Membrane Topology of Aquaporin CHIP

ANALYSIS OF FUNCTIONAL EPITOPE-SCANNING MUTANTS BY VECTORIAL PROTEOLYSIS*

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CHIP is the archetypal member of the aquaporins, a widely expressed family of membrane water channels. The NH2- and COOH-terminal halves of CHIP are sequence-related, and hydropathy analysis predicted six membrane-spanning domains with five connecting loops (A–E). Here, we determined the membrane topology of CHIP expressed in Xenopus oocytes using biologically active recombinant channels. CHIP is glycosylated at Asn-42, indicating loop A is exofacial. An epitope from the coronavirus E1 glycoprotein was inserted into CHIP and localized to the outer or inner leaflet of the membrane by α-chymotrypsin digestion of intact oocytes or inside-out membrane vesicles. The E1 epitope at Thr-120 was protease-sensitive in intact oocytes, indicating that loop C is exofacial. The E1 epitope at Lys-267 was protease-sensitive in inside-out membrane vesicles, confirming the cytoplasmic location of the NH2 and COOH termini and loop D. Insertions into loops B and E did not produce active water channels, but their cleavage patterns were consistent with inner (loop B) and outer (loop E) leaflet locations. This study indicates that the functional CHIP molecule is a unique structure with two internal repeats oriented 180° to each other within the membrane.

CHIP is a 28-kDa channel-forming integral membrane protein first identified and purified from red cells (Denker et al., 1988; Smith and Agre, 1991), and the cDNA has been isolated (Preston and Agre, 1991). CHIP functions as a water-selective pore, a discovery first made by expression of the cDNA in Xenopus oocytes (Preston et al., 1992) and directly verified by reconstitution of highly purified CHIP protein into proteoliposomes (Zeidel et al., 1992). The known mercury inhibition of water transport (Macey, 1984) is due to a single residue in CHIP, Cys-189 (Preston et al., 1993). These studies were subsequently confirmed by others (van Hoek and Verkman, 1992; Zhang et al., 1993a, 1993b). The related mammalian kidney protein WCH-CD (Fushimi et al., 1992) and the plant tonoplast intrinsic protein γ-TIP (Maurel et al., 1993) were recently shown to form water-selective channels, and these proteins are now referred to as the "aquaporins" (Agre et al., 1993a, 1993b). The aquaporins belong to a larger gene family including MIP, the major intrinsic protein of lens (Gorin et al., 1984), and other proteins expressed in animals, plants, Drosophila, yeasts, and bacteria (Pao et al., 1991). The functions of most MIP homologs remain undefined, but several may function as pores selective for water or possibly other small uncharged molecules.

CHIP protein is also expressed in the water-permeable epithelium of many mammalian tissues including renal proximal tubules (Denker et al., 1988; Nielson et al., 1993b; Sabolic et al., 1992) cornea, ciliary body, choroid plexus, biliary ductules, and nonfenestrated capillary and lymphatic endothelia (Nielson et al., 1993a). CHIP transcripts are expressed in at least three different developmental patterns in fetal rats (Bondy et al., 1993). These CHIP transcripts and proteins are the products of a single gene (Genome Data Base symbol AQP1) that has been isolated and localized to human chromosome 7p14 (Moon et al., 1993).

Red cell CHIP is a homotramer (Smith and Agre, 1991); however, each monomer may contain a separate water pore (van Hoek et al., 1991; Preston et al., 1993).1 Biochemical and immunohistochemical studies demonstrated the cytoplasmic locations of the NH2 and COOH termini (Smith and Agre, 1991; Nielson et al., 1993b). Hydrophathy analysis of the CHIP cDNA predicted six membrane-spanning domains connected by five loops (A–E), and the NH2- and COOH-terminal halves of the polypeptide are sequence-related internal repeats (Preston and Agre, 1991). The purpose of this study was to define further the structure of CHIP by establishing the membrane topology of functional epitope-tagged CHIP molecules.

