Characterization of the Transmembrane Orientation of Aquaporin-1 Using Antibodies to Recombinant Fusion Proteins†

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ABSTRACT: Aquaporin-1 (AQP1) is a member of a family of integral membrane proteins, the aquaporins, which function as molecular channels for the movement of water across the plasma membrane. While the primary structure of AQP1 has been obtained from the cloning of its cDNA, its secondary structure is less certain. In this study, antibodies have been generated to defined regions of AQP1 in order to characterize its secondary structure. The antibodies were produced in chickens against glutathione *S*-transferase fusion proteins which represented loops C and E, and the carboxyl terminus of AQP1 as defined in the six-transmembrane model of Preston and Agre [(1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 11110]. Characterization of the antibodies showed that they recognized their corresponding fusion proteins as well as native AQP1 in erythrocytes and recombinant AQP1 expressed in COS7 cells. They differed, however, with respect to the specific conditions required for recognition. Thus, the anti-C-terminal antibodies recognized COS7 cells transfected with AQP1 that were fixed and permeabilized but did not recognize live cells (unpermeabilized). Conversely, antibodies to loop C labeled both live and fixed cells, while antibodies to loop E labeled live cells but not fixed. The data indicate that the carboxyl terminus of aquaporin-1 is intracellular and that loops C and E are extracellular. Furthermore, antibody recognition of loop E is very sensitive to the labeling conditions which may reflect an active role in the functional protein.

Cells live in an aqueous environment, and the movement of water across cell membranes is a fundamental biological process. In general, water crosses the cell membrane in two ways: either by simple diffusion or by a channel-mediated process. For simple diffusion, the movement of water across the membrane is limited by the hydrophobic nature of the lipid bilayer. The movement of water through channels, however, is much more efficient, and in membranes which contain such channels, the movement of water across the membrane is up to 10-fold greater than by simple diffusion (Macey & Farmer, 1970; Finkelstein, 1987).

Although channel-mediated water movement was postulated some time ago (Sidel & Solomon, 1957; Hays & Leaf, 1962; Macey & Farmer 1970; Finkelstein, 1987), the water channel proteins themselves have only recently been characterized. The first of these, aquaporin-1 (AQP1),[1] was initially purified as a protein of unknown function (Denker *et al*., 1988) and was subsequently cloned and expressed and was found to be a water-selective channel [see review by Agre *et al*. (1993)]. Water can move freely through AQP1 with its direction being dictated by the prevailing ionic gradient across the membrane.

The molecular structure of AQP1 is starting to be understood. Biochemical evidence indicates that AQP1 is present in the plasma membrane as a homotetramer, with one of the four subunits being glycosylated and each subunit containing a functional pore (Smith & Agre, 1991; Verbavatz *et al*., 1993; Shi *et al.*, 1994). In addition, biochemical and immunological experiments have provided evidence which indicates that both the carboxyl and amino termini are intracellular (Smith & Agre, 1991; Nielsen *et al*., 1993); however, there is disagreement about the transmembrane organization of the rest of the molecule. One model (Preston & Agre, 1991; Jung *et al*., 1994), shown in Figure 1A, proposes that there are six transmembrane (6-TM) domains which give rise to three loops which are extracellular (A, C, and E) and two which are intracellular (B and D). Evidence in favor of this arrangement was provided by mutagenesis experiments which further showed the importance of loops B and E in the transport of water (Jung *et al*., 1994).

A second model for AQP1 has recently been proposed in which 16 β-sheets form a β-barrel similar to the bacterial

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[1] Abbreviations: AQP1, aquaporin-1; DNA, deoxyribonucleic acid; TM, transmembrane; PCR, polymerase chain reaction; GST, glutathione *S*-transferase; DMSO, dimethyl sulfoxide; TME, Tris/magnesium/EDTA; EDTA, (ethylenedinitrilo)tetraacetic acid disodium salt; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; NBT/BCIP, nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt; TBS-T, Tris-buffered saline−Tween-20; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified Eagle's medium; BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate.

