Dual Functional Characteristic of Human Aquaporin 10 for Solute Transport

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Key Words

Aquaporin 10 • Glycerol • Urea • Channel • Carrier • Transporter

Abstract

Background/Aims: Although aquaglyceroporins have been generally believed to operate in a channel mode, which is of nonsaturable nature, for glycerol as well as for water, we recently found that human aquaporin 9 (hAQP9) operates in a carrier-mediated mode, which is of saturable nature, for glycerol. Based on the finding, we assumed that such a characteristic might be shared by the other aquaglyceroporins and examined the functional characteristics of hAQP10, which is an intestine-specific aquaglyceroporin. **Methods:** Transport assays were conducted using *Xenopus laevis* oocytes expressing hAQP10 derived from the microinjected cRNA. **Results:** The transport of glycerol by hAQP10 was found to be highly saturable with a Michaelis constant of 10.4 μ M and specifically inhibited by several glycerol analogs such as monoacetin. Furthermore, when glycerol was preloaded in hAQP10-expressing oocytes, its efflux was *trans*-stimulated by extracellular glycerol. These results indicate the involvement of a carrier-mediated mechanism in glycerol transport by hAQP10.

Interestingly, a channel mechanism was also found to be involved in part in hAQP10-mediated glycerol transport. **Conclusion:** The present study unveiled the uniquely dual functional characteristic of hAQP10 as a carrier/channel for solute transport, providing a novel insight into its operation mechanism, which would help further elucidate its physiological role.

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Introduction

It is known that aquaporins (AQPs), which are primarily characterized as water channel proteins, include a group of members (AQP3, AQP7, AQP9 and AQP10) that can also transport glycerol and are called aquaglyceroporins [1-4]. Although they are of great interest for their roles in the disposition of glycerol [3], which is involved in various physiological and pathological processes as an intermediate of energy metabolism [5], the characteristics of their glycerol transport functions have little been clarified, being generally believed to operate in a channel mode, which is of nonsaturable nature, for glycerol as well as for water. However, we

recently found that human AQP9 (hAQP9), which is mainly expressed in the liver, transports glycerol in a highly saturable manner, indicating the involvement of a carrier-mediated mechanism rather than a channel mechanism [6]. The saturability of transport is generally assumed to be characteristic of a carrier-mediated mechanism that involves binding of a substrate to the recognition site and its subsequent translocation across the membrane by conformational change of the transporter. To the contrary, transport through a channel is assumed not to be saturable, as it does not involve such translocation. Consistent with the indication of the involvement of a carrier-mediated mechanism, hAQP9-mediated glycerol transport was found to be inhibited by glycerol analogs such as monoacetin and monobutyrin, indicating potential competition in substrate binding. This carrier-mediated mechanism of hAQP9 was in addition found not to require Na⁺ for operation, suggesting that it may be of facilitative type.

Based on the finding on hAQP9, we assumed that the carrier-mediated characteristic in glycerol transport might be shared by the other aquaglyceroporins. AQP10 is an aquaglyceroporin mainly expressed in the small intestine and one of those with little information about glycerol transport function [7, 8]. Although its apically localized expression in the intestinal epithelial cells may indicate its involvement in the intestinal uptake of glycerol and also some other small molecular solutes [9], its physiological role have not been fully clarified yet. Therefore, in the present study, we examined the functional characteristics of its human ortholog (hAQP10), using the *Xenopus laevis* oocyte expression system. Clarifying its functional characteristics would help further elucidate its physiological role.

Materials and Methods

Chemicals

[2-³H]Glycerol (37.0 GBq/mmol) was obtained from GE Healthcare (Little Chalfont, Buckinghamshire, UK) and [¹⁴C]urea (2.07 GBq/mmol) was from Moravsek Biochemicals (Brea, CA, USA). Unlabeled glycerol and urea were obtained from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were of analytical grade and commercially available.