EXPERIMENTAL PROCEDURES

Site-directed and Insertional Mutagenesis—The BamHI site in the polylinker of the CHIP expression vector (Preston et al., 1992) was removed by digesting the DNA with BamHI, followed by T4 DNA polymerase to fill in the overhangs and T4 DNA ligase. This construct served as the template for the site-directed and insertional mutagenesis reactions using the Muta-Gene phagemid in vitro mutagenesis kit (Bio-Rad). Table I lists the CHIP site-directed and BamHI insertional mutants used in this study. Fig. 1 shows the locations of amino acids targeted for mutagenesis relative to the predicted topology of the CHIP monomer (Preston and Agre, 1991). The 6-base pair BamHI sites were inserted between the second and third base of a codon, resulting in the insertion of the amino acids Asp and Pro. The BamHI insertion at Phe-92 changed this residue to Leu (L92-Bam1, which is the naturally occurring amino acid in the rat and mouse CHIP cDNA sequences (Deen et al., 1992; Lanahan et al., 1992). None of the other BamHI insertions changed the coding specificity of the preceding residue. The BamHI insertion between Lys-267 and Pro-268 (K267-Bam) required only a 3-base pair insertion. Mutations were confirmed by enzymatic nucleotide sequencing (United States Biochemical Corp.).

A 90-base pair BamHI sequence encoding the E1 tag (Maclachlan and Rose, 1987) was isolated from pSM224 and ligated into the BamHI sites of the insert constructs. A 123-base pair BglII sequence encoding the hemagglutinin tag (Tysers et al., 1992) was isolated from pSM492 and ligated into the BamHI sites of the insert constructs, pSM224 and...
pSM492 were kindly provided by P. Chen, C. Berkowski, and S. Michaelis (The Johns Hopkins University School of Medicine, Baltimore). Restriction analysis and enzymatic nucleotide sequencing confirmed the correct orientation and reading frame of all insertions. The resulting amino acid insertions are as follows: E1 tag, DPMFYaketQDVTDGEASATVSSLKTYMDP and HA tag, DLGRIFYPYDVPDYDPDP.

Preparation of Oocytes and Measurement of P2—Female Xenopus laevis were anesthetized on ice, and skin and the right hind limb were excised. The blastula stage embryos were transferred to 15-mm flat-bottom tissue culture dishes containing 900 μl of MBS. α-Chymotrypsin (50 units/mg; CooperBiomedical, Inc.) was freshly dissolved in ice-cold MBS at 1 mg/ml and added to the oocytes at 10–100 μg/ml. After 60 min at 32°C with gradual shaking, the MBS/chymotrypsin solutions was removed, and the oocytes were washed five times with 1 ml of ice-cold MBS containing 2 × protease inhibitors. Oocyte membranes were then isolated in the presence of 2 × protease inhibitors and immediately analyzed by SDS-PAGE immunoblotting.

Membranes for α-chymotrypsin digestion were isolated from oocytes lysed in hypotonic buffer containing 20 μg/ml phenylmethylsulfonyl fluoride and gently washed once in hypotonic buffer without phenylmethylsulfonyl fluoride. The membrane pellets were resuspended in 8 μl of hypotonic buffer/oocyte. α-Chymotrypsin was dissolved at 1 mg/ml in 2 mM HCl and diluted in 2 mM HCl. Reactions containing 40 μl of membranes, 5 μl of 20 mM Tris, pH 8.0, and 5 μl of diluted α-chymotrypsin in 2 mM HCl (or 5 μl of 2 mM HCl) were incubated at 37°C for 60 min. The reactions were quenched with 450 μl of ice-cold hypotonic buffer containing 2 × protease inhibitors; the membranes were recovered by centrifugation at 16,000 × g for 30 min at 4°C, washed once, and analyzed by SDS-PAGE immunoblotting. Control reactions with membrane pellets resuspended in isomisc MBS and digested with α-chymotrypsin under the same conditions gave identical results (data not shown). Endoglycosidase H digestions of isolated oocyte membranes were performed as described (Preston et al., 1993).