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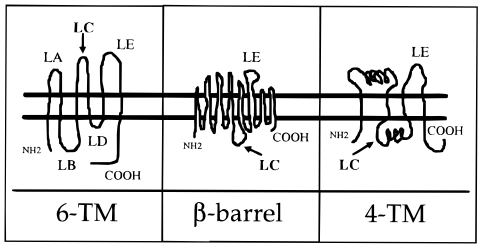

FIGURE 1: Three topographical models for aquaporin-1. Model A (left) is the 6-transmembrane, α-helical model (Preston & Agre, 1991); model B (middle) is the 16-transmembrane, β-barrel model (Fischbarg, 1995); and model C (right) is the 4-transmembrane model (Skach, 1994). Abbreviations: loop A (LA), loop B (LB), loop C (LC), etc.

porins (Fischbarg *et al.*, 1995). This model (shown in Figure 1b) was developed out of concerns about the theoretical folding of the 6-TM model and is supported by a variety of secondary structure algorithms; however, the β-barrel model has not been tested experimentally. In contrast to the 6-TM model in which loop C is extracellular, in the β-barrel model the amino acids corresponding to loop C are predicted to be intracellular.

A third model (Figure 1C) for the orientation of AQP1 in the membrane has also been proposed (Skach *et al*., 1994). By engineering nine reporter protease cleavage sites into the cDNA encoding AQP1, a unique four-transmembrane (4-TM) model was hypothesized. This 4-TM model was also supported by cell-free translation of five truncated AQP1 cDNAs and by epitope tagging of the amino terminus. Like the β-barrel model, the 4-TM model has the amino acids corresponding to loop C of the 6-TM model on the inside of the cell.

To further test these models, we have developed antibodies against specific epitopes of AQP1. These epitopes, defined in reference to the original 6-TM of Preston and Agre (1991), were expressed as recombinant fusion proteins with glutathione *S*-transferase and correspond to two of the putative extracellular loops (C and E) and the carboxyl terminus of AQP1. Antibodies were raised to these fusion proteins and were used in immunocytochemical studies with COS7 cells that were transiently transfected with AQP1. Our findings support a model in which loops E and C are extracellular and the carboxyl terminus is cytoplasmic and are consistent with the 6-TM model for AQP1.

EXPERIMENTAL PROCEDURES

Construction of the Expression Plasmids and Purification of Fusion Proteins. The polymerase chain reaction (PCR) was used to generate the following fragments of human AQP1: a 128 base pair (bp) fragment encoding 38 amino acids of the carboxyl terminus; a 122 bp fragment encoding 36 amino acids, of loop C; and a 149 bp fragment encoding 45 amino acids of loop E. The primer sets used for these PCR reactions included sense primers corresponding to nucleotides 694−716 (for the carboxyl tail), 316−336 (for loop C), and 523−543 (for loop E) of aquaporin-1 and antisense primers corresponding to nucleotides 796−820 (for the carboxyl tail), 412−437 (for loop C), and 646−671 (for loop E). Shown below are the nucleotide sequences with changes made (underlined) to introduce *Bam*HI and *Eco*RI restriction sites into the sense and antisense primers, respectively:

AQP1-C sense:
5′-GCCGGATCCAGCAGTGACCTCAC-3′

AQP1-C antisense:
5′-CAGGAATTCTCTATTTGGGCTTCA-3′

AQP1-LC sense:
5′-ATCGGATCCACCGCCATCCTC-3′

AQP1-LC antisense:
5′-GTCGAATTCTACTCGATGCCCAGGCC-3′

AQP1-LE sense:
5′-TCTGGATCCCTTGGACACCTC-3′

AQP1-LE antisense:
5′-ACAGAATTCTATCCCCCGATGAATGG-3′

The pcrDNA products were digested with *Bam*HI and *Eco*RI and were cloned in-frame behind the gene for glutathione *S*-transferase (GST) in the bacterial expression vector pGEX-2T. The resulting plasmids, pGEX/AQP1-C, pGEX/AQP1-LC, and pGEX/AQP1-LE, when expressed in *E. coli*, yielded the following respective fusion proteins, GST/AQP1-C, GST/AQP1-LC, and GST/AQP1-LE. PCR conditions and the isolation, restriction, and subcloning of the pcrDNA products were as previously described (Vanscheeuwijck *et al.*, 1993). *E. coli* strain XL-1 Blue (Stratagene, La Jolla, CA) was used to express the fusion proteins, as previously described (Vanscheeuwijck *et al.*, 1993).

Immunization of Chickens and Purification of Antibodies to AQP1. Mature hens (White Leghorns) were injected intramuscularly with 50−100 μg of the GST/AQP1 fusion proteins in Freund's adjuvant and were given subsequent injections with fusion protein in incomplete adjuvant at 2 week intervals. Eggs were collected, and crude antibody fractions were prepared from the yolks using (polyethylene glycol) precipitation as described (Vanscheeuwijck *et al.*, 1993). Antibodies specific for each of the fusion proteins were affinity-purified by first passing crude IgG fractions through columns of GST−agarose and then through columns of the immobilized fusion proteins. Antibodies were eluted with 100 mM glycine (pH 2.8) and were neutralized with 1.0 M Tris/HCl (pH 9.0).

