Aquaporin-3 (AQP3) is a water channel found in the basolateral cell membrane of principal cells of the renal collecting tubule as well as in other epithelia. To examine the selectivity of AQP3, the permeability to water ($P_w$), urea ($P_\text{urea}$), and glycerol ($P_\text{gly}$) of *Xenopus* oocytes injected with cRNA encoding AQP3 was measured. Oocytes injected with cRNA encoding either human or rat aquaporin-1 (AQP1) were used as controls. Although both aquaporins permit water flow across the cell membrane, only AQP3 was permeable to glycerol and urea ($P_\text{gly} > P_w$). The uptake of glycerol into oocytes expressing AQP3 was linear up to 165 mJ. For AQP3 the Arrhenius energy of activation for $P_w$ was 3 kcal/mol, whereas for $P_\text{gly}$ and $P_\text{urea}$ it was $>12$ kcal/mol. The sulfhydryl reagent p-chloromercuriphenylsulfonate (1 mM) abolished $P_w$ of AQP3, whereas it did not affect $P_\text{gly}$. In addition, phloretin (0.1 mM) inhibited $P_w$ of AQP3 by 35%, whereas it did not alter $P_\text{gly}$ or $P_\text{urea}$. We conclude that water does not share the same pathway with glycerol or urea in AQP3 and that this aquaporin, therefore, forms a water-selective channel.

Aquaporin-3 (AQP3) is a protein located in the kidney exclusively in the basolateral cell membrane of the principal cells of the collecting tubule, and it is also found in cells of other epithelia like the stomach and colon (1–3). AQP3 is a member of the MIP family of proteins. This family includes two groups of proteins: the aquaporins, which constitute cell membrane channels selective for water, and a group of homologs that do not transport water but that may serve as transporters for small solutes or have functions that are not yet well defined (4, 5). AQP3 differs from other aquaporins in that it has features found in both of these groups because, in addition to being a water channel, it transports glycerol and urea (1, 2). From studies of the uptake of water, glycerol, and urea into *Xenopus* oocytes expressing AQP3, it has been concluded that water and these solutes most likely share the same pore as they move across the protein (1). This view implies that the selectivity of the channel is based not only on the molecular size but also on other physical properties of the transported molecules. However, it could be hypothesized that water and glycerol do not share the same pore in AQP3. In support of this proposal it could be mentioned that, within the MIP family, AQP3 has the largest homology with the glycerol facilitator (GlpF) of *Escherichia coli* (1, 2). GlpF transports glycerol and other small solutes across the inner membrane of the bacteria by a pore-type mechanism (6), but it excludes water (7). Thus, it is possible that some MIP homologs (the aquaporins) form one type of pore that is a water-selective channel, whereas other homologs form a pore that is permeable only to some small uncharged solutes. AQP3 could have components of each of these two groups and form in the cell membrane two different permeation pathways, one for water and another for glycerol and possibly other solutes. To test this hypothesis, we have examined in *Xenopus* oocytes injected with AQP3 cRNA the effects of inhibitors of the permeability to water and to some small solutes, on the assumption that if water and solutes share the same pore the inhibitors should affect the permeability to both. As controls we have used oocytes injected with cRNA encoding rat or human aquaporin-1 (AQP1, also called AQP-CHIP) because this protein forms a channel selective for water when incorporated in liposomes (8).

**Materials and Methods**

Expression of Water Channels in Xenopus Oocytes—The rat cDNAs for AQP3 and AQP1 and human AQP1 cDNA were ligated into the expression vectors pSPORT 1 (2), pBluescript SK (9), and the *Xenopus* expression construct pXβ (10). Capped cRNA encoding rat AQP3 and rat or human AQP1 were synthesized using the mCAP mRNA kit (Stratagene). *Xenopus laevis* oocytes were isolated, microinjected with either 50 nl of water or 50 nl of water containing 5 ng of aquaporin cRNA, and incubated as described (11) during 3–4 days.

Oocyte Permeability Measurements—The oocyte osmotic water permeability coefficient ($P_w$) was calculated from the rate of volume increase, measured with videomicroscopy every 10 s for 2 min, upon exposure to a hypotonic Barth’s solution (11). Glycerol or urea permeabilities ($P_\text{gly}$ and $P_\text{urea}$, respectively) were calculated from the initial rate of uptake of these solutes into the oocytes. Two methods were used to measure the uptake. In the first one (volumetric method), we calculated the uptake from the rate of oocyte swelling, measured with videomicroscopy, upon exposure for 2 min to an isosmotic modified Barth’s solution in which 165 mM of either glycerol or urea substituted for 88 mM NaCl. In the second method (isotopic method), we measured the $^{14}$C uptake per oocyte during incubation for 2 min, or in a few cases 5 min, in Barth’s solution containing either 7.5 μCi/ml of $^{14}$Cglycerol and 1.05 mM glycerol or 16.6 μCi/ml of $^{14}$Curea and 1.3 mM urea. Subsequently, the oocytes were rapidly rinsed twice in ice-cold modified Barth’s solution and each one transferred to a vial containing 0.2 ml of 0.1 g/ml SDS for liquid scintillation counting. Oocytes exposed to label-containing medium for only 2 s had negligible $^{14}$C uptake, indicating that the washing procedure had adequately removed the extracellular contamination with incubation medium. No attempt was made to compare in the same batch of oocytes the permeability values obtained with both methods. In water-injected oocytes, the values were quite comparable with both methods. However, in the cRNA-injected oocytes, the permeability values varied somewhat from batch to batch, depending in part on the efficiency of expression of the protein encoded by the injected cRNA. In addition, the isotopic method gave generally lower values, probably due to partial loss of the labeled solute during the oocyte