**TABLE I**

| Mutant         | Amino acid      | Codon   | Amino acid(a) | Codon   |
|----------------|-----------------|---------|---------------|---------|
| N42Q           | Asn-42          | AAC     | Gln-42        | CAA     |
| N210Q          | Asn-210         | ACG     | Gln-205       | CAA     |
| K5-Bam         | Lys-6           | AAG     | Lys-Asp-Pro   | AAAGGatGC |
| Q88-Bam        | Gln-88          | CAG     | Gln-Asp-Pro   | CAAGGatGC |
| L92-Bam        | Phe-92          | TTC     | Leu-Asp-Pro   | TTGatGC |
| T120-Bam       | Thr-120         | ACT     | Thr-Asp-Pro   | ACTGatGC |
| R162-Bam       | Arg-162         | CGT     | Arg-Asp-Pro   | CGGatGC |
| V201-Bam       | Val-201         | GTG     | Val-Asp-Pro   | CTGatGC |
| K267-Bam       | Lys-267         | AACCGC  | Lys-Asp-Pro   | AAAGatCCG |

**RESULTS**

**N-Glycosylation Sites**—Red cell CHIP is a homotetramer with a complex glycan attached by N-linkage to one of the four subunits (Smith and Agre, 1991). When CHIP is expressed in oocytes, complex glycosylated subunits of 35–50 kDa and non-glycosylated core subunits of 28 kDa are detected. Oocytes also express a 30-kDa CHIP polypeptide containing an N-linked high mannose oligosaccharide that can be removed by digestion with endoglycosidase H, a characteristic of incomplete glycan maturation. The relative abundance of the core, high mannose, and complex glycosylated CHIP polypeptides varied between experiments; however, mutant forms of CHIP lacking water channel activity exist predominantly as high mannose polypeptides without detectable complex glycosylated subunits (Preston et al., 1993).

The two consensus sites for N-glycosylation of CHIP at Asn-42 and Asn-205 were both predicted to be extracellular (Fig. 1); therefore, Asn-42 and Asn-205 were mutated individually or in combination to Glu. CHIP is N-glycosylated at Asn-42 in oocytes since mutation of this residue alone eliminated detectable high mannose and complex N-glycosylated polypeptides (Fig. 2, top). Confirming the exofacial location of loop A, N-Glycosylation at only the first potential site has been demonstrated in other glycoproteins with multiple potential N-glycosylation sites. The N-glycosylation site mutants had wild-type water channel activities (Fig. 2, top), indicating that N-glycosylation is not required for channel folding, oligomerization, or cell-surface expression. It remains to be determined whether CHIP is O-glycosylated in red cells or oocytes.

**BamHI and Epoxyte Insertions**—BamHI sites were inserted at selected locations of the CHIP coding region. The effects of the two amino acid insertions on osmotic water permeability and protein expression were assessed (Fig. 3A). Three of the insert mutants (Q88-Bam, T120-Bam, and R162-Bam) had 

<sup>2</sup>The abbreviations used are: P<sub>v</sub>, osmotic water permeability; MBS, modified Barth’s solution; PAGE, polyacrylamide gel electrophoresis.

<sup>3</sup>C. Landolt and R. Reithmeier, personal communication.
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**FIG. 1. Proposed membrane topology of aquaporin CHIP.** Indicated in the model are the regions of CHIP recognized by anti-NH2-terminal peptide and anti-CHIP antibodies (Smith and Agre, 1991), the proposed locations of amino acids targeted for mutagenesis in this study, the location of the mercury-sensitive residue Cys-189 (C189) (Preston et al., 1993), membrane-spanning domains (heavy dark lines), exofacial (OUT) and cytoplasmic (IN) domains, and the highly conserved Asn-Pro-Ala motifs (NPA; boxed) located in the first and second inverted tandem repeats.

**FIG. 2. N-Glycosylation is not required for expression or function of CHIP.** Top, $P_f$ values for oocytes injected with 10 ng of CHIP cRNA or the indicated mutant RNA. After 72 h, some of the oocytes were used for osmotic swelling experiments (see “Experimental Procedures”). Shown are the means ± S.D. (n = 3–4). Bottom, contact print of an immunoblot of membrane proteins isolated from the remaining oocytes. Each lane contains the equivalent of membranes from 2.5 oocytes run on a 12% SDS-polyacrylamide slab. The blot was incubated with affinity-purified anti-CHIP (see “Experimental Procedures”). Complex N-glycosylated CHIP polypeptides migrate as a 35–50-kDa smear (CHIP and N205Q) relative to the mobility of prestained SDS-PAGE standards shown on the right.

Values and protein levels resembling oocytes expressing wild-type CHIP. The L92-Bam mutant had reduced protein expression or stability, which abolished CHIP-mediated $P_f$. The V201-Bam mutant was expressed at wild-type levels with an altered protein glycosylation pattern and lacked CHIP-enhanced $P_f$.