Isolation of hAQP10 cDNA

The cDNA of hAQP10 was cloned from the human small intestine total RNA (Clontech, Mountain View, CA, USA) by reverse transcription and subsequent polymerase chain reaction (PCR). In brief, a reverse transcription reaction was carried out using 3 µg of the total RNA, an oligo(dT) primer and

ReverTra Ace (Toyobo, Tokyo, Japan) as a reverse transcriptase. The cDNA of hAQP10 was isolated from the human small intestine cDNA mixture by PCR amplification using KOD plus polymerase (Toyobo) and the following primers: forward primer, 5'-GTC AAG CTC CCC ATT TAC ATC T-3'; reverse primer, 5'-TGA GTG AAG TGA GGG TTG TCC-3'. These primers were designed based on the sequence in GenBank (accession number, AB066105). PCR was performed using the following conditions: 94°C for 2 min; 32 cycles of 1) 94°C for 20 s, 2) 55°C for 20 s, and 3) 72°C for 1 min. The amplified cDNA product was subcloned into pTA2 vector (Toyobo). The coding sequence of hAQP10 was transferred into pGH vector [10] at the *EcoRI* and *HindIII* sites. The sequence of the amplified cDNA product was determined with an automated sequencer (ABI PRISM 3100; Applied Biosystems, Foster City, CA, USA) and confirmed to be identical to that in GenBank.

Expression of hAQP10 in *Xenopus laevis* oocytes

The capped cRNA of hAQP10 was synthesized from the pGH vector carrying hAQP10 cDNA, using AmpliCap-MAX T7 High Yield Message Maker kit (Epicentre Biotechnologies, Madison, WI, USA). Stage V and VI oocytes were isolated from mature female *Xenopus laevis* toads by treatment with 2 mg/ml collagenase (type IA; Sigma-Aldrich, St. Louis, MO, USA) at 18°C for 2 h in Ca²⁺-free ND96 solution (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, and 5 mM HEPES, pH 7.4) and then maintained in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES, pH 7.4). The oocytes were microinjected either with the cRNA of hAQP10 (5 ng/50 nl) or an equivalent amount of water and maintained at 18°C for 2 days in ND96 solution supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml).

Transport experiments in *Xenopus laevis* oocytes expressing hAQP10

Uptake assays were started by placing oocytes into 0.25 ml of ND96 solution containing [³H]glycerol (0.4 µM) or [¹⁴C]urea (2.4 µM) on 24-well plates at 25°C. Unlabeled glycerol and urea, for adjusting the concentration, and inhibitors were added to the solution, as required. NaCl in the solution was substituted with KCl, choline Cl, Na gluconate or mannitol in experiments to examine the effect of extracellular ions. Assays were stopped by transferring each oocyte into ice-cold ND96 solution (2.5 ml) in a counting vial, and the oocytes were washed twice with 2.5 ml of the same solution. Each oocyte was transferred to another counting vial and solubilized in 0.5 ml of 0.2 M NaOH solution containing 0.5% SDS at room temperature for 1 h, and the associated radioactivity was determined by liquid scintillation counting, using 3 ml Clear-sol I (Nacalai Tesque, Kyoto, Japan) as a scintillation fluid, for the evaluation of uptake. Oocytes injected with water were used to determine the basal transport activity in the absence of hAQP10 for control. The specific uptake of each substrate by hAQP10 was estimated by subtracting the basal uptake from the uptake in hAQP10-expressing oocytes.

In experiments to examine *trans* stimulation of glycerol transport, oocytes were microinjected with 50 nl of ND96 solution containing [³H]glycerol (10 µM) and placed in ND96

solution (0.25 ml) with or without unlabeled glycerol (2 mM) on 24-well plates at 25°C for the initiation of the assays of [³H]glycerol efflux. Assays were stopped by transferring each oocyte into ice-cold ND96 solution (2.5 ml) in a counting vial. The oocyte was briefly rinsed in the solution and then transferred to another counting vial for solubilization and liquid scintillation counting of the remaining radioactivity by the procedure described above for uptake assays. The amount of [³H]glycerol that disappeared from the oocyte was estimated for the evaluation of its efflux.

Data analysis

The uptake rate (v) was calculated by dividing the uptake by time during the initial uptake phase (30 min), where uptake was in proportion to time. For both glycerol and urea, the amount in the fluid adhering to the oocyte and that initially adsorbed to the oocyte were negligible. The hAQP10-specific uptake was estimated by subtracting the uptake in water-injected oocytes, though only minimal, from that in hAQP10-expressing oocytes.