Construction of pBC/AQP1 and Heterologous Expression in COS7 Cells. The eukaryotic expression vector pBC12BI and a plasmid encoding AQP1 (pCHIPev; Preston *et al*., 1992) were digested with *Hin*dIII and *Bam*HI. The digests were electrophoresed in a 1.1% agarose gel, and a 1.15 kb fragment corresponding to the coding region of AQP1 and a 3.9 kb fragment of the expression vector were excised from the gel and purified using Geneclean (Bio101, La Jolla, CA). The fragments were ligated using T4 DNA ligase (Gibco/BRL, Grand Island, NY), and XL-1 Blue cells (Stratagene) were transformed and positive clones were selected using ampicillin resistance.

COS7 cells were plated and grown until 60−70% confluent on 15 cm culture dishes. The cells were transfected with pBC/AQP1 (50 μg/plate) using the DEAE-dextran procedure with DMSO shock as previously described (Vanscheeuwijck *et al.*, 1993).

Membrane Preparation and Immunoblot Analysis. COS7 cells grown on 15 cm culture plates were scraped into 30 μL of TME buffer (50 mM Tris, 10 mM $MgCl_2$, 1 mM

EDTA, pH 7.5), homogenized using a Polytron homogenizer, and centrifuged at 2500*g* for 10 min. The supernatant was poured into a fresh tube and centrifuged at 37000*g* for 20 min. The resulting pellet was resuspended in 1 mL of TME buffer with a Duall homogenizer and centrifuged at 13000*g* for 6 min. The pellet was suspended in 30 μL of loading buffer (4% SDS, 125 mM Tris, 20% glycerol), electrophoresed into a 12% polyacrylamide gel with 0.1% SDS, and blotted onto nitrocellulose using the Transblot system (Bio-Rad, Hercules, CA) as previously described (Stamer *et al*., 1994). The blots were preincubated for 2 h at 22 °C in blocking buffer [Tris-buffered saline, containing 5% non-fat powdered milk and 0.2% Tween (TBS-T)] and were probed with affinity-purified fusion protein antibodies (1:500 dilution) overnight at 4 °C. The blots were washed (3 × 5 min) in TBS-T and were incubated for an additional 10 min in TBS-T with rotation. Blots were incubated in blocking buffer containing goat anti-chicken/alkaline phosphatase-conjugated secondary antibody (1:1000 dilution) (Sigma, St. Louis, MO) for 2 h at 22 °C with rotation and were visualized using NBT/BCIP (Gibco/BRL) per manufacturer's instructions.

Dot Blot Analysis. Membrane preparations from COS7 cells that were transiently transfected with pBC/AQP1 or with the original plasmid, pBC12BI, were solubilized in 30 μL of 1% NP-40 in TME, and 10 μL (20 μg of total protein) of each preparation was applied onto strips of nitrocellulose (BioRad) as a single dot. The blots were allowed to air-dry and were incubated with blocking buffer for 1 h at 22 °C with rotation. Anti-fusion protein antibodies were added (1:500 dilution), and the blots were incubated overnight at 4 °C and were washed 3 × 5 min in TBS-T and incubated for 10 min in TBS-T with rotation at 23 °C. Following a 5 min preincubation with blocking buffer, alkaline phosphatase-conjugated rabbit anti-chicken (Sigma) secondary antibody (1:1000 dilution) was added, and the blots were incubated for 2 h at 22 °C with rotation, washed as before, and developed as described above. The reaction was quenched with an excess of distilled water, and blots were digitized and analyzed by densitometry using the AMBIS system (San Diego, CA).