Two different epitope tags were evaluated at K6-Bam and K267-Bam (see “Experimental Procedures”). The 3.3-kDa E1 values and protein levels resembling oocytes expressing wild-type CHIP. The L92-Bam mutant had reduced protein expression or stability, which abolished CHIP-mediated $P_f$. The V201-Bam mutant was expressed at wild-type levels with an altered protein glycosylation pattern and lacked CHIP-enhanced $P_f$.

**FIG. 3. Introduction of Asp-Pro (BamHI sites) and viral protein epitopes into CHIP.** BamHI site insertional mutagenesis was performed at selected points within CHIP (A); E1 and HA epitopes were then inserted at K6-Bam and K267-Bam sites (B). The $P_f$ (top) was measured, and levels of CHIP protein (bottom) were determined. In each experiment, oocytes were injected with 10 ng of the indicated cRNA. After 48 h, osmotic swelling experiments (mean ± S.D., n = 3–4) and immunoblot analysis were performed as described for Fig. 2 and under “Experimental Procedures.”
were isolated and subjected to immunoblot analysis following electrophoresis of two oocyte membrane equivalents on a 14% SDS-polyacrylamide slab. The relative mobility of prestained SDS-PAGE standards is shown on the left of the contact print. At the right is a diagram representing the digestion of T120-E1 with α-chymotrypsin at an exofacial domain. Indicated in the model are the site of a-chymotrypsin digestion (arrow pointing to X), the location of the E1 tag (circle), and the CHIP fragment recognized on the blot by the anti-CHIP antibody following digestion (thick black line).

FIG. 4. Effects of E1 insertion mutagenesis on osmotic water permeability and α-chymotrypsin sensitivity of CHIP in intact oocytes. A, the $P_f$ of oocytes injected with 10 ng of the indicated cRNA was assessed 72 h after injection. Shown are the means ± S.D. (n = 4–5). B, oocytes injected with 10 ng of the indicated cRNA were maintained for 72 h. Groups of four oocytes were incubated for 60 min at 32 °C in the absence (−) or presence (+) of 10 μg/ml (data not shown) or 100 μg/ml α-chymotrypsin. The oocytes were then washed, and their membranes were isolated and subjected to immunoblot analysis following electrophoresis of two oocyte membrane equivalents on a 14% SDS-polyacrylamide slab. The relative mobility of prestained SDS-PAGE standards is shown on the left of the contact print. At the right is a diagram representing the digestion of T120-E1 with α-chymotrypsin at an exofacial domain. Indicated in the model are the site of a-chymotrypsin digestion (arrow pointing to X), the location of the E1 tag (circle), and the CHIP fragment recognized on the blot by the anti-CHIP antibody following digestion (thick black line).

The E1 epitope tag encodes 25 amino acids from the COOH terminus of the avian coronavirus E1 glycoprotein, which is not involved in either glycosylation or membrane binding (Machamer and Rose, 1987). The 4.8-kDa HA epitope tag from the influenza hemagglutinin HA1 peptide encodes 9 amino acids in a triple tandem cassette (Tyers et al., 1992). Introduction of the E1 epitope at either site did not affect CHIP protein expression or $P_f$ (Figs. 3D and 4). In contrast, insertion of the HA epitope at either site significantly reduced protein expression and $P_f$ (Fig. 3B).

The E1 epitope was then inserted into the BamHI sites at Gln-88, Thr-120, Arg-162, and Val-201. Oocytes expressing T120-E1 or R162-E1 had $P_f$ values and protein expression levels similar to those of wild-type CHIP (Fig. 4). V201-E1 exhibited a low $P_f$, although the protein was expressed at nearly wild-type levels. Insertion of the E1 epitope at Gln-88 resulted in greatly reduced levels of protein expression and a low $P_f$.