The uptake clearance (CL_{up}) was calculated by dividing v by the substrate concentration (C). The hAQP10-specific uptake of glycerol consisted of a saturable component, which conformed to the Michaelis-Menten kinetics, and a nonsaturable component. The expression of CL_{up} for that is as follows: $CL_{up} = V_{max}/(K_m + C) + CL_{ns}$. The kinetic parameters of maximum transport rate (V_{max}), the Michaelis constant (K_m) and the clearance of nonsaturable transport (CL_{ns}) were estimated by fitting this equation to the experimental data profile of CL_{up} vs. C by using a nonlinear least-squares regression analysis program, WinNonlin (Pharsight, Mountain View, CA, USA), and the reciprocal of variance as the weight. The parameters are presented as computer-fitted ones with SE. The plot of CL_{up} vs. logarithmic C was used because it was more suitable to handle data for a wider concentration range that was needed in the data analysis. The CL_{up} represents simply v normalized to C and, hence, its reduction with an increase in C means that an increase in v is less than proportional to C , indicating saturable nature of transport.

Experimental data are presented as means $\pm$ SE. Statistical analysis was performed by using Student's t -test or, when multiple comparisons were needed, ANOVA followed by Dunnett's test, with $P < 0.05$ considered significant.

Results

Kinetic characteristics of glycerol transport by hAQP10

We first examined the time courses of the uptake of glycerol at a trace concentration of 0.4 μ M and a higher concentration of 20 μ M (Fig. 1). At the both concentrations, the uptake of glycerol was much greater in oocytes injected with the cRNA of hAQP10 than in control oocytes injected with water, indicating the prevailing contribution of efficient glycerol uptake that

Fig. 1. Time courses of the uptake of glycerol in *Xenopus laevis* oocytes expressing hAQP10. Data represent means $\pm$ SE ($n = 5$ or 6). The uptake of glycerol was evaluated at 25°C and pH 7.4 at its concentration of 0.4 μ M ($\bullet$, $\circ$) and 20 μ M ($\blacktriangle$, $\triangle$) in hAQP10-expressing oocytes ($\bullet$, $\blacktriangle$) and water-injected control oocytes ($\circ$, $\triangle$).

Fig. 2. Concentration dependence of glycerol uptake by hAQP10 expressed in *Xenopus laevis* oocytes. Data represent means $\pm$ SE ($n = 6$). The specific uptake of glycerol was evaluated at 25°C and pH 7.4 for 30 min. The V_{max} , K_m , and CL_{ns} are 0.748 ± 0.192 pmol/min/oocyte, 10.4 ± 3.7 μ M and 0.00890 ± 0.00020 μ l/min/oocyte, respectively, as the computer-fitted parameters with SE ($n = 9$). A plot in the format of the Michaelis-Menten equation, shown in the inset, was also constructed using the rate of carrier-mediated uptake (v_c) estimated by subtracting the rate of nonsaturable transport ($CL_{ns} \times C$) from the total uptake rate ($v = CL_{up} \times C$). The data points for higher concentrations (1 and 10 mM) were excluded from the plot because the clearances of total uptake were comparable with those of nonsaturable transport, indicating that the contributions of the carrier-mediated uptakes were negligibly small. The solid lines represent the computer-fitted profiles.

specifically involves hAQP10 derived from the cRNA in the former, and the uptake increased in proportion to time at least up to 30 min. We, therefore, set a 30-min uptake

Fig. 3. Effect of various compounds on glycerol uptake by hAQP10 expressed in *Xenopus laevis* oocytes. Data represent means $\pm$ SE ($n = 5$ or 6). The specific uptake of glycerol ($0.4 \mu\text{M}$) was evaluated at 25°C and pH 7.4 for 30 min in the presence or absence of a test compound. The control value was 1.07 pmol/oocyte . * Significantly different from the control at $P < 0.05$. G3P, glycerol-3-phosphate.