Live Cell Immunocytochemistry. COS7 cells were plated onto glass coverslips in 15 cm culture plates (Falcon, Lincoln Park, NJ) and were grown in DMEM (Gibco/BRL) containing 5% FBS (Summit, Ft. Collins, CO), penicillin G sodium (100 units/mL), and streptomycin sulfate (100 μg/mL) in humidified air containing 5% CO_2 at 37 °C. After reaching 60−70% confluency, the cells were transfected as described above. Cells were grown for an additional 48 h, placed on ice, washed twice with ice-cold phosphate-buffered saline (PBS), and kept on ice for 15 min. Cells were labeled using a modification of a previously described procedure (Pappas *et al*., 1995) in which the primary anti-fusion protein antibodies (1:100 dilution) were preincubated with secondary antibodies (FITC-conjugated anti-chicken, 1:100 dilution, Pierce, Rockford, IL) in PBSA buffer (4% goat serum and 2% BSA in PBS) for 30 min at 4 °C in the dark. Coverslips with cells were inverted onto 40 μL of antibody mixture for 20 min at 4 °C in the dark. Cells were washed 4 times with ice-cold PBS and were fixed in 4% paraformaldehyde in PBS for 5 min. Cells were incubated for 5 min in 100 mM glycine (pH 7.5), washed twice with PBS, and mounted in glycerol containing *p*-phenylenediamine. Cells were visualized for fluorescence labeling with a Leica TCS-4D confocal microscope with SCANWARE version 4.2a.

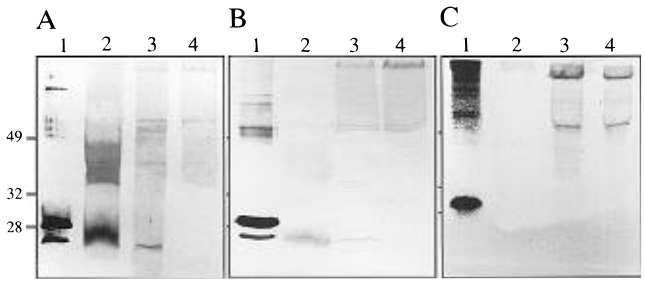

FIGURE 2: SDS−polyacrylamide gel electrophoresis and immunoblotting of AQP1 fusion proteins (lane 1), membranes prepared from erythrocytes (lane 2), aquaporin-1-transfected COS7 cells (lane 3), or mock-transfected COS7 cells (lane 4). Panel A was probed with antibodies against the carboxyl terminus, panel B with antibodies against loop C, and panel C with antibodies against loop E of AQP1. Conditions for immunoblotting are described under Experimental Procedures. The positions of the molecular size markers are indicated on the left ($\times 10^3$ daltons). Shown are representative blots for each of the antibodies that have been repeated a total of 6 times.

Immunocytochemistry. Aquaporin-1-transfected COS7 cells were seeded onto glass coverslips and were grown for 3 days as described above. Cells were fixed with 4% paraformaldehyde in PBS for 15 min at 22 °C and washed 3 times with PBS. Cells were incubated with sodium borohydride (1 mg/mL) for 10 min and washed 3 times with PBS. Cells were then permeabilized with saponin buffer (0.05% saponin in PBS containing 10% goat serum) for 30 min at 22 °C and were inverted onto 40 μL of 0.05% saponin buffer with primary antibody (1:400 dilution) for 2 h at 22 °C. Cells were washed 3 times with PBS and were incubated with 40 μL of flourescein-conjugated rabbit anti-chicken (1:1000 dilution in 0.05% saponin buffer; Pierce) for 2 h at 22 °C. Cells were then washed 3 times in PBS, mounted, and examined by confocal microscopy. In all experiments, the specificity of labeling was determined by either omitting the primary antibodies, omitting both primary and secondary antibodies, or preincubating the primary antibodies with a 10-fold excess of corresponding fusion proteins.

RESULTS

Immunoblot Analysis. Sense and antisense oligonucleotide primers were designed to regions flanking loops C and E and the carboxyl terminus of AQP1. Using these primers with pCHIPev (Preston *et al*., 1992) as a template, the polymerase chain reaction (PCR) gave products of expected sizes. These fragments were subcloned into the bacterial expression vector (pGEX2T) to yield plasmids encoding fusion proteins of ~31 500 daltons (26 500 Da for GST plus ~5000 Da for the AQP1 segments). The purified fusion proteins were used to generate antibodies in chickens and were characterized initially by immunoblot analyses. Figure 2 shows the results of Western blot analysis and indicates that the antibodies to the carboxyl terminus (panel A) and loop C (panel B) recognized their corresponding fusion proteins (lane 1). These antibodies also recognized native AQP1 in erythrocyte membrane preparations (lane 2), and in membrane preparations from transiently transfected COS7 cells (recombinant AQP1, lane 3). In contrast, antibodies