Identification of Exofacial Domains of CHIP in Intact Oocytes

The orientation of the E1 epitope in plasma membranes was assessed with anti-E1 peptide antiserum (Pluta et al., 1992). Control oocytes, oocytes expressing wild-type CHIP, and oocytes expressing the functional mutant K6-E1, T120-E1, or K267-E1 were incubated with rabbit anti-E1, followed by 125I-protein A. Similar protein A binding was obtained with control oocytes (3100 ± 125 dpm, mean ± S.D., n = 4) and with oocytes expressing wild-type CHIP (3125 ± 210 dpm), K6-E1 (3375 ± 355 dpm), or K267-E1 (3010 ± 410 dpm). In contrast, oocytes expressing T120-E1 had 60% more 125I-protein A binding than control oocytes (5010 ± 1145 dpm), suggesting an exofacial location for Thr-120.

The resistance of CHIP in intact red cells to protease digestion (Smith and Ager, 1991) and the presence of protease-sensitive residues in the E1 epitope were exploited to identify more definitively exofacial domains of CHIP. Intact oocytes expressing wild-type CHIP or one of the six E1 epitope-tagged CHIP proteins were digested with α-chymotrypsin, washed, and studied by immunoblot with the COOH-terminal specific anti-CHIP antibody (Fig. 4B). As expected, wild-type CHIP was resistant to protease digestion in intact oocytes.

Four of the E1 epitope-tagged CHIP proteins formed functional water channels (Fig. 4A). K6-E1, R162-E1, and K267-E1 contain the epitope at sites expected to be cytoplasmic (Fig. 1, and all three of these proteins were resistant to protease digestion in the intact oocytes (Fig. 4B). In contrast, loop C was predicted to be exofacial. Consequently, T120-E1 was partially cleaved by α-chymotrypsin digestion to a 17-kDa polypeptide (Fig. 4B). The >35-kDa complex glycosylated polypeptides were almost completely digested, whereas the 34-kDa high mannose and 32-kDa core polypeptides were relatively resistant. Membrane channel proteins overexpressed in Xenopus oocytes are known to have only a small percentage of the expressed protein in the plasma membrane (Nishimura et al., 1993). Thus, these results suggest that most complex glycosylated CHIP is transported to the plasma membrane. Similar results were obtained with trypsin and with lower concentrations of α-chymotrypsin; higher concentrations of protease were deleterious to the oocytes. Surprisingly, the same $P_f$ values were obtained for undigested and chymotrypsin-digested T120-E1-expressing oocytes, suggesting that cleavage of the protein at this location does not destroy function (data not shown).

Expression of two E1 epitope-tagged CHIP proteins resulted in only a small increase in $P_f$ relative to buffer controls (Fig. 4A). Oocytes expressing V201-E1 were expected to have a low $P_f$ since insertion of the BamHI site alone at this location reduced the $P_f$. V201-E1 was expressed at nearly wild-type levels, but no protease sensitivity was detected in intact oocytes (Fig. 4B). It is likely that these insertions at Val-201 perturb protein folding, resulting in significantly reduced cell-surface expres-
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**Fig. 5. Effects of E1 insertional mutagenesis on α-chymotrypsin sensitivity of CHIP in oocyte membrane vesicles.** Oocytes injected with 10 ng of the indicated cRNA (40 ng for Q88-E1) were maintained for 72 h prior to membrane isolation. Membranes were incubated at 37 °C for 60 min in the absence (0 lanes) or presence of 1 (1 lanes) or 10 (10 lanes) μg/ml α-chymotrypsin. The membranes were recovered by centrifugation, washed, and subjected to immunoblot analysis with the anti-NH2-terminal peptide antibody following electrophoresis of three oocyte membrane equivalents on a 14% SDS-polyacrylamide slab. The relative mobility of prestained SDS-PAGE standards is shown. Exposure times for the immunoblots varied from 2 to 60 min. Shown below the contact prints are models of CHIP and the different E1 insertional proteins. Indicated are the regions recognized by the anti-NH2-terminal peptide antibody (left of CHIP model), E1 tags (circles), locations of α-chymotrypsin digestion in inside-out membrane vesicles (X), and the CHIP fragments recognized by the antibody after digestion (thick black lines).