Fig. 4. Effect of various alcohols on glycerol uptake by hAQP10 expressed in *Xenopus laevis* oocytes. Data represent means $\pm$ SE ($n = 5$). The specific uptake of glycerol ($0.4 \mu\text{M}$) was evaluated at 25°C and pH 7.4 for 30 min in the presence of an alcohol (10 mM) or in its absence. The control value was 1.07 pmol/oocyte . * Significantly different from the control at $P < 0.05$.

period in subsequent experiments for the evaluation of the hAQP10-specific transport of glycerol in the initial uptake phase. It is notable that glycerol uptake normalized to the concentration was reduced at the higher concentration of $20 \mu\text{M}$ in hAQP10-expressing oocytes, indicating the involvement of a saturable mechanism in hAQP10-specific glycerol transport.

We then examined the concentration dependence of the hAQP10-specific uptake of glycerol in more detail to characterize the saturable mechanism. As shown in Fig. 2, the clearance of hAQP10-specific glycerol uptake decreased extensively with an increase in concentration, conforming to the Michaelis-Menten kinetics with a V_{max} of $0.748 \text{ pmol/min/oocyte}$ and a K_m of $10.4 \mu\text{M}$. It is notable that a nonsaturable transport component, which was minor but significant with the clearance of $0.00890 \mu\text{l/min/oocyte}$, was also observed. This finding indicates that hAQP10 functions not only as a carrier, which mediates transport in a saturable manner, but also as a channel, which provides a nonsaturable transport pathway. Thus, interestingly, hAQP10 was suggested to have dual mechanisms for glycerol transport. The kinetic analysis indicated that the carrier-mediated mechanism is prevailing at around the lowest concentration of $0.4 \mu\text{M}$, where the clearance of carrier-mediated transport can be approximated with V_{max}/K_m ($0.0719 \mu\text{l/min/oocyte}$) and about 10 times greater than the CL_{ns} . The channel mechanism was, on the other hand, indicated to be prevailing at around 1 mM or higher. The carrier-mediated

uptake rate, which was estimated by subtracting the rate of nonsaturable uptake from the total uptake rate, was also inspected and confirmed to obey the Michaelis-Menten equation (Fig. 2), supporting the kinetic analysis.

Effect of various compounds on glycerol transport by hAQP10

A carrier-mediated mechanism can be inhibited by some compounds analogous to the primary substrate by competition, whereas such a competitive inhibition would not be expected for a channel mechanism. To examine this aspect and also the structural requirements to be recognized by the carrier-mediated mechanism, we assessed the effect of various compounds on the hAQP10-specific transport of glycerol at its concentration of $0.4 \mu\text{M}$, where the carrier-mediated mechanism, according to the kinetic analysis, accounts for about 90% of total uptake and operates in a manner most efficient and sensitive to competitive inhibitors. The results are shown in Figs. 3 and 4 and the chemical structures of glycerol and tested compounds are shown in Fig. 5. As shown in Fig. 3, monoacetin and monobutylin, which are glycerol esters, inhibited the hAQP10-specific uptake of glycerol most potently, and diglycerol, an ether type of glycerol derivative, also did potently. However, the inhibitory effects of glycerol-3-phosphate, a glycerol derivative, and chloramphenicol, a drug having a glycerol-like moiety in its molecule, were only minimal or insignificant. Urea and theophylline, which are not

structurally related to glycerol, were found not to inhibit the hAQP10-specific uptake. Among various alcohols (Fig. 4), only 1,2-ethanediol (ethylene glycol), 1,2-propanediol (propylene glycol) and S-(+)-1,2-propanediol inhibited the hAQP10-specific uptake. Thus, it was found that some of glycerol derivatives and polyols analogous to glycerol can specifically inhibit the hAQP10-specific uptake of glycerol, indicating their possible competition with glycerol for hAQP10 in its operation in the carrier-mediated mode.

