Communication

Expression of mRNA Coding for Kidney and Red Cell Water Channels in Xenopus Oocytes*

(Received for publication, May 30, 1990)

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The existence and identity of protein water transporters in biological membranes has been uncertain. Osmotic water permeability (P\textsubscript{t}) was measured in defolliculated Xenopus oocytes microinjected with water or mRNA from kidney cortex, kidney papilla, reticulocyte, brain, and muscle. P\textsubscript{t} was measured by quantitative image analysis from the time course of oocyte swelling in response to an osmotic gradient. When assayed at 10°C, P\textsubscript{t} in water-injected oocytes increased from (3.6 ± 0.9) × 10\textsuperscript{-4} cm/s (S.D., n = 16) to 74 × 10\textsuperscript{-4} cm/s with addition of amphoterin B, showing absence of unstirred layers. At 48-72 h after injection of 50 ng of unfraccionated mRNA, P\textsubscript{t} (in cm/s × 10\textsuperscript{-4}) was: 4.0 ± 1.5 (rabbit brain, n = 15), 4.2 ± 1.8 (rabbit muscle, n = 10), 18.4 ± 6.3 (rabbit reticulocyte, n = 20), 16.1 ± 5.6 (rat renal papilla, n = 24), 12.9 ± 6.3 (rat renal cortex, n = 20), 14.4 ± 6.1 (rabbit renal papilla, n = 15), and 11.8 ± 3.4 (rabbit renal cortex, n = 8). In oocytes injected with mRNA from rat renal papilla, P\textsubscript{t} was inhibited reversibly by 0.3 mM HgCl\textsubscript{2} (4.1 ± 1.8, n = 10); expressed water channels from kidney and red cell had activation energies of ≈ 4 kcal/mol. These results show functional oocyte expression of water channels from red cell, kidney proximal tubule (cortex), and the vasopressin-sensitive kidney collecting tubule (papilla), indicating that water channels are proteins, and providing an approach for the expression cloning of water channels.

There is strong evidence that facilitated pathways exist for water movement through plasma membranes of kidney proximal tubule (1–4), vasopressin-stimulated kidney collecting tubule (5–7), and red blood cell (8, 9). Water transport in these cell types is strongly inhibited by mercurial sulfhydryl reagents, and has a low activation energy (E\textsubscript{a}) and a ratio of osmotic-to-diffusional water permeability (P\textsubscript{t}IP\textsubscript{d}) >> 1 (10, 11). The nature and molecular identity of water transporter(s) is unknown. It has been proposed that water transporters are proteins, organized lipids, or specialized protein-lipid assemblies (10–12).

The molecular identification and cloning of water channels is a difficult task because of the lack of selective water transport inhibitors, the relatively high water permeability of membranes not containing water channels, and the ubiquitous nature of water, making ligand protection approaches impossible (10). Whereas plasma membrane water channels in proximal tubule and red cells are probably fixed and not subject to physiological regulation, water permeability in cells containing the vasopressin-sensitive water channel is regulated by the exocytic-endocytic trafficking of vesicles containing functional water channels (10, 13–15). The identification and molecular cloning of water channels is important for tissue localization and for definitive biochemical and biophysical studies of water transporting mechanisms.

We report here the functional expression of mRNA coding for kidney and red cell water transporters in Xenopus oocytes. Oocyte water permeability was remarkably increased by microinjection of mRNA from kidney and red cells, but not by water or mRNA from brain or muscle. The expressed water channel from rat renal papilla had characteristics of the vasopressin-sensitive water channel.

EXPERIMENTAL PROCEDURES

Materials—The following materials were obtained from the indicated sources: guanidinium thiocyanate, Fluka; CsCl, Bethesda Research Laboratories; mRNA isolation kit, Pharmacia LKB Biotechnology Inc. All other chemicals were purchased from Sigma.

mRNA Isolation—Fresh brain and skeletal muscle from New Zealand White rabbits were removed and frozen rapidly in liquid N\textsubscript{2}. Fresh kidneys from Sprague-Dawley rats and New Zealand White rabbits were removed, dissected to give superficial cortex and papilla, and frozen. To isolate reticulocytes, rabbits (1–1.5 kg) were made anemic (hematocrit 20-23 volume %) by removal of 30–40 ml of blood on days 1, 2, and 3. Rabbits were exsanguinated on day 6 by cardiac puncture at which time the reticuloocyte count was >6%. Whole blood was washed and the red cell/reticuloocyte layer was suspended in buffer A (4 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% sarcosyl, 0.1 mM EDTA, pH 7). Total RNA was isolated by a modification of the method of Sambrook et al. (16). 1–2 g of frozen tissue or 5–10 g of red cells/reticuloocytes in 10 ml of buffer A were homogenized with a Brinkmann Polytron homogenizer for 30–60 s on ice. 1 ml of 2 M sodium acetate (pH 4.4), 10 ml of phenol, 2 ml of chloroform, and 0.04 ml of isomyl alcohol were added to 10 ml of the homogenate. The mixture was incubated for 45 min on ice and spun at 10,000 × g for 30 min. An equal volume of isopropanol was added and RNA was precipitated overnight at −20 °C. The precipitate was spun, resuspended in 0.1 mM EDTA (pH 7.5), and spun at 140,000 × g for 16 h at 25 °C. To obtain mRNA (poly(A\textsuperscript{+}) RNA), the RNA pellet was suspended in elution buffer from the Pharmacia mRNA isolation kit and purified by affinity chromatography on oligo(dT)-cellulose. mRNA was dissolved at a concentration of 1 µg/µl in water and stored at −70 °C.

