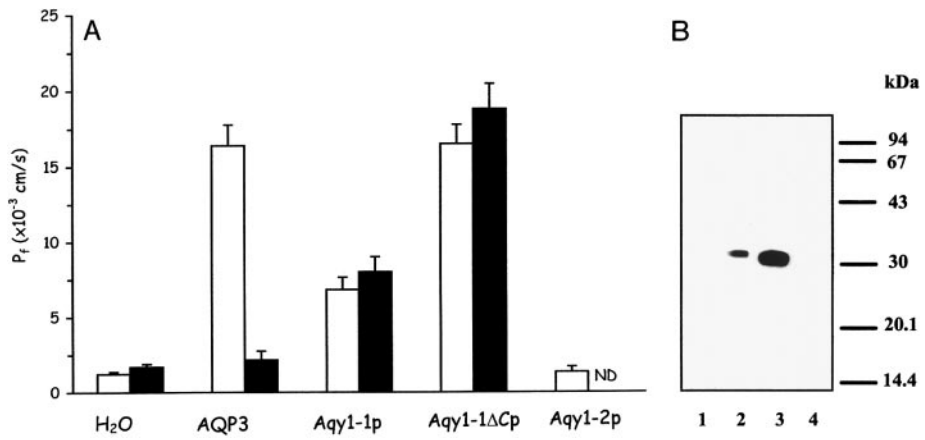

FIG. 2. Osmotic water permeability measurements and membrane expression of Aqy1p in *X. laevis* oocytes. A, P_f values (21°C) of oocytes injected with water or cRNA-encoding proteins as indicated. Data are expressed as means ± SE (in white: $-HgCl_2$, n = 14; in black: $+HgCl_2$, n = 4). ND, non-determined. B, Immunoblot of whole membranes probed with anti-Aqy1p immunopurified serum; lane 1: Aqy1-2p, lane 2: Aqy1-1p, lane 3: Aqy1-1ΔCp, lane 4: H_2O.

pressing the truncated Aqy1-1ΔCp exhibited a 13.8-fold increase in P_f ($(1.65 \pm 0.13) \times 10^{-2}$ cm/s (n = 14), similar to AQP3 P_f) when compared to controls, and, consequently, a 2.7-fold increase when compared to oocytes expressing Aqy1-1p, after substraction of control P_f value. No inhibition with 1 mM $HgCl_2$ was observed for both forms of Aqy1-1p (Fig. 2A, black bars). Oocytes expressing Aqy1-2p did not show a significant P_f increase ($P_f = (0.13 \pm 0.03) \times 10^{-2}$ cm/s, n = 14).

Solute permeabilities. Oocytes injected with the same cRNAs as above were tested for their glycerol and urea permeabilities at 18°C. As expected, AQP3 greatly enhanced both glycerol and urea permeabilities ($(3.90 \pm 0.08) \times 10^{-5}$ cm/s and $(1.60 \pm 0.18) \times 10^{-5}$ cm/s, respectively, compared to $(1.72 \pm 0.60) \times 10^{-6}$ cm/s and $(1.00 \pm 0.27) \times 10^{-6}$ cm/s, respectively, for controls, n = 5) (data not illustrated). In contrast, neither Aqy1-1p nor Aqy1-2p seemed to transport these two solutes in our experimental conditions, since the permeability values were close to controls. Confirming phylogenetic analyses [9], our data indicate that yeast Aqy1p is not an aquaglyceroporin but a water-specific aquaporin *sensus stricto.*

Western blot analysis. In order to control the expression level of the various exogenous proteins in oocytes, a Western blot analysis was carried out, using a purified anti-Aqy1p antibody raised against 7 amino acids in the Aqy1p N-terminal sequence. No signal was detected in the membranes of Aqy1-2p oocytes (Fig. 2B, lane 1), indicating that the protein is poorly expressed or unstable in these cells. In contrast, a signal at 35 kDa (lane 2) and 33 kDa (lane 3) indicated a proper expression of Aqy1-1p and Aqy1-1ΔCp, respectively. A higher expression level for the truncated form of the protein was observed, consistent with the higher water permeability conferred by Aqy1-1ΔCp.

Quantification of Oocyte Intramembrane Particles

In order to correlate the functional difference between Aqy1-1p and Aqy1-1ΔCp to a number of expressed channels, we estimated this number from the density of intramembrane particles inserted in the plasma membrane of oocytes [18]. The method is based on the fact that control water-injected oocytes exhibit a very low density of endogenous particles, and that heterologously expressed channels increase the density of particles only on the protoplasmic (P) fracture face. Results are summarized in Table I, and a typical illustration is presented in Fig. 3. The surfaces of P membrane used for counting were 44 μm^2 for water-injected oocytes, 25 μm^2 for Aqy1-2p, 42 μm^2 for Aqy1-1p and 26 μm^2 for Aqy1-1ΔCp. Oocytes expressing Aqy1-1ΔCp exhibited a density, measured after substraction of H_2O-injected oocyte density, of 9.40×10^{10} particles/cm^2, while oocytes expressing Aqy1-1p exhibited 3.62×10^{10} particles/cm^2. The ratio of particle density, equal to 2.6, is very close to the ratio of P_f values between Aqy1-1ΔCp over Aqy1-1p (= 2.7), indicating a good correlation between the water channel activity and the number of intramembrane particles inserted in the oocyte plasma membrane. From the ratio of P_f to particle density, the functional permeability per monomer (p_f) was estimated to be 4.32×10^{-15} cm^3/s for Aqy1-1p (assuming each particle is a tetramer), i.e., lower than those previously reported for AQP1 [23,24]. Interestingly, both Aqy1-1p and Aqy1-1ΔCp exhibit similar unitary p_f values. This clearly indicates that the full length Aqy1-1p is ~3 times less expressed and/or localized to the plasma membrane of the oocyte than the truncated form.