Notably, although inhibitions by monoacetin, monobutylin, diglycerol and S-(+)-1,2-propanediol were extensive at their concentration of 10 mM, there still remained about 10% of the control uptake persistently. Taking it into account that the remaining uptake was comparable in clearance with the nonsaturable transport component found in the kinetic analysis, they could be equivalent and represent transport by the channel mechanism, which can be neither saturated by glycerol nor inhibited by glycerol analogs. Particularly for monoacetin and monobutylin, the half inhibition concentrations (IC_{50}) would be estimated to be smaller than 0.1 mM because the percentages of inhibition were greater than 50% at their concentration of 0.1 mM. Assuming competition for saturable transport by the carrier-mediated mechanism, these glycerol derivatives would be able to inhibit carrier-mediated glycerol transport almost completely at their concentration of 10 mM, which would be more than 100 times greater than the IC_{50} values. Thus, the remaining uptake would most likely represent the transport that cannot be inhibited by these glycerol derivatives. This is consistent with the characteristic expected for the channel mechanism.

Effect of extracellular ions on glycerol transport by hAQP10

The hAQP10-specific uptake of glycerol (0.4 μ M) was not altered when NaCl in the medium was substituted with KCl, choline Cl or mannitol (Table 1), indicating that the carrier-mediated mechanism as well as the channel mechanism does not require Na^+ for operation. The insignificant effect of mannitol as a substitute for NaCl to eliminate Cl^- as well as Na^+ indicates that Cl^- is not involved in hAQP10 operation, either. Although an alternative substitution of NaCl with Na gluconate to eliminate Cl^- caused a slight but significant reduction in hAQP10-specific glycerol uptake, it may suggest a weak sensitivity of hAQP10 to gluconate rather than Cl^- requirement in hAQP10 operation. At least, it is apparent that Cl^- is not an indispensable element for hAQP10

Fig. 5. Chemical structures of glycerol and various compounds tested for their inhibitory effect on glycerol transport by hAQP10.

Medium condition	Uptake (% of control)
NaCl (control)	100.0 $\pm$ 2.4
KCl	115.8 $\pm$ 7.8
Choline Cl	103.1 $\pm$ 4.0
Na gluconate	76.7 $\pm$ 4.3*
Mannitol	86.5 $\pm$ 4.1

Table 1. Effect of extracellular ions on glycerol uptake by hAQP10 expressed in *Xenopus laevis* oocytes. Data represent means $\pm$ SE ($n = 4-6$). The specific uptake of glycerol (0.4 μ M) was evaluated at 25°C and pH 7.4 for 30 min. NaCl in ND96 solution was substituted as indicated. The control value was 1.07 pmol/oocyte. * Significantly different from the control at $P < 0.05$.

operation. Furthermore, hAQP10-specific glycerol uptake was found not to be altered when pH was lowered from pH 7.4 to pH 5.5 (0.727 ± 0.033 and 0.618 ± 0.094 pmol/oocyte, respectively, at pH 7.4 and pH 5.5 as means $\pm$ SE, $n = 5$), indicating that H^+ does not have any significant influence on hAQP10 operation. All these results suggest that the carrier-mediated mechanism may be of facilitative type.

Trans stimulation of glycerol transport by hAQP10

To further gain an evidence for the carrier-mediated mechanism suggested for glycerol transport by hAQP10, we examined the *trans* effect of unlabeled glycerol on the transport of [^3H]glycerol. As shown in Fig. 6, only in hAQP10-expressing oocytes, the efflux of [^3H]glycerol after its injection (10 μM , 50 nl) into oocytes was stimulated by unlabeled glycerol (2 mM) in the extracellular medium. This *trans* stimulation indicates an increased availability of hAQP10 for [^3H]glycerol efflux due to its enhanced turnover caused by the influx of unlabeled glycerol (counter transport effect). It is generally assumed that this phenomenon involves translocation cycle of the substrate binding site between the positions facing the outside and inside of the membrane and, hence, can be expected for carrier-mediated mechanisms, which involve such translocation cycle, but not for channel mechanisms, which lack that. This finding strongly supports the suggested involvement of a carrier-mediated mechanism in hAQP10-mediated glycerol transport.