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**References**

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4. The abbreviations used are: AQP3, aquaporin-3; $P_w$, osmotic water permeability; $P_\text{gly}$, permeability to urea; $P_\text{urea}$, permeability to glycerol; pCMBS, sodium p-chloromercuriphenylsulfonate; DIDS, diisothiocyanosulfonyl stilbene.
Permeabilities of AQP3 and AQP1 expressed in _Xenopus oocytes_. Oocytes were microinjected with either water or cRNA encoding rat AQP3, rat AQP1, or human AQP1. The oocyte permeabilities were measured with the volumetric method (see “Materials and Methods”) after 4 days of incubation at 20 °C. Bars are means ± S.E., and the number of oocytes measured per group is given in parentheses. A, osmotic water permeability (Pf) values are represented. B, values of permeability (P) to urea (black bars) and glycerol (open bars) are shown.

Water permeation was also included in the medium during the permeability measurements. The uptake of water or solutes was measured at 10, 20, and 30 °C in water- or AQP3 cRNA-injected oocytes to estimate the Arrhenius energy of activation (Ea) of the permeabilities (11).

**RESULTS**

Oocytes injected with cRNA encoding AQP3 had a 10-fold larger Pf than that of water-injected oocytes (Fig. 1A), confirming results previously reported (1, 2). As expected, oocytes injected with either rat or human AQP1 cRNA also showed a larger Pf than controls (Fig. 1A). In contrast, only the oocytes expressing AQP3 showed increased Pf and Z (measured with the volumetric method) relative to the values found in water-injected oocytes (Fig. 1B). Therefore, AQP3 is permeable to water, glycerol, and slightly to urea, although these solute permeabilities are approximately 3 orders of magnitude lower than that to water.

To examine the effect of inhibitors of water flow on the solute permeabilities, it was necessary to measure directly the rate of glycerol and urea uptake using the isotopic method (see “Materials and Methods”). Pf of AQP3 cRNA-injected oocytes (14.6 (±1.0) × 10⁻⁶ cm/s) was again significantly larger than that of water-injected oocytes (3.8 (±0.4) × 10⁻⁶ cm/s), whereas that of AQP1 cRNA-injected cells (1.2 (±0.2) × 10⁻⁶ cm/s) was not increased. Similarly, Pf of AQP3 cRNA-injected cells (1.1 (±0.1) × 10⁻⁶ cm/s) relative to that of water-injected cells (0.3 (±0.1) × 10⁻⁶ cm/s) while that of AQP1 cRNA-injected oocytes (0.4 (±0.1) × 10⁻⁶ cm/s) remained unchanged. These results, then, confirm that only AQP3 is permeable to glycerol and urea.

The sulfhydryl group reagent pCMBS blocks the Pf rendered to oocytes by the expression of AQP3 (2) without altering the cell membrane conductance to the major ions of intra- or extracellular fluids (11). We tested, therefore, whether exposure to 1 mM pCMBS for 30 min prior to and during the measurement. A, Pf values are shown. B, glycerol permeability values (Pf glycerol), measured isotopically, are represented.

**FIG. 2. Effect of pCMBS on water and glycerol permeabilities of AQP3 expressed in oocytes.** Oocytes were injected with either water or cRNA encoding AQP3, and their permeabilities were measured after 3–4 days of incubation. Bars are means ± S.E., and the number of oocytes per group is shown in parentheses. Open bars represent values measured in oocytes not exposed to pCMBS. Stippled bars show values obtained in oocytes exposed to 1 mM pCMBS for 30 min prior to and during the measurement. A, Pf values are shown. B, glycerol permeability (Pf glycerol), measured isotopically, are represented.
oocytes that expressed AQP3 it inhibited $P_f$ by 35% while $P_{gly}$ was unchanged (Fig. 3, A and B). These results, therefore, are also consistent with the view that water and glycerol move through AQP3 by separate pathways.

Phloretin caused a small increase in $P_{ur}$ in water-injected oocytes and had no significant effect on $P_{ur}$ in AQP3 cRNA-injected oocytes (Fig. 3C). These results might indicate that phloretin partially inhibits $P_{ur}$ of AQP3. However, the unexpected increase in $P_{ur}$ without changes in $P_{gly}$ or $P_f$ in water-injected oocytes produced by phloretin is similar to the increase in oocyte basal $P_{ur}$ elicited by HgCl$_2$ (1). This indicates that the endogenous urea transport is unspecifically altered by these compounds and, therefore, given the small magnitude of the AQP3-mediated $P_{ur}$, it is difficult to assess whether phloretin inhibits $P_{ur}$ in oocytes injected with AQP3 cRNA.