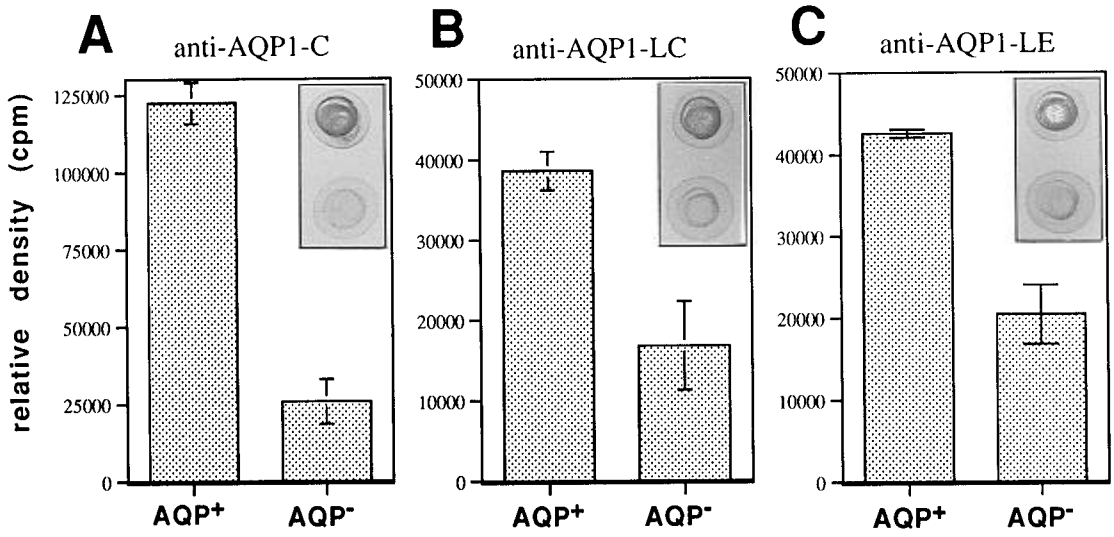

FIGURE 3: Immunoblot (dot blot) analysis of membranes that were prepared from COS7 cells and probed with anti-aquaporin-1 antibodies. Cells were transfected with an expression plasmid, pBC12BI, that either contains the cDNA that encodes aquaporin-1 (AQP+) or does not (AQP−). Membranes were prepared as described under Experimental Procedures, solubilized in 1% NP-40, and blotted onto nitrocellulose (20 μg total protein). Blots were probed with antibodies that were raised against the carboxyl terminus (panel A), loop C (panel B), or loop E (panel C). Blots were quantified by densitometry and expressed as an average of three independent experiments (±SEM). A representative example of the developed blots is shown as an insert to each panel.