Characteristics of urea transport by hAQP10

Among those which did not show a significant effect on glycerol uptake and, therefore, did not seem to be recognized by the carrier-mediated mechanism, urea was of particular interest. Because this compound has been reported to be permeable through AQP10 [8], we assumed that it might be transported only by the channel mechanism. As shown in Table 2, urea was indeed found to be transported by hAQP10 in a nonsaturable manner, as indicated by the concentration independence of the uptake/concentration, being about 0.25 $\mu\text{l}/\text{oocyte}$ for the uptake period of 30 min. Furthermore, the specific uptake of urea at a trace concentration of 2.4 μM was not inhibited by glycerol at a high concentration of 10 mM (0.317 ± 0.015 and 0.323 ± 0.013 $\mu\text{l}/\text{oocyte}$, respectively, as the uptake/concentration in the absence and presence of glycerol, $n = 5$), indicating that urea does not share the carrier-mediated mechanism that is supposed to be completely inhibited by the high concentration of glycerol as the primary substrate with a high affinity. Thus, urea was suggested to be transported only by the channel mechanism, as we assumed. Finally, the specific uptake of urea was comparable with that of glycerol by the channel mechanism (0.277 ± 0.009 $\mu\text{l}/\text{oocyte}$ as the uptake/concentration estimated at 10 mM glycerol, $n = 6$), indicating no major difference in selectivity between these two solutes.

Fig. 6. *Trans* stimulation of [^3H]glycerol efflux from *Xenopus laevis* oocytes expressing hAQP10. Data represent means $\pm$ SE ($n = 6$). The efflux of [^3H]glycerol in 5 min was evaluated in the presence of unlabeled glycerol (2 mM) in the extracellular medium or in its absence after its microinjection (10 μM , 50 nl) into the oocytes. * Significantly different from the value in the absence of extracellular glycerol in oocytes without hAQP10 at $P < 0.05$; † Significantly different from the value in the absence of extracellular glycerol in hAQP10-expressing oocytes at $P < 0.05$.

Concentration (mM)	Uptake/concentration ($\mu\text{l}/\text{oocyte}$)
2.4×10^{-3}	0.252 ± 0.019
1	0.274 ± 0.016
10	0.249 ± 0.014

Table 2. Concentration independence of urea uptake by hAQP10 expressed in *Xenopus laevis* oocytes. Data represent means $\pm$ SE ($n = 4$ or 5). The specific uptake of urea was evaluated at 25°C and pH 7.4 for 30 min.

Discussion

It was successfully demonstrated that hAQP10-mediated glycerol transport involves a carrier-mediated mechanism, as we assumed that the mechanism previously found for hAQP9 [6] might be shared by the other aquaglyceroporins. hAQP10-mediated glycerol transport was highly saturable (Fig. 2) and suggested to be specific to glycerol and analogous compounds (Figs. 3 and 4), as found for hAQP9-mediated one. hAQP10 was also similar to hAQP9 in operating in a Na^+ -independent manner (Table 1), indicating that the carrier-mediated mechanism might be of facilitative type. As an additional and stronger evidence for the suggested carrier-mediated type of mechanism, we were successful in observing *trans*

stimulation of glycerol transport in the present study (Fig. 6). It is notable, however, that transport by a channel mechanism, which is nonsaturable and was not observed for hAQP9, was suggested to be additionally involved in hAQP10-specific glycerol uptake (Fig. 2). Thus, hAQP10 was suggested to be unique in operating by dual mechanisms. Furthermore, only the channel mechanism was suggested to be shared by urea, based on the nonsaturable and glycerol-insensitive characteristics of its hAQP10-specific transport (Table 2). The channel mechanism was also suggested to recognize glycerol and urea similarly without a major difference in selectivity. It may be likely that the channel mechanism is less strict in recognizing substrates and provides a pathway of transport for some more wide variety of solutes than the carrier-mediated mechanism. The structural feature of hAQP10 behind the dual functional characteristic should be of interest. A possibility is that each hAQP10 molecule might have dual sites, one for recognizing glycerol and analogs to induce the conformational change for the carrier-mediated process and the other for sensing glycerol and urea as well as water to introduce them into the channel pathway. Alternatively, hAQP10 molecules for the channel mode and those for the carrier-mediated mode might be in conformational equilibrium. hAQP10 could be present as tetramers in the plasma membrane, as suggested for hAQP1 [1, 11, 12]. Although it has also been suggested that the monomers could function independently in the case of hAQP1, hAQP10 monomers may operate interactively to manifest the dual functional characteristic.