TABLE I

Particle Density and Unitary Water Permeability of Aqy1p

Oocyte	P_f (cm/s)[a] $\times 10^2$	Particle density (particles/cm^2) $\times 10^{-10}$	p_f/particle (cm^3/s)[b] $\times 10^{14}$	p_f/monomer (cm^3/s)[c] $\times 10^{15}$
H_2O	0.12	1.72	—	—
Aqy1-1p	0.68	5.35	1.73	4.32
Aqy1-1ΔCp	1.65	11.11	1.80	4.52
Aqy1-2p	0.13	2.34	—	—

[a] Mean P_f, n = 14 (see text).
[b] Control (water-injected oocytes) water permeability and particle density were first subtracted before calculating permeability per particle.
[c] Assuming each particle is a tetramer.

Stopped Flow Experiments

Finally, *in situ* functional experiments were performed on whole yeast membranes. Spheroplasts from Σ1278b and FL100 (control) strains were prepared and mixed in a stopped flow apparatus with a hyperosmotic solution of sorbitol to induce cell shrinking. The corresponding increase in 90° scattered light was recorded as a function of time (Fig. 4), and P_f was calculated as described in Material and methods. The P_f of Σ1278b spheroplasts was ~10 times higher than that of FL100 spheroplasts (at 10°C, $P_f = 1.7 \times 10^{-2}$ cm/s and 1.8×10^{-3} cm/s, respectively). The temperature dependence of water flux between 10°C and 30°C indicated an activation energy value of 6.6 kcal/mol for Σ1278b and 17.7 kcal/mol for FL100 (data not shown). P_f was inhibited at 47% in the presence of 0.5 mM $HgCl_2$ in Σ1278b spheroplasts ($P_f = 9.9 \times 10^{-3}$ cm/s), without having any effect on FL100 spheroplast P_f (not illustrated). The above biophysical features of water flow in yeast spheroplasts confirmed the existence of functional water channels in the Σ1278b strain. Since Aqy1-1p-mediated water flow into oocytes was Hg-

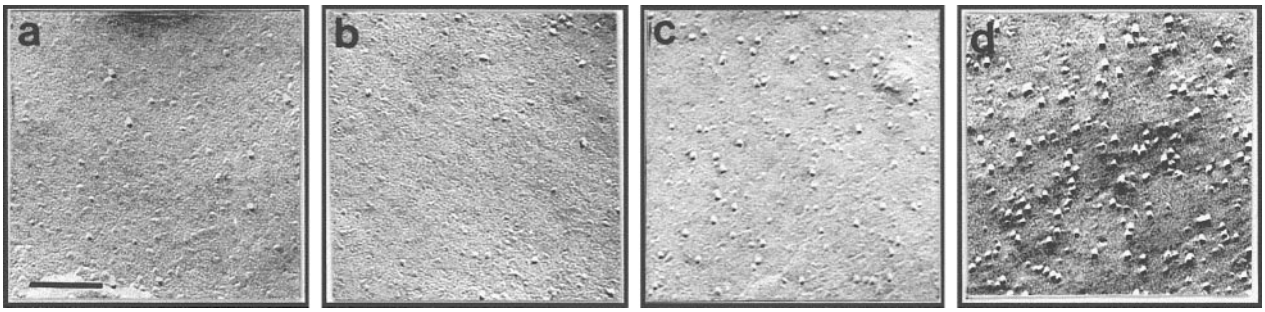

FIG. 3. Freeze-fracture micrographs of oocyte plasma membranes. P fracture face of oocytes injected with water (a) or expressing Aqy1-2p (b), Aqy1-1p (c) and Aqy1-1ΔCp (d). Magnification: ×94600, calibration bar: 100 nm.

resistant, this suggests the presence of another functional aquaporin, that would be Hg-sensitive.

