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# Acetazolamide inhibits osmotic water permeability by interaction with aquaporin-1

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**Abstract**

Water channel proteins, known as aquaporins, are transmembrane proteins that mediate osmotic water permeability. In a previous study, we found that acetazolamide could inhibit osmotic water transportation across *Xenopus* oocytes by blocking the function of aquaporin-1 (AQP1). The purpose of the current study was to confirm the effect of acetazolamide on water osmotic permeability using the human embryonic kidney 293 (HEK293) cells transfected with pEGFP/AQP1 and to investigate the interaction between acetazolamide and AQP1. The fluorescence intensity of HEK293 cells transfected with pEGFP/AQP1, which corresponds to the cell volume when the cells swell in a hyposmotic solution, was recorded under confocal laser fluorescence microscopy. The osmotic water permeability was assessed by the change in the ratio of cell fluorescence to certain cell area. Acetazolamide, at concentrations of 1 and 10 μM, inhibited the osmotic water permeability in HEK293 cells transfected with pEGFP/AQP1. The direct binding between acetazolamide and AQP1 was detected by surface plasmon resonance. AQP1 was prepared from rat red blood cells and immobilized on a CM5 chip. The binding assay showed that acetazolamide could directly interact with AQP1. This study demonstrated that acetazolamide inhibited osmotic water permeability through interaction with AQP1.


*Keywords:* Acetazolamide; Aquaporin-1; Osmotic water permeability; Transfected cell; Interaction; Surface plasmon resonance

The water channel proteins, namely aquaporins (AQPs),[1] are channel-forming transmembrane glycoproteins originally discovered due to their abilities to mediate water entry or release through cell membranes driven by a transmembrane osmotic gradient [1,2]. Recent studies have implicated AQPs in many illnesses, including kidney dysfunction, brain edema, cirrhosis, congestive heart failure, glaucoma, preeclampsia, starvation, and arsenic toxicity [3,4]. To further elucidate the exact mechanism of AQPs' involvement in these diseases and to investigate the function of water channels, specific and high-affinity inhibitors are necessary. Mercury compounds, known to have an affinity for AQPs, traditionally have been used to test for the presence of AQPs in plants and animals. Other studies

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[1] *Abbreviations used:* AQP, aquaporin; TEA, tetraethylammonium; AQP1, aquaporin-1; CHIP28, channel-like integral protein, 28 kDa; BIA, biomolecular interaction analysis; SPR, surface plasmon resonance; HEK293, human embryonic kidney 293; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GFP, green fluorescent protein; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethyl sulfonyl fluoride; DEAE, diethylaminoethyl; T-TBS, blocking buffer containing 5% nonfat dry milk and 0.05% Tween 20 in Tris-buffered saline; SDS, sodium dodecyl sulfate; NHS, *N*-hydroxysuccinimide; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; HBS–EP, running buffer containing 10 mM Hepes, 150 mM NaCl, 3 mM EDTA, and 0.005% surfactant P-20; DMSO, dimethyl sulfoxide; ANOVA, analysis of variance; RU, response units.

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have tested tetraethylammonium (TEA) and silver compounds as potential inhibitors for AQPs [5]. However, these compounds are notoriously toxic and/or nonspecific at the high concentrations to be used. So far, there is no effective inhibitor for AQPs.

Acetazolamide, a carbonic anhydrase inhibitor, exerts its inhibitory activities through direct binding with $Zn^{2+}$ and by forming the carbonic anhydrase–acetazolamide complex [6]. A previous study in our laboratory demonstrated that acetazolamide inhibited osmotic water permeability through aquaporin-1 (AQP1) expressed in *Xenopus* oocytes [7]. AQP1, formerly CHIP28 (channel-like integral protein, 28 kDa), is the best characterized one among the 13 members of the mammalian AQP family [8,9]. However, the mechanism whereby acetazolamide inhibits water permeability through AQP1 still was unknown.

Biomolecular interaction analysis (BIA), a biosensor technology based on the principle of surface plasmon resonance (SPR), has been shown to be a powerful and well-established tool for investigating the binding behavior of biomolecules [10,11]. It offers several advantages over traditional interaction analysis techniques [12,13], including free label and real-time detection. In addition, this analytical technique can provide quantitative information such as kinetic parameters and equilibrium constants for complex formation [14].