The glycerol analogs that inhibited hAQP10-specific glycerol uptake all have a 1,2-diol structure with 2- or 3-carbon backbone (Figs. 3 and 4). This characteristic, which can be typically found in 1,2-ethanediol and 1,2-propanediol, is also shared by monoacetin, monobutyrin and diglycerol, which are all modified at the hydroxyl group attached to the 3rd position of the glycerol-derived carbon backbone. However, those which did not inhibit hAQP10-specific glycerol uptake do not have such a structure, with an only exception of R-(-)-1,2-propanediol. Therefore, the 1,2-diol structure with 2- or 3-carbon backbone may play an important role in recognition by hAQP10 in its operation in the carrier-mediated mode. It is also notable that the inhibition by S-(+)-1,2-propanediol was more potent than that by R-(-)-1,2-propanediol, indicating an enantioselective characteristic in recognition by hAQP10. All these characteristics were also similar to those previously found for the carrier-mediated mechanism of hAQP9 [6], although 1,2-ethanediol and

R-(-)-1,2-propanediol inhibited hAQP9 some more potently as minor differences.

The K_m of hAQP10 for glycerol (10.4 μ M) was comparable with that of hAQP9 (9.2 μ M). With the small K_m relatively close to the glycerol level in the systemic circulation, which could be as low as several tens of μ M [13], the carrier-mediated mechanism may be effective in its operation for cellular uptake of glycerol from the systemic circulation, as expected for hAQP9 in the liver. However, the presence of hAQP10 has not been reported in any major organ other than the small intestine. It is reported to be localized to the brush border membrane in the small intestine and, hence, presumed to be involved in glycerol uptake from the lumen [7-9]. However, the concentration of glycerol liberated from dietary fat (triglycerides) could be as high as mM level in the intestinal lumen. At such high concentrations, the contribution of the carrier-mediated mechanism would be reduced to be almost negligible in its operation for glycerol transport and the channel mechanism would be the major mechanism for that. Thus, the role of the carrier-mediated mechanism of hAQP10 is unclear. It may work in the distal part of the small intestine in picking up glycerol remaining at low concentrations after being mostly absorbed in the proximal part. But future studies will be necessary to clarify its role in glycerol absorption or, if any, in some other aspects of glycerol disposition in the other organs.

Glycerol uptake in the rat small intestine involves a carrier-mediated mechanism and a nonsaturable mechanism, as we reported previously [14, 15]. However, the carrier-mediated mechanism in the rat intestine is quite different from that of hAQP10 in that it is Na^+ -dependent and has a much lower affinity (K_m of 0.77 mM) for glycerol. The K_m is rather close to the glycerol level expected in the intestinal lumen, indicating that the mechanism could be effectively involved in glycerol uptake. The intestinal carrier-mediated mechanism is also different from hAQP10 in that it is not very sensitive to polyols, including 1,2-ethanediol and 1,2-propanediol, to which hAQP10 is sensitive. Thus, the transporter involved in the intestinal carrier-mediated uptake of glycerol seems to be different from AQP10. On the other hand, the clearance of glycerol transport by the nonsaturable mechanism is comparable with that of urea transport, which is nonsaturable, in the rat intestine [15]. It is in agreement with the characteristic found for the channel mechanism of hAQP10, indicating a possibility that it may be responsible for that. We also indicated the presence of an unidentified glycerol transporter in the HCT-15

human colon cancer cell line [16, 17]. However, the transporter is also different from hAQP10 in its Na⁺ dependence, although it is similar to hAQP10 with regard to K_m (15 μ M for glycerol) and specific sensitivity to glycerol derivatives, such as monoacetin, monobutylin and diglycerol, and to 1,2-ethanediol and 1,2-propanediol among polyols.

In conclusion, hAQP10 was found to operate in a carrier-mediated mode, which is Na⁺-independent and may be of facilitative type, as well as in a channel mode. The carrier-mediated mechanism was suggested to be specific to glycerol and analogous compounds, whereas the channel mechanism was suggested to be less strict in substrate recognition, recognizing urea, which was not recognized by the carrier-mediated mechanism, as well

as glycerol. Thus, the present study unveiled the uniquely dual functional characteristic of hAQP10 as a carrier/channel for solute transport, redefining the functional characteristic of this protein, which has been almost implicitly presumed to mediate solute transport solely in a nonsaturable manner as a member of AQP channels, and providing a novel insight into its operation mechanism, which would help further elucidate its physiological role.