DISCUSSION

The cloning and water transport studies described in the present work showed the existence of a genetic heterogeneity for the yeast aquaporin *AQY1* among the *S. cerevisiae* strains, confirming our preliminary report [25], and in good agreement with the work of Bonhivers et al. [9]. The gene cloned from the Σ1278b strain encodes Aqy1-1p shown to increase the oocyte P_f, in contrast with the same gene cloned from the other tested strains, encoding Aqy1-2p, unable to significantly increase the P_f of oocytes. A very low expression level of Aqy1-2p in the oocyte plasma membrane, revealed by immunoblot and freeze-fracture analyses, could explain this result. Moreover, we cannot exclude that, if Aqy1-2p was correctly expressed and addressed to the plasma membrane, it could act as a functional water channel. In the case of Aqy1-1p, we took advantage of the low density of endogenous proteins present in the plasma membrane of control oocytes, as well as the ability of these cells to express a large number of copies of exogenous proteins in their plasma membrane, to measure the particle density and calculate the unitary p_f of Aqy1-1p. The unitary p_f of Aqy1-1p is in the same range than that of Nodulin 26 [26], and remains lower than the one of human AQP1 [23] or mammalian AQP1-5 [24]. Interestingly, oocytes expressing the C-terminal truncated form of Aqy1-1p exhibited a 2.7-fold increase of P_f when compared to oocytes expressing the full-length protein. This functional difference is in good correlation with the 2.6-fold increase obtained after quantification of oocyte plasma membrane particle density. As a consequence, calculated water unitary p_f are the same for Aqy1-1p and its truncated form. A sequence present in the last 21 residues of the Aqy1-1p C-terminal extension could therefore control the number of water channels delivered to the membrane. A mechanism of density regulation was reported for the human epithelial sodium channel, in which the C-terminal domain was shown to control channel density in the oocyte plasma membrane [27]. Another explanation could be a different maturation or transport of the protein in the cell. The mechanism by which the C-terminal extremity of Aqy1-1p could be involved in such a regulation remains to be elucidated.

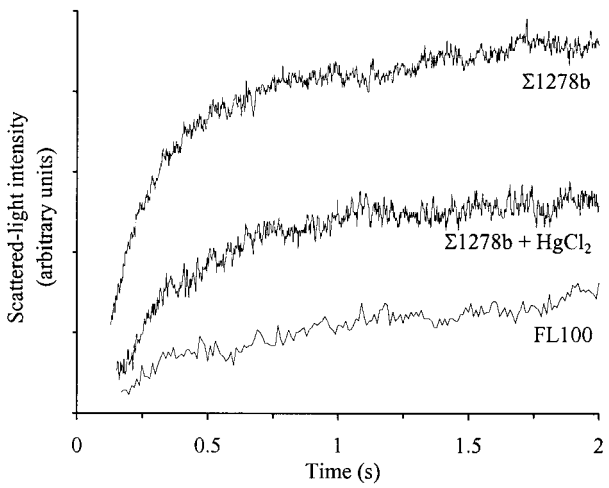

FIG. 4. Water transport in spheroplasts from Σ1278b and FL100 strains. Shrinking time courses were recorded after mixing the spheroplasts with a hyperosmotic sorbitol solution at 10°C. Fitted exponential rate constants (k) are 5.40 s^{-1} for Σ1278b spheroplasts, 0.57 s^{-1} for FL100 spheroplasts and 3.08 s^{-1} for Σ1278b spheroplasts preincubated 10 min with 0.5 mM $HgCl_2$.

Finally, spheroplasts prepared from Σ1278b cells (*AQY1-1*) exhibited a higher P_f and a lower activation energy than spheroplasts prepared from FL100 cells (*AQY1-2*), demonstrating the presence of functional water channels in the membranes of Σ1278b strain. Moreover, the P_f of Σ1278b spheroplasts appeared to be partially inhibited by $HgCl_2$. We speculated that Aqy1-1p was responsible in Σ1278b for the mercury-insensitive water permeability, supported by oocyte studies. No data are yet available showing that Aqy1-1p is localized in the yeast plasma membrane. A mercury-sensitive water permeability was also observed, suggesting the presence of a second functional

aquaporin in the Σ1278b strain and we hypothesized that this could be the protein encoded by *AQY2*.

The aquaporin gene heterogeneity among *S. cerevisiae* is unexpected. Strain Σ1278b is extensively studied because of its ability to form pseudohyphae under nitrogen starvation condition [28], as many wild-type strains. Based on this criterion, Bonhivers et al. [9] classified Σ1278b as a wild-type strain and they hypothesized that the genetic heterogeneity for *AQY1* observed among *S. cerevisiae* could be due to laboratory growth conditions. Our cloning results demonstrated that *AQY1* heterogeneity is not restricted to the laboratory environment, since the product of the three wine strains used in this study is Aqy1-2p. The question of a possible implication of Aqy1p in the yeast osmotic response arises. Evidence was provided that osmotic stress is better tolerated by yeasts lacking functional *AQY1* (*AQY1-1* null Σ1278b), than by yeasts expressing a functional one (wild-type Σ1278b) [9]. This result contrasts with the one described for the *E. coli* homologue AQPZ [29]. Further experiments will be necessary to clarify and establish the role of Aqy1p in yeasts.

ACKNOWLEDGMENTS

The authors thank Dr. P. Thuriaux who kindly provided FL100, X2180-1A, and Σ1278b strains and for interesting discussions. This work was supported by the Commission of the European Union via Grant FMRX-CT97-0128 and BIO4-CT98-0024. Vincent Laizé acknowledges the Wenner-Gren Foundation and Dr. J. Rydström for postdoctoral training support. Our colleague C. Maurel is greatly acknowledged for critically reading the manuscript.

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