The relevance of acetazolamide as an AQP1 channel blocker outside the *Xenopus* expression system has not been examined. There is always the possibility that differences may exist in the properties of a membrane channel protein expressed in oocytes as compared with those in mammalian cells [15]. Therefore, in the current study, we investigated the effect of acetazolamide on osmotic water permeability in human embryonic kidney 293 (HEK293) cells transfected with AQP1 and investigated the direct binding between acetazolamide and AQP1 using SPR. Our results indicated that acetazolamide inhibited osmotic water permeability through AQP1. This inhibition may be achieved through direct binding between acetazolamide and AQP1.

## Materials and methods

### *Determination of osmotic water permeability*

The determination of osmotic water permeability was performed using HEK293 cells stably transfected with pEGFP/AQP1 according to the method described previously [16]. In brief, HEK293 cells stably transfected with pEGFP/AQP1 or pEGFP (named HEK293/AQP1 or HEK293/vector cells, respectively), which have been constructed in our laboratory, were plated in the middle of confocal wells in Dulbecco's modified Eagle's medium nutrient mixture (DMEM, Sigma Chemical, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere with 5% $CO_2$ for 24 h. The cultures were treated with acetazolamide (1, 10, or 100 μM with purity >99%, Sigma Chemical), 10 μM $HgCl_2$, or control solution for 30 min at room temperature. Osmotic water permeability was determined by the time course of the change in cell fluorescence intensity in response to a hypotonic solution by a twofold dilution of extracellular DMEM with distilled water under a confocal laser scanning microscope (LSM410, Zeiss, Oberkochen, Germany) equipped with a Krypton–Argon laser using the 100× objective lens. Green fluorescent protein (GFP) fluorescence was excited at 488 nm and detected using a 515- to 565-nm bandpass filter. The fluorescence intensity of a single cell was scanned every 0.6 s for 2 min. A certain area of the cell was selected as the investigational target, and a polygon was drawn onto the digitized image. The change of fluorescence intensity in the selected area was recorded. Image reconstructions were done with Zeiss LSM software.

Osmotic water permeability was calculated by assessing the maximal slope of the time course of relative fluorescence intensity in a certain area of the cell. To eliminate the variance in the amount of initial fluorescence intensity, relative fluorescence intensity was used to evaluate cell volume change in response to the hypotonic stimulation and was expressed as $F_t/F_o$, where $F_o$ is the initial fluorescence intensity of the cell area and $F_t$ is the real-time fluorescence intensity of the same cell area after cell swelling. Thus, a rise in $F_t/F_o$ represents a decrease in cell volume, whereas a drop in $F_t/F_o$ indicates an increase in cell volume.

### *AQP1 preparation from rat red blood cell*

Seven male Sprague–Dawley rats weighing 250–300 g were used. They were sacrificed with an overdose of pentobarbital (100 mg/kg, i.m.). Animal handling and study protocols were reviewed and approved by animal care and use review committees at Peking University Health Science Center. The entire procedure of AQP1 preparation was conducted at 4 °C or on ice unless otherwise indicated. Erythrocytes obtained by cardiac puncture were washed in a 10-fold volume of 0.9% sodium chloride by centrifugation at 320*g* for 10 min three times.

AQP1 was prepared based on the previous methods with some modifications [17]. Erythrocytes were incubated for 1 h with a 10-fold volume of hypotonic lysis solution [18] containing 7.5 mM phosphate buffer (pH 7.5), 1 mM ethylenediaminetetraacetic acid (EDTA)–Na, and 20 μg/ml phenylmethyl sulfonyl fluoride (PMSF), with 2 μg/ml pepstain A added just before use, and were centrifuged at 10,000*g* for 10 min. The pellet was suspended in the solution, 2-fold of the initial erythrocyte volume, containing 1% (w/v) sodium *N*-lauroylsarcosine, 1 mM $NH_4HCO_3$, 1 mM $NaN_3$, 1mM dithiothreitol, and 0.5 mM PMSF, and was incubated for 2 h. The suspended solution was saturated with ammonium sulfate for 2 h and centrifuged for 10 min at 8000*g*.

The pellet was dissolved with diethylaminoethyl (DEAE) buffer, containing 1% (v/v) Triton X-100, 20 mM Tris–HCl (pH 7.8), 1 mM $NaN_3$, and 1 mM dithiothreitol, and was filtered through a 0.22-μm filter and then loaded