FAST AND SELECTIVE AMMONIA TRANSPORT BY AQUAPORIN-8

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Running Title: Gas transport by AQP8

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The transport of ammonia/ammonium is fundamental to nitrogen metabolism in all forms of life. So far no clear picture has emerged as to whether a protein channel is capable of transporting exclusively neutral NH₃ while excluding H⁺ and NH₄⁺. Our research is the first stoichiometric study to show the selective transport of NH₃ by a membrane channel. The purified water channel protein, aquaporin-8, was reconstituted into planar bilayers and the exclusion of NH₄⁺ or H⁺ was established by ensuring a lack of current under voltage clamp conditions. The single channel water permeability coefficient of 1.2x10⁻¹⁴ cm³/subunit/s was established by imposing an osmotic gradient across reconstituted planar bilayers and resulting minute changes in ionic concentration close to the membrane surface were detected. It is more than twofold smaller than the single channel ammonia permeability (2.7 x 10⁻¹⁴ cm³/subunit/s) that was derived by establishing a transmembrane ammonium concentration gradient and measuring the resulting concentration increases adjacent to the membrane. This permeability ratio suggests that electrically silent ammonia transport may be the main function of AQP8.

Aquaporins are commonly believed to mediate fast and selective water transport (1). However, some members of the protein family may have other functions. The intracellular acid sensing aquaporin-6 (AQP6), for example, serves as an anion channel (2). The function of aquaporin-8 (AQP8) is under dispute. First, it was suggested that AQP8-mediated water transport may be particularly important for the rapid expansion of mitochondrial volume (3). In a contrasting study, it was concluded that the rapid volume equilibration in mitochondria in response to an osmotic gradient was due to its small size (high surface-to-volume ratio) rather than to AQP-mediated high membrane water permeability (4). Moreover, only mild phenotype differences between wild-type and AQP8-deficient mice were found (5).

In addition, AQP8 was anticipated to participate in ammonia transport. AQP8 was able to rescue the growth of yeast defective in ammonium uptake suggesting that the protein is involved in NH₃ transport in humans. Increased acidification of the oocyte medium containing NH₄⁺ was in accordance with NH₃ diffusion through the protein (6). Voltage clamp experiments suggested that AQP8 conducts NH₄⁺ as well (7). Because growth complementation could be an indirect effect of AQP8 expression, light-scattering experiments with reconstituted vesicles were conducted. They revealed AQP8 permeability to formamide suggesting that the protein may transport ammonium in vivo and physiologically contribute to the acid-base equilibrium (8). However, comparative phenotype studies in wildtype vs. AQP8 null mice revealed no significant or only very small differences in serum ammonia, colonic ammonia absorption, renal ammonia clearance, and liver ammonia accumulation (9). It is difficult to interpret these results as evidence against physiologically significant AQP8-facilitated NH₃ transport in mice. Due to the importance of ammonium homeostasis several ammonium transport pathways are likely to exist. This is crucial for the urine pH adjustment and the acid-base equilibrium of body fluid. One of the alternative NH₃ transport pathways was identified in terms of RhBG and RhCG, the non-erythroid members of the Rh family (10). These
proteins were also knocked out in mice and neither distal tubular acidosis nor hyperammonemia were detected (11).

The assumption that a double knock out of both AQP8 and Rh proteins leads to a detectable phenotype remains to be tested. This expectation is based on the observation that the apical membranes of AQP8 expressing cells must maintain large chemical and osmotic gradients and therefore be effectively impermeable to small molecules including NH$_3$ and water. Tightening of the lipid matrix and, thus, a reduced permeability is achieved by high concentrations of glycosphingolipids and sphingomyelin in the outer membrane leaflet (12). It is therefore not surprising that, for example, the expression of hepatic AQP8 was associated with apical microdomain fractions enriched in cholesterol and sphingolipids (13). With respect to the low basal NH$_3$ permeability of the epithelial membrane, the requirement for controllable proteinaceous NH$_3$ transport machinery becomes obvious. AQP8 is the ideal candidate because it is largely localized in intracellular vesicles and can be redistributed to plasma membranes via a microtubule-depending, cAMP-stimulated mechanism (14).