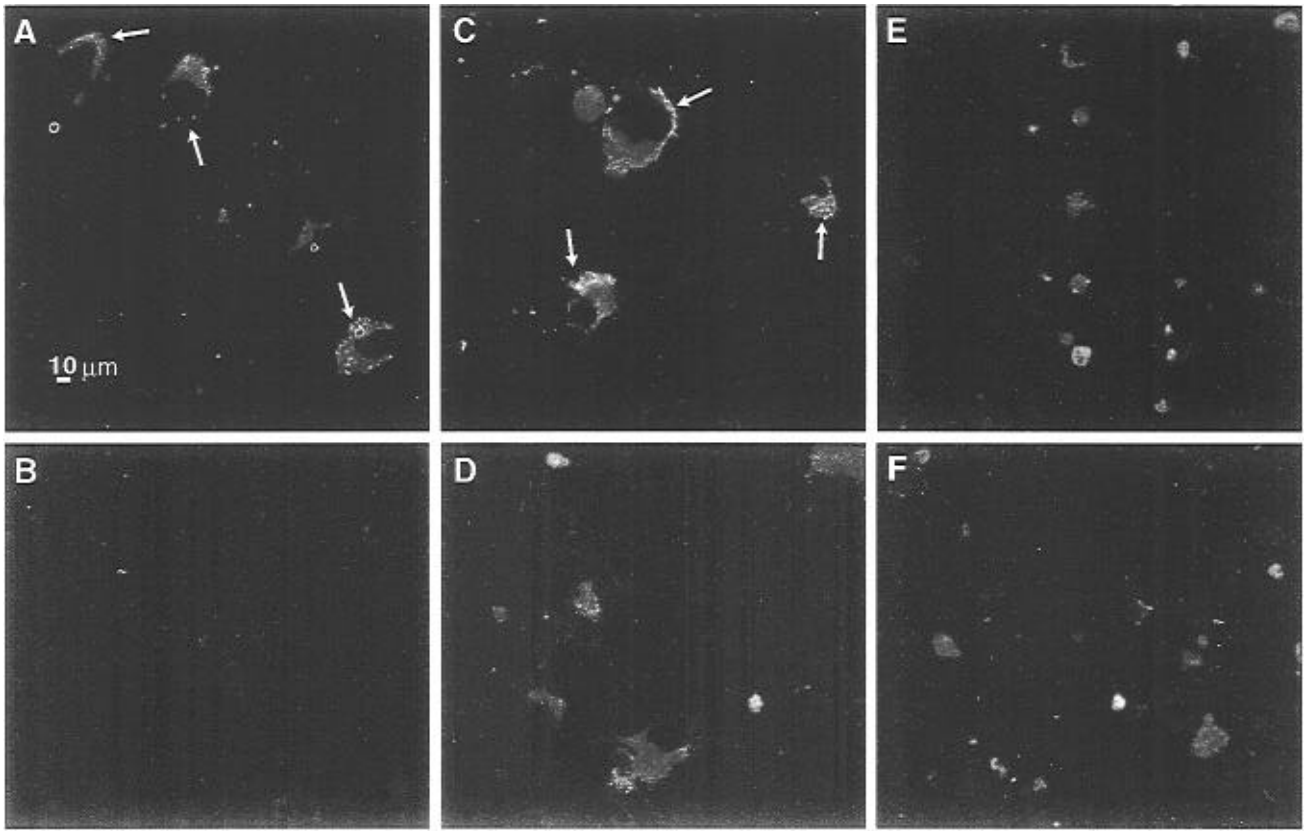

FIGURE 4: Immunofluorescence microscopy of live COS7 cells that were transfected with full-length cDNAs that encode AQP1 and were probed with anti-AQP1 antibodies. Antibodies were raised against the GST/AQP1-LE (panels A, B), GST/AQP1-LC (panels C, D), and GST/AQP1-C (panel E) fusion proteins. Cells were transfected as previously described (Vanscheeuwijck *et al*., 1993). Transfected cells were placed on ice, washed with ice-cold PBS, and probed with an antibody cocktail of primary plus secondary antibodies as described under Experimental Procedures. Panels A and C demonstrate specific labeling (arrows) of AQP1 expressed in COS7 cells using antibodies generated to GST/AQP1-LE or GST/AQP1-LC fusion proteins, respectively. Panel E shows that antibodies to GST/AQP1-C did not react with live COS7 cells that were transfected with AQP1. Panels B and D show the non-specific labeling that was obtained after preincubation of primary antibodies with a 10-fold excess of corresponding fusion proteins for 16−18 h at 4 °C. Panel F shows background fluorescence when primary antibody is omitted. Shown is a representative experiment that has been repeated a total of 4 times. Bar = 10 μm.

to loop E recognized the GST/LE fusion protein (panel C, lane 1), but did not recognize the native or recombinant AQP1 (lanes 2−3). None of the antibodies showed positive immunoreactivity to COS7 cells transfected with the expression vector alone (lane 4), and they did not cross-react with noncorresponding fusion proteins (data not shown).