Glycerol intrinsic protein (GLIP), a cloned MIP family member with a deduced amino acid sequence very similar to that of AQP3, is permeable to glycerol but not to urea or water (12). GLIP-mediated glycerol transport was inhibited by 88% by 0.2 mM DIDS (12). Hence, with the aim of finding an inhibitor of solute transport via AQP3, we examined the effect of exposure to 0.2 mM DIDS on oocytes injected with AQP3 cRNA. $P_{gly}$ was measured isotopically in groups of 10 oocytes. Control $P_{gly}$ in water-injected and in AQP3 cRNA-injected oocytes was 2.4 $(\pm 0.3) \times 10^{-6}$ and 8.1 $(\pm 0.3) \times 10^{-6}$ cm/s, respectively, and the corresponding values for the DIDS-treated oocytes were 2.6 $(\pm 0.5) \times 10^{-6}$ and 8.5 $(\pm 0.8) \times 10^{-6}$ cm/s. Thus, no change in $P_{gly}$ of AQP3 was observed with DIDS.

To better characterize the mode of transport of urea and glycerol by AQP3, we measured the values of $E_f$ for $P_{gly}$, $P_{ur}$, $E_{f, gly}$ and $E_{f, ur}$ for $P_f$ was 3 kcal/mol, a low value, therefore consistent with AQP3 being a water channel. For $P_{ur}$ and $P_{gly}$ the $E_{f, ur}$ values were much higher, 19.7 and 12.2 kcal/mol, respectively, and thus, indicative of a transport mechanism that involves a strong interaction between AQP3 and urea or glycerol.

To examine whether glycerol transport by AQP3 expressed in oocytes is saturable, we measured uptake as a function of glycerol concentration. Barth's solutions containing various glycerol concentrations were prepared by replacing NaCl with glycerol maintaining osmolality constant. A higher than 165 mM glycerol concentration was not used because it would have exposed the oocytes to a hyperosmotic medium, introducing conditions that were not comparable to the rest of the measurements. As shown in Fig. 4, glycerol uptake was linear in AQP3 cRNA- and in water-injected oocytes. Thus, in the concentration range examined, AQP3-mediated glycerol transport was not saturable.

**DISCUSSION**

The results of this study confirm reports of others that human AQP1 (13) and rat AQP1 (12) expressed in oocytes are not permeable to glycerol. In addition, rat AQP1 was not permeable to urea, and therefore, it constitutes a water-selective channel (8, 15). Among the known aquaporins, $\gamma$-tonoplast intrinsic protein ($\gamma$-TIP) from Arabidopsis thaliana (13), AQP4 (12), and AqpZ from E. coli (14) have not been found to promote increased $P_{gly}$ when they were expressed in the oocyte. Thus, in the MIP family, only GlpF, AQP3, and GLIP (if this homolog is indeed different from AQP3) are permeated by glycerol.

The present results and those of others (7, 12–14) show that the cell membrane of control Xenopus oocytes is slightly permeable to glycerol. Not previously reported is the observation that this permeability is practically completely inhibited by pCMBS (Fig. 2B). This suggests that glycerol crosses the oocyte membrane by facilitated diffusion via a transporter that may resemble that of E. coli because GlpF is inhibited by sulfhydryl-modifying reagents as well (6, 7). However, since pCMBS does not affect the transport of glycerol mediated by AQP3 (Fig. 2B), the endogenous transport system differs from AQP3.
Comparison of the amino acid sequence of AQP3 to that of rat AQP1 and of E. coli GlpF shows differences that may help locate segments potentially involved in the formation of the glycerol transport site in AQP3. An alignment of AQP1, AQP3, and GlpF, based on that proposed by Reizer et al. (4) for the entire MIP family before the cloning of AQP3, is shown in Fig. 5. White letters in a black background highlight those residues that are most highly conserved in the family (4). Identity of AQP3 residues with those of either AQP1 or GlpF is indicated by rectangles. The larger degree of homology of AQP3 with GlpF than with AQP1 is shown. AQP3 and GlpF contain two rather homologous segments, corresponding to the residues 130–150 and 227–239 of AQP3, which in Fig. 5 are underlined as segments I and II, respectively. Segment II, in particular, is largely identical (62%) with the corresponding one in GlpF. These segments have no homology in AQP1, AQP2, AQP4, or AQP5. Both segments are mostly polar and are probably located in extracellular loops of AQP3. It is, therefore, possible that one or both of these segments contribute to the formation of the glycerol transport pathway. Since at present it is unknown even whether AQP3 exists in the cell membrane as a homotetramer like AQP1 (16) or in some other configuration, a more specific mode of transport for glycerol cannot be proposed at this time. Certainly the absence of saturation of the rate of glycerol transport in the concentration range studied (1–165 mM) is more consistent with passage through a pore than via a transporter. However, the exact nature of the transport system remains to be elucidated.

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