Microinjection of mRNA in Oocytes—Fully grown oocytes (1.2–1.3 mm diameter, stages 5 and 6) were isolated from adult Xenopus laevis as described (17) and stored in Barth’s buffer (in mM: NaCl, 88; KCl, 1; MgSO\textsubscript{4}, 0.62; Ca(NO\textsubscript{3})\textsubscript{2}, 0.33; CsCl, 0.41; NaHCO\textsubscript{3}, 2.4; HEPES\textsuperscript{1} 10; pH 7.4, 200 mosm) containing benzyl penicillin (10 µg/ml) and streptomycin (10 µg/ml). The follicular cell layer was removed by incubation of oocytes with 2 µg/ml collagenase (type 1A, Sigma) in Barth’s buffer containing 10 mM glucose and 0.5% Sarcosyl, 0.1 mM EDTA, pH 7.

\textsuperscript{1} The abbreviation used is: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

* This work was supported by Grants DK35124, DK39934, and HL42368 from the National Institutes of Health and by a grant from the National Cystic Fibrosis Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Barth’s buffer for 2 h at 25 °C with gentle continuous agitation (18). Oocytes were washed 6 times with 10 ml of Barth’s buffer. 50 nl of water or mRNA in water (1 µg/µl) were microinjected into oocytes using an automatic Eppendorf microinjection system with sterile Femtotips (~1 µm diameter). Oocytes were maintained at 18 °C in Barth’s buffer with daily buffer changes.

(Osmotic Water Permeability Assay) Oocyte water permeability was assayed by a real-time quantitative imaging method as described recently (19). Oocytes were removed from Barth’s buffer and placed in a 20-fold dilution of Barth’s buffer with distilled water (10 mosm) in a temperature-jacketed blown glass chamber. Oocytes were viewed by transmitted monochromatic light (500 nm) on a Nikon diaphot microscope through a 4× objective. The oocyte was imaged on a Cobu CCD camera operating at fixed gain. 512 × 512 8-bit images were recorded on an 80286 computer through Data Translation hardware boards. Relative oocyte volume (V/V0) was calculated in 1- s intervals from the relative oocyte area (A/A0) in the focal plane, V/V0 = (A/A0)0.5. The osmotic water permeability coefficient (Pf cm/s) was calculated from oocyte surface area (S = 0.046 cm2), initial oocyte volume (V0 = 9 × 10-4 cm3), ω1 (18 cm2/mol), and the initial rate of oocyte swelling, d(V/V0)/dt, by the relation,

\[ P_f = \frac{V_0 d(V/V_0)/dt}{[S V_0 (\text{osm}_{\text{in}} - \text{osm}_{\text{out}})]} \tag{1} \]

where osm_{in} is 10 mosm and osm_{out} is 200 mosm. Pf in rabbit red blood cells was measured by 90° light scattering at 500 nm as described previously (12). A 0.5% (v/v) suspension of red cells in phosphate-buffered saline was subjected to a 100 mosm inwardly directed gradient of NaCl in a Hi-Tech SF50 stopped-flow apparatus. In four experiments, Pf in rabbit red cells was 0.035 ± 0.002 cm/s at 25 °C, higher than Pf in human red blood cells (8). These results show that red cells from rabbit have water channels similar to those of human red cells.

RESULTS

Fig. 1 shows the time course of oocyte volume in response to transfer of oocytes from Barth’s buffer to a 20-fold dilution of Barth’s buffer with distilled water at 10 °C. A low assay temperature was used to maximize the relative increase in oocyte Pf due to expression of water channels; the activation energy of the endogeneous lipid pathway for water transport in oocytes is 10.2 kcal/mol (19), whereas that predicted for an exogenous water channel is 2-5 kcal/mol (11). A large osmotic gradient was used to maximize signal-to-noise ratio. In the absence of an osmotic gradient, oocyte volume remained constant for ~60 min under the assay conditions (not shown).