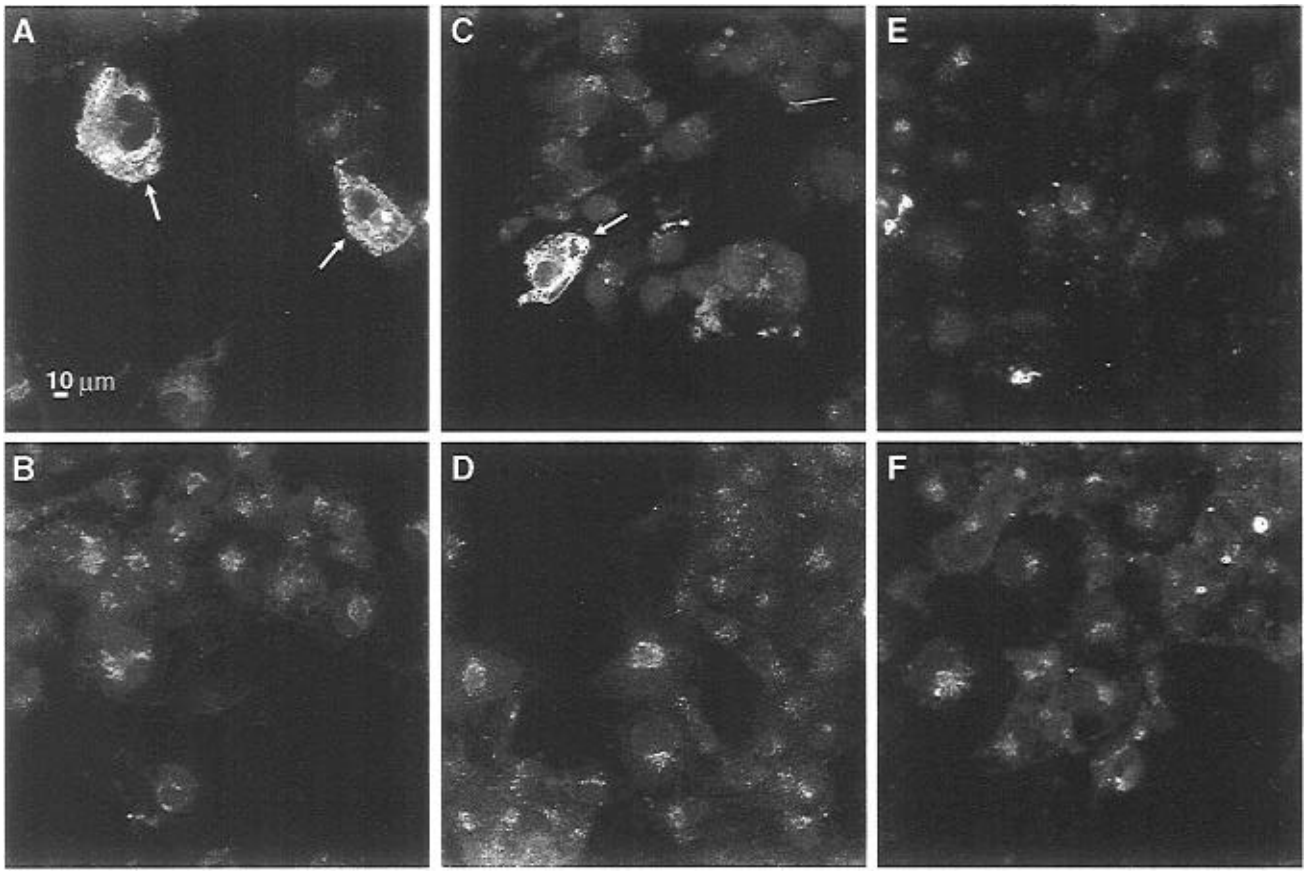

FIGURE 5: Immunofluorescence microscopy of fixed and permeabilized COS7 cells that were transfected with full-length cDNAs that encode aquaporin-1 and were probed with anti-aquaporin-1 antibodies. Antibodies were raised against the GST/AQP1-C (panels A, B), GST/AQP1-LC (panels C, D), and GST/AQP1-LE (panel E) fusion proteins. Cells were transfected and were labeled as described under Experimental Procedures. Panels A and C demonstrate specific labeling (arrows) of AQP1 in transfected COS7 cells using antibodies generated to GST/AQP1-C and GST/AQP1-LC fusion proteins, respectively. Panel E shows that antibodies to GST/AQP1-LE did not react with transfected COS7 cells that were fixed and permeabilized. Panels B and D show the nonspecific labeling that was obtained after preincubation of primary antibodies with a 10-fold excess of corresponding fusion proteins for 16−18 h at 4 °C. Panel F shows background fluorescence when primary antibody is omitted. Shown is a representative experiment that has been repeated a total of 8 times. Bar = 10 μm.

Since the antibodies to loop E recognized the fusion protein but did react with AQP1 following SDS−PAGE, this epitope may be altered during SDS−PAGE. To explore this further, the antibodies were characterized by dot blot analysis and by live cell versus fixed cell immunofluorescence microscopy. For dot blot analysis, COS7 cells were solubilized with 1% NP-40, were blotted onto nitrocellulose, and were probed sequentially with the primary antibodies to AQP1 followed by alkaline phosphatase-conjugated secondary antibodies. Figure 3 shows a representative blot for each of the fusion antibodies as well as a summary of their relative density as analyzed by densitometry. In each case, the antibodies showed significantly higher immunoreactivity with the AQP1-transfected cell membranes (AQP+) over those of control (expression vector alone, AQP−). Moreover, it appeared that antibodies to the carboxyl-terminal fusion protein (panel A) were more reactive than the antibodies to loop C or loop E.