**Discussion**

CHIP is a widely expressed integral membrane protein and the archetypal member of the aquaporins (Agre et al., 1993a, 1993b), a family of water-selective pores also found in mammalian renal collecting ducts (Fushimi et al., 1993) and in plant tonoplasts (Maurel et al., 1993). The sequences of the aquaporins are related to the major intrinsic protein of lens, MIP, a membrane channel with undefined specificity (Gorin et al., 1984), and to several other proteins from widely divergent species (Pao et al., 1991). The aquaporins are freely permeated by water, but fail to pass protons, other ions, or uncharged solutes. The explanation for water-selective transport is unknown since only limited structural information exists. Although MIP and CHIP are homotetramers (Aerst et al., 1990; Smith and Agre, 1991), radiation inactivation studies (van Hoek et al., 1991) and pharmacological analyses of coexpressed CHIP and the mercury-resistant CHIP mutant C189S (Preston et al., 1993) suggest that each individual subunit contains an aqueous pore. Near- and far-UV circular dichroism of MIP revealed ~50% α-helix and ~20% β-structure (Horwitz and Bok, 1987). Hydropathy analysis of the deduced amino acid sequences of CHIP and the other MIP homologs predicted six bilayer-spanning domains (Preston and Agre, 1991; Gorin et al., 1984), and the proteins contain two sequence-related tandem repeats (Pao et al., 1991; Wistow et al., 1991).

The goal of this study was to establish experimentally the
membrane topology of CHIP by mutating N-glycosylation sites or inserting E1 viral protein epitope tags with protease cleavage sites (Fig. 1). The CHIP mutants were expressed in Xenopus oocytes, and the ability of each to transport water was determined since preservation of this function is necessary to confirm the relevance of the deduced topology to that of the native CHIP molecule. This study demonstrated exofacial locations of Asn-42 (loop A) and Thr-120 (loop C) and cytoplasmic locations of Lys-6 (NH$_2$ terminus), Arg-162 (loop D), and Lys-267 (COOH terminus) in water-transporting CHIP mutants. This study also suggests that Gln-88 (loop B) is cytoplasmic and Val-201 (loop E) is exofacial, although these mutants exhibited only marginally increased P$_r$ values. Previous studies have documented the existence of the single mercury-sensitive residue at Cys-189 (loop E) (Preston et al., 1993a) which is thought to lie near the exofacial leaflet of the lipid bilayer (Macey, 1984). These structural determinations are most likely relevant to the structures of the other aquaporins and MIP homologs. For example, WCH-CD contains a single potential N-glycosylation site that is likely to be exofacial since the location corresponds to loop C. The studies reported here are all consistent with the topology model of CHIP (Fig. 1). Therefore, the two internal tandem repeats, corresponding to the NH$_2$ and COOH-terminal halves of the molecule (CHIP-1 = residues 14 - 113 and CHIP-2 = residues 140 - 231), are oriented 180° to each other as originally proposed (Preston et al., 1992).

This study has advanced our understanding of the structure of CHIP subunits and provides a hypothesis for further investigating the structure of CHIP and other aquaporins. Mutations in loops B and E were less well tolerated than elsewhere in the CHIP molecule (Figs. 3a and 4), and preliminary analysis of a series of CHIP mutants containing single amino acid substitutions throughout loops B and E revealed that most mutants exhibited markedly reduced water channel activity. Loops B and E contain the Asn-Pro-Ala motifs that are conserved in the aquaporins and in all mammalian and plant homologs of MIP (Pao et al., 1991; Wistow et al., 1991). Moreover, loops B and E are both very hydrophobic and may extend into the lipid bilayer. Loops B and E are therefore candidate structures of the pore-forming domains for CHIP and the other aquaporins. Such a structure is consistent with the recognized reciprocal transport of water into and out of cells containing water channels. Further studies will concentrate upon loops B and E to define further the structure and function of these domains.