Addition of the pore-forming agent amphotericin B caused a ~20-fold increase in oocyte Pf, indicating that the rate of oocyte swelling is sensitive to the addition or expression of exogenous water channels. The large increase in Pf demonstrates absence of significant unstirred layer effects (20). In oocytes injected with mRNA from kidney papilla and rabbit reticulocyte, the rate of oocyte swelling increased remarkably compared to that in oocytes injected with water or mRNA from brain, a tissue not expected to have water channels. There was a submaximal increase with injection of mRNA from kidney cortex. The data from a series of oocytes in Fig. 1B support this conclusion and provide information about the variability of water channel expression.

To evaluate the characteristics of the expressed water channel in mRNA from rat kidney papilla, effects of the water channel inhibitor HgCl2 and temperature were examined. Fig. 2A shows that HgCl2 decreased the rate of swelling in oocytes injected with mRNA from kidney papilla to a value near that in water-injected oocytes; the inhibition by HgCl2 was reversed partially by the sulfhydryl reagent mercaptoethanol.

Fig. 2B shows the time course of oocyte Pf following injection of mRNA from rat kidney papilla. At an assay temperature of 25 °C, the Pf of water-injected oocytes measured 3 days after injection was 9.2 × 10-4 cm/s. In mRNA-injected oocytes, there was no measurable increase in Pf within 4 h after injection (0 days). Pf began to increase at 1 day and peaked at days 2-4. By comparison of Pf values in Figs. 1B and 2B, it is notable that an increase in assay temperature from 10 to 25 °C increased Pf in water-injected oocytes by a greater factor than in oocytes injected with mRNA from rat papilla.

The data (mean ± S.D.) for a large series of oocytes is summarized in Fig. 3. When assayed at 10 °C, oocyte Pf increased 4.5-fold (rat kidney papilla), 4.0-fold (rabbit kidney papilla), and 5.1-fold (rabbit reticulocyte) over that in water-injected oocytes. There was no significant increase in Pf in oocytes injected with mRNA from brain or muscle. There was a smaller increase with mRNA from kidney cortex. HgCl2 strongly inhibited the increase in oocyte Pf due to injection of mRNA from reticulocyte and kidney papilla. When Pf was assayed at 25 °C, there was a maximal 2.3-fold increase in Pf, giving activation energies for the expressed reticulocyte and kidney water channels of <4 kcal/mol. As above, HgCl2 strongly and reversibly inhibited the Pf of expressed water channels. There was no effect of a permeable CAMP analogue (8-Br-cAMP) on oocyte Pf. There was no effect of 0.25 mM phloretin on Pf of water- or mRNA-injected oocytes (not shown).

DISCUSSION

The purpose of this study was to determine whether heterologous mRNA from cells known to contain specialized water transporters could be expressed measurably in Xenopus oocytes. mRNA from kidney papilla and reticulocyte caused...
a 4-5-fold increase in oocyte water permeability when assayed at 10 °C at 48-72 h after microinjection. Microinjection of water or of mRNA from brain and muscle, tissues thought not to contain water transporters, did not increase water permeability beyond that measured in native oocytes. These results provide strong evidence that the water channel is a protein; however, it cannot be formally ruled out that the mRNA encodes for a protein which catalyzes the synthesis of a specialized lipid component of the water channel.

Because most cells derived from kidney papilla are vasopressin-responsive (21), it is likely that the expressed water transporter is the vasopressin-sensitive water channel. The water transport function expressed in oocytes injected with mRNA from rat papilla was inhibited by HgCl
2 and the inhibition was reversed by mercaptoethanol. These results are in agreement with data for the vasopressin-sensitive water channel from reticulocyte and rat papilla. The increase in oocyte water permeability when assayed at 10 °C at 48-72 h after injection is -15 X 10^{-4} cm/s. Assum- ing a single channel Pr of -10^{-13} cm/s based on a maximum of 10^6 water channels per red cell (9), and an oocyte surface area of 0.046 cm², there are -6 X 10^5 expressed water channels per oocyte. This number might be considerably lower if the single channel Pr is higher.

Fischbarg et al. (24) have proposed that glucose transporters carry water in some cell types. Microinjection of 10-25 ng of pure mRNA coding for glucose transporters gave a small (<1.6-fold) increase in oocyte osmotic water permeability (25). Because -4 X 10^9 glucose transporters were expressed per oocyte, the single channel water permeability of the glucose transporter would probably be less than that of the vasopressin-sensitive red cell and kidney water transporters by several orders of magnitude. The role of glucose transporters in cells where high rates of water transport do not occur remains unclear.

The functional expression of mRNA coding for red cell and kidney water transporters into Xenopus oocytes provides strong evidence that the water channel is a protein suitable for expression cloning. The expressed proteins may serve as pores or channels for water, or they may organize other membrane components, such as lipid, to form a transmembrane conduit for water.

Acknowledgments—We thank Drs. V. Lingappa and E. Sigal at the...
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University of California, San Francisco for advice on mRNA expression cloning in oocytes.

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Expression of mRNA coding for kidney and red cell water channels in Xenopus oocytes.
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