Live Cell Versus Fixed Cell Immunofluorescence Microscopy. Transfected COS7 cells were incubated with antibodies while in culture at 4 °C (live and unpermeabilized, Figure 4). In panel A, ∼20% of the cells incubated with antibodies directed against the loop E fusion protein were immunoreactive (arrows). Preincubation of these antibodies with the loop E fusion protein blocked labeling (panel B). Similarly, panel C shows that antibodies directed against loop C also labeled transfected COS7 cells (arrows) upon incubation in culture, and that the loop C fusion protein blocked labeling (panel D). As predicted by all three models, antibodies against the carboxyl terminus of AQP1 did not label transfected COS7 cells that were incubated with the antibodies in culture (panel E). Panel F demonstrates the background fluorescence in the absence of primary antibodies.

Figure 5 shows photomicrographs of COS7 cells that were transfected with AQP1 and fixed before labeling. Panel A shows strong immunofluorescence labeling (arrows) following exposure to antibodies against the carboxyl terminus. This labeling could be blocked following preincubation with its fusion protein (panel B). Likewise, panel C shows the specific labeling of transfected cells using antibodies to loop C which also was blocked by preincubation with the loop C fusion protein (panel D). In contrast to the results obtained with the labeling of cultured cells, antibodies against loop E did not recognize AQP1-transfected COS7 cells following fixation (panel E). Finally, panel F shows the background fluorescence in the absence of the primary antibodies.

DISCUSSION

Using antibodies to three defined regions of aquaporin-1, we tested topographical models of AQP1. The regions comprised two of the putative extracellular sites (loops C

Table 1: Summary of Reactivity of Fusion Antibodies to AQP1

	AQP1-C	AQP1-LC	AQP1-LE
live cell	−	+	+
fixed/permeabilized	+	+	−
Western blot	+	+	−
dot blot	+	+	+

and E) and one intracellular site (the carboxyl terminus), as defined by the 6-TM model of Preston and Agre (1991). Using AQP1-transfected COS7 cells, these antibodies revealed differential immunoreactivity when the labeling was done on live cells as compared with cells that were fixed and permeabilized (Table 1). Thus, the antibodies to loops C and E showed positive immunoreactivity on live cells, but the antibodies to the carboxyl terminus did not. This is consistent with an extracellular orientation of loops C and E and an intracellular localization of the carboxyl terminus. When the labeling was done with fixed and permeabilized cells, positive immunoreactivity was obtained with the antibodies to the carboxyl terminus and with the antibodies to loop C, but not with the antibodies to loop E. This unexpected result with the antibodies to loop E indicates that this region of the protein, previously exposed in the live cells, is masked or altered during fixation.

As it concerns the various models that have been proposed for the orientation of AQP1 in the membrane, our data are consistent with the 6-TM model proposed by Preston and Agre (1991) in which loops C and E are extracellular and the carboxyl terminus is intracellular. Our data for the orientation of loop C (arrows, Figure 1), however, are at odds with both the β-barrel model of Fischbarg *et al*. (1995) and the 4-TM model of Skach *et al*. (1994). In both of the latter models, the region corresponding to loop C of the 6-TM model is placed on the intracellular aspect of the plasma membrane which is inconsistent with the labeling that we obtained with the live cells. These models, however, do not differ from the 6-TM model with respect to the orientation of loop E and the carboxy terminus. In this regard, our data with the antibodies to loop E and the carboxyl terminus are consistent with all three models.

The labeling observed with antibodies to loop E is interesting and shows a sensitivity which is very dependent upon the conditions of the labeling. Antibodies to loop E labeled AQP1 in live cells, indicating that this epitope is accessible in the native state. Once perturbed, however, by either fixation or SDS−PAGE, this epitope was masked. It would appear, therefore, that the native conformation of AQP1 is important for antibody recognition of loop E. It has been proposed, in the context of the 6-TM model, that loop E forms part of the pore structure of the channel and that it may fold into the membrane through hydrophobic interactions with the TM domains. We predict on the basis of our present findings that in the native state the pore is relatively open but that it may collapse onto loop E following fixation or SDS−PAGE. This sensitivity would be consistent with a proposed role for this domain in the functional operation of the water channel.

The present studies have addressed questions concerning the orientation of aquaporin-1 in the membrane. The results clearly support a 6-TM model over the alternative models for aquaporin-1 and indicate that loop E is not freely accessible to antibody recognition and may associate with TM domains. Recently, a cationic conductance has been identified as a property of aquaporin-1 that is activated by a cAMP-dependent mechanism (Yool *et al*., 1996). Future immunocytochemical and functional studies will be needed to further define the topography of aquaporin-1 and to understand how its various domains interact to enable the regulated movement of both water and ions.

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