REFERENCES

Aerts, T., Xia, J.-Z., Slageren, H., de Block, J., and Clauwaert, J. (1990) J. Biol. Chem. 265, 8675-8680
Agre, P., Sassaki, S., and Chrapeels, M. J. (1993) Am. J. Physiol. 265, F461
Agre, P., Preston, G. M., Smith, B. L., Jung, J. S., Raina, S., Moon, C., Guggino, W. B., and Nielsen, S. (1993a) Am. J. Physiol. 265, F463-F476
Bundy, C., Chin, E., Smith, B. L., Preston, G. M., and Agre, P. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4500-4504
Deen, P. J. T., Dempster, J. A., Weiering, B., and Van Os, C. H. (1992) Biochem. Biophys. Res. Commun. 188, 1267-1273
Denker, B. M., Smith, B. L., Kuhadsa, F. P., and Agre, P. (1988) J. Biol. Chem. 263, 15834-15843
Fushimi, K., Uchida, S., Haru, Y., Hirata, Y., Marumo, F., and Sassaki, S. (1993) Nature 361, 549-552
Gorin, M. B., Yanoye, S. B., Cloie, J., Revel, J.-P., and Horwitz, J. (1984) Cell 30, 49-55
Horwitz, J., and Bok, D. (1987) Biochemistry 26, 8092-8098
Laemmli, U. K. (1970) Nature 227, 680-685
Lanahan, A., Williams, J. B., Sanders, L. K., and Nathans, D. M. (1992) Mol. Cell. Biol. 12, 3919-3929
Lo, L., Montrose-Rafizadeh, C., Hwang, T.-C., and Guggino, W. B. (1990) Biochips. J. 37, 1117-1123
Macey, R. I. (1984) Am. J. Physiol. 246, C192-C203
Machamer, C. E., and Roso, J. K. (1987) J. Cell Biol. 108, 1205-1214
Maurel, C., Reizer, J., Schroeder, J. I., and Chrapeels, M. J. (1993) EMBO J. 12, 2241-2247
Moon, C., Preston, G. M., Griffin, C. A., Jakob, E. W., and Agre, P. (1993) J. Biol. Chem. 268, 17772-17778
Nielsen, S., Smith, B. L., Christensen, E. I., and Agre, P. (1993a) Proc. Natl. Acad. Sci. U. S. A. 90, 7275-7279
Nielsen, S., Smith, B. L., Christensen, E. I., Knepper, M. A., and Agre, P. (1993b) J. Cell. Biol. 120, 371-383
Nishimura, H., Pallardo, F. V., Seidner, G. A., Vannucci, S., Simpont, I. A., and Birnbaum, M. J. (1993) J. Biol. Chem. 268, 8514-8520
Pao, G. M., Wu, L.-F., Johnson, K. D., Hill, B., and Agre, P. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7275-7279
Pao, G. M., Wu, L.-F., Johnson, K. D., Hofte, H., Chrapeels, M. J., Sweet, G., Sandal, N. N., and Steier, M. H., Jr. (1991) Mol. Microbiol. 5, 35-37
Pluta, A. F., Sztek, N., Goldenberg, I., and Earmahaw, W. C. (1992) J. Cell. Biol. 116, 1081-1093
Preston, G. M., and Agre, P. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11110-11114
Preston, G. M., Carroll, T. P., Guggino, W. B., and Agre, P. (1992) Science 256, 385-387
Preston, G. M., Jung, J. S., Guggino, W. B., and Agre, P. (1993) J. Biol. Chem. 268, 17-20
Sabolic, I., Valenti, G., Verbavatz, J. M., van Hoek, A. N., Verkman, A., A. S., A. S., D. A., and Brown, D. (1992) Am. J. Physiol. 263, C1225-C1233
Smith, B. L., and Agre, P. (1991) J. Biol. Chem. 266, 6407-6415
Tyers, M., Tokiwa, G., Nash, R., and Patcher, B. (1992) EMBO J. 11, 1773-1784
van Hoek, A. N., and Verkman, A. S. (1992) J. Biol. Chem. 267, 18267-18297
van Hoek, A. N., Aermark, M. L., Luthroth, L. H., de Jong, M. D., Dempster, J. A., and van Os, C. H. (1991) J. Biol. Chem. 266, 10653-10655
Wistow, G. J., Piasio, M. M., and Chepelinsky, A. B. (1993) Trends Biochem. Sci. 16, 179-171
Zeidel, M. L., Ambekdcar, S. V., Smith, B. L., and Agre, P. (1992) Biochemistry 31, 7436-7440
Zhang, R., Logue, K. A., and Verkman, A. S. (1990) J. Biol. Chem. 265, 15375-15379
Zhang, R., Skach, W., Hasegawa, H., van Hoek, A. N., and Verkman, A. S. (1993a) J. Cell. Biol. 120, 359-369
Zhang, R., van Hoek, A. N., Biewers, J., and Verkman, A. S. (1993b) Biochemistry 32, 2938-2941