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References

- Agre P, Bonhivers M, Borgnia MJ: The aquaporins, blueprints for cellular plumbing systems. *J Biol Chem* 1998;273:14659-14662.
- Gomes D, Agasse A, Thiébaud P, Delrot S, Gerós H, Chaumont F: Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. *Biochim Biophys Acta* 2009;1788:1213-1228.
- Hibuse T, Maeda N, Nagasawa A, Funahashi T: Aquaporins and glycerol metabolism. *Biochim Biophys Acta* 2006;1758:1004-1011.
- Morishita Y, Sakube Y, Sasaki S, Ishibashi K: Molecular mechanisms and drug development in aquaporin water channel diseases: aquaporin superfamily (superaquaporins): expansion of aquaporins restricted to multicellular organisms. *J Pharmacol Sci* 2004;96:276-279.
- Brisson D, Vohl M-C, St-Pierre J, Hudson TJ, Gaudet D: Glycerol: a neglected variable in metabolic processes? *Bioessays* 2001;23:534-542.
- Ohgusu Y, Ohta K, Ishii M, Katano T, Urano K, Watanabe J, Inoue K, Yuasa H: Functional characterization of human aquaporin 9 as a facilitative glycerol carrier. *Drug Metab Pharmacokinet* 2008;23:279-284.
- Hatakeyama S, Yoshida Y, Tani T, Koyama Y, Nihei K, Ohshiro K, Kamiie J, Yaoita E, Suda T, Hatakeyama K, Yamamoto T: Cloning of a new aquaporin (AQP10) abundantly expressed in duodenum and jejunum. *Biochem Biophys Res Commun* 2001;287:814-819.
- Ishibashi K, Morinaga T, Kuwahara M, Sasaki S, Imai M: Cloning and identification of a new member of water channel (AQP10) as an aquaglyceroporin. *Biochim Biophys Acta* 2002;1576:335-340.
- Mobasheri A, Shakibaei M, Marples D: Immunohistochemical localization of aquaporin 10 in the apical membranes of the human ileum: a potential pathway for luminal water and small solute absorption. *Histochem Cell Biol* 2004;121:463-471.
- Inoue K, Fei YJ, Zhuang L, Gopal E, Miyauchi S, Ganapathy V: Functional features and genomic organization of mouse NaCT, a sodium-coupled transporter for tricarboxylic acid cycle intermediates. *Biochem J* 2004;378:949-957.
- van Os CH, Deen PM, Dempster JA: Aquaporins: water selective channels in biological membranes. *Molecular structure and tissue distribution. Biochim Biophys Acta* 1994;1197:291-309.
- Verkman AS, van Hoek AN, Ma T, Frigeri A, Skach WR, Mitra A, Tamarappoo BK, Farinas J: Water transport across mammalian cell membranes. *Am J Physiol* 1996;270:C12-C30.
- Roberts RA, Griffin SE: Glycerol: biochemistry, pharmacokinetics and clinical and practical applications. *Sports Med* 1998;26:145-167.
- Kato T, Hayashi Y, Inoue K, Yuasa H: Functional characterization of the carrier-mediated transport system for glycerol in everted sacs of the rat small intestine. *Biol Pharm Bull* 2004;27:1826-1830.
- Yuasa H, Hamamoto K, Dogu S, Marutani T, Nakajima A, Kato T, Hayashi Y, Inoue K, Watanabe J: Saturable absorption of glycerol in the rat intestine. *Biol Pharm Bull* 2003;26:1633-1636.
- Fujimoto N, Inoue K, Hayashi Y, Yuasa H: Glycerol uptake in HCT-15 human colon cancer cell line by Na⁺-dependent carrier-mediated transport. *Biol Pharm Bull* 2006;29:150-154.
- Fujimoto N, Inoue K, Hayashi Y, Yuasa H: Effect of glycerol-related compounds on carrier-mediated glycerol uptake in HCT-15 human colon cancer cell line. *Drug Metab Pharmacokinet* 2008;23:216-220.