The molecular mechanism for ammonia transport by AQP8 has not yet been resolved. Evidence was reported showing both NH$_3$ and NH$_4^+$ transport (7), although it was not possible to differentiate whether NH$_4^+$ transport occurred through the aquaporin itself or if secondary effects related to rapid NH$_3$ transport took place. We have addressed this question by reconstituting purified AQP8 into planar lipid bilayers mimicking the lipid composition of epithelial plasma membranes. Functional reconstitution was confirmed by water flux measurements. Simultaneous ion and ammonia flux measurements revealed perfect NH$_3$ selectivity, i.e. ammonia transport by AQP8 is electrically silent. We found that ammonia permeability exceeds water permeability twofold suggesting that ammonia transport may be the main function of AQP8.

**EXPERIMENTAL PROCEDURES**

Expression and purification of AQP8 from yeast

Rat AQP8 was expressed in pep4A S. cerevisiae and purified as previously described (8). In brief, after induction with 2% (w/w) galactose harvested by centrifugation, and three French press cycles, the membrane fraction was recovered from the supernatant by ultracentrifugation. AQP8 protein was solubilized in n-octyl-β-D-glucoside. The 10-His tagged protein was absorbed by a nickel column. Its purity was better than 90% as revealed by the comparison of coomassie-stained SDS gels with bands obtained by immunoblotting.

Protein reconstitution into planar bilayers

Purified AQP8 proteins were first reconstituted into proteoliposomes by dialysis (15). In brief, the reconstitution mixture was prepared at room temperature by sequentially adding 100 mM MOPS-Na, pH 7.5 (Fluka, Buchs, Switzerland), 1.25% (w/v) octylglucoside, purified AQP8 (final concentration 0.5–1 mg/ml), and 20–50 mg/ml of preformed lipid vesicles. The latter consisted of a 3 : 2 : 1 mixture of cholesterol, *E.coli* lipid extract (Avanti Polar Lipids, Alabaster, AL), and sphingomyelin. It was loaded into SPECTRA/POR 2.1 dialysis tubing, molecular mass cut-off 15,000 (Spectrum Laboratories, Laguna Hills, CA), and dialyzed against 100 volumes of assay buffer for 48 hours at 4 °C. Proteoliposomes were harvested by ultracentrifugation (60 min at 100,000 x g) and were resuspended into assay buffer (compare Fig. 1) at a concentration of 5–10 mg/ml.

At the air-water interface of vesicle suspensions monolayer were formed spontaneously (16). Two such monolayers were combined to form a planar bilayer in the 150-µm-diameter aperture of a 25-µm-thick polytetrafluoroethylene septum separating the two aqueous phases of the chamber (15;17). The septum was pretreated with a hexadecane-hexane mixture (volume ratio of 1:200).
**Water flux measurements**

Transmembrane osmotic water flow was derived from solute dilution in the immediate membrane vicinity (18). The solute concentration at the interface, \( C_s \), increases with the distance, \( x \), to the membrane:

\[
C(x) = C_s \exp\left(-\frac{vx}{D} + bx^3/3D\right),
\]

where \( v \) and \( b \) are - the linear drift velocity of the osmotic volume flow, and the stirring parameter, respectively. In the steady state, \( v \) was obtained by fitting the concentration distribution of Na\(^+\) ions to this equation. \( v \) is related to Pf by

\[
P_f = \frac{v}{C_{\text{osm}} V_w}
\]

\( (19) \).

**Theoretical model for NH\(_3\) transport**

In the model, the membrane flux of NH\(_4^+\) is neglected. This assumption is justified by the lack of any incremental conductivity after AQP8 reconstitution (Fig. 2). For acidic pH the experimental results are analysed precisely by solving the complete system of differential equations which takes into account all relevant chemical reactions in the immediate membrane vicinity (20;21):

\[
J_i = -D_i dc_i / dx_i, \ i = 1,...,6
\]

\( (1) \)

\[
dJ_i / dx = R_i(c), \ c = (c_1,...,c_6)
\]

\( (2) \)

\( J_i, D_i, c_i(x) \) denote, respectively, flux, diffusion coefficient, and concentration of the \( i \)th species, where 1 = H\(^+\), 2 = NH\(_3\), 3 = NH\(_4^+\), 4 = OH\(^-\), 5 = MES\(^-\), 6 = H-MES. \( R_i(c) \) is the specific local rate of expenditure of the \( i \)th species in the chemical reactions:

\[
\begin{align*}
H^+ + NH_3 &\rightleftharpoons NH_4^+; \\
H^+ + OH^- &\rightleftharpoons H_2O
\end{align*}
\]

\( (3) \)

\[
H^+ + \text{MES}^- &\rightleftharpoons H\text{-MES}
\]

\( (4) \)

At the membrane-water interface, the fluxes of all species are required to be equal to zero, except for \( J_2 \), so that

\[
J_1 = J_3 = J_4 = \ldots = J_8 = 0; \ J_2 = J
\]

\( (5) \)

For other boundary conditions, please compare Antonenko et al (21). The numerical solutions are derived, assuming that the rates of chemical reactions (like dissociation/recombination of water, buffer, and NH\(_3\)) are very high compared to the rate of diffusion through the USL, so that the local chemical equilibrium is maintained.

**RESULTS**

To demonstrate functional reconstitution, we measured osmotic water flow through purified AQP8 channels by imposing an osmotic gradient across reconstituted planar bilayers and detected resulting minute changes in ionic concentration close to the membrane surface (15;17). We formed planar membranes which mimicked the composition of epithelial cells (cholesterol : E.coli lipid extract : sphingomyelin = 3 : 2 : 1). In line with previous experiments (12), these membranes exhibited a very low osmotic water permeability, \( P_f \), of only \( (11.0 \pm 1.5) \mu m/s \). Reconstitution of AQP8 at lipid : protein mass ratio, \( r_m = 100 \), resulted in a...
threefold increase in $P_f$ (Fig. 1). The incremental water permeability, $P_{f,c}$, allowed calculation of the hydraulic permeability coefficient of a single channel, $p_f$, from the absolute hydraulic conductivity of all channels $P_{f,c}$ and the number of channels, $n$ (19). $n$ is anticipated to be equal to the total number of lipid molecules, $L$, in the bilayer divided by the molar lipid to protein ratio, $r$, where $L$ is derived from two times (for both leaflets) the membrane area, $A$, divided by the area, $b$, per lipid molecule:

$$p_f = P_{f,c} A / n = P_{f,c} Ar / 2L = P_{f,c} br_i M_p / 2M_L$$

where $M_F$ and $M_L$ are the molecular masses of the protein (30 kDa) and the lipid (700 Da), respectively. For $b = 70$ A$^2$, $p_f$ of AQP8 is found to be $(2.4 \pm 0.2) \times 10^{-14}$ cm$^3$ subunit$^{-1}$ s$^{-1}$. The result was obtained assuming that protein incorporation is 100% efficient. The real efficiency is, most probably, smaller. Reconstitution of AQP0, for example, was about 50% efficient (22). If our value is in the same range, $p_f$ adopts a value of about $(1.2 \pm 0.1) \times 10^{-14}$ cm$^3$ subunit$^{-1}$ s$^{-1}$. That is in reasonable agreement with results reported previously using a Xenopus oocyte expression system (23).

$pf$ allows calculation of the AQP8 turnover numbers for water, $T_w$:

$$T_w = N_A p_f / V_w = 4 \cdot 10^8 \text{ s}^{-1}$$

where $N_A$ and $V_w$ are the Avogadro number and the molecular volume of water, respectively.

Demonstration of functional reconstitution was followed by probing NH$_4^+$ transport through AQP8. A hundredfold augmentation of the NH$_4$Cl bulk concentration did not alter the current voltage characteristics of bare lipid bilayers or membranes reconstituted with AQP8 (Fig. 2). The similarity of ion conductivities indicated that AQP8 excluded NH$_4^+$ ions. Even if the entire conductivity of $G = 5$ nS cm$^{-2}$ was attributed to the protein, the total ion flux, $j_{ion}$, did not exceed $10^{15}$ mol cm$^{-2}$ s$^{-1}$ as calculated according to the equation:

$$j_{ion} = \frac{RT}{z^2F^2} G$$

Consequently, the ion : water selectivity was better than 1 : $10^9$ (compare 17;24).

Subsequently, transport of neutral NH$_3$ molecules by AQP8 was tested. At acidic pH, a transmembrane NH$_3$ flux, $J_{NH_3}^M$, was expected to give rise to a NH$_4^+$ flux through the near-membrane aqueous stagnant layers (20):

$$J_{NH_3}^M = -D_{NH_3} \frac{d[NH_3]}{dx} - D_{NH_4^+} \frac{d[NH_4^+]}{dx}$$

$$J_{NH_3}^M = D_{NH_4^+} \frac{d[NH_4^+]}{dx} (1 + \alpha)$$

$D_{NH_3}$, $D_{NH_4^+}$ and $x$, are the aqueous diffusion coefficients of NH$_3$, NH$_4^+$, and the distance to the membrane, respectively. $\alpha$ is equal to $10^{pH-pK}$. A NH$_4^+$ concentration gradient was imposed across the planar bilayer and the resulting small changes in NH$_4^+$ concentration close to the membrane were detected by scanning ammonia selective microelectrodes. The difference in NH$_4^+$ polarisation adjacent to bare (Fig. 3a) and reconstituted planar bilayers (Fig. 3b) indicated NH$_3$ transport by aquaporins. Plotting $J_{NH_3}^M$ versus the NH$_4^+$ transmembrane concentration gradient reveals a linear dependence (Fig. 3c). Calculation of $J_{NH_3}^M$ was performed taking into account the accompanying chemical reactions of ammonia and buffer. The set of differential equations (see Experimental Procedures) was solved numerically to fit the experimental profiles for 0 < $x$ < 50 µm (20;21). NH$_3$ permeabilities of 16 and 105 µm/s were computed for bare and AQP8 containing bilayers, respectively.

Protein inhibition by Hg$^{2+}$ was testable; although the ammonia selective microelectrode was less sensitive in the presence of Hg$^{2+}$ (a tenfold change in NH$_4^+$ concentration corresponded to a change in microelectrode potential of only 27 mV instead of the usually measured 52 mV). In the particular experiment shown in Fig. 4, Hg$^{2+}$ reduced the transmembrane NH$_3$ flux from 0.2 nmol cm$^{-2}$ s$^{-1}$ to 0.06 nmol cm$^{-2}$ s$^{-1}$. Thus, the NH$_3$ permeability of the planar bilayer reconstituted with AQP8 decreased from 50 µm/s to 15 µm/s, indicating complete AQP8 inhibition.

For further proof of NH$_3$ conductance by AQP8, flux dependence on membrane protein abundance was measured. Reconstitution of increasing amounts of AQP8 was accompanied...
by an increasing NH₄⁺ concentration polarization in the immediate membrane vicinity (Fig. 5). As before, the set of differential equations (see materials and methods) was solved numerically for different $P_{NH₃}^{M}$. For each AQP8 concentration the parameter was changed iteratively till the deviation of the theoretical profile from the experimental one was minimal. Plotting $P_{NH₃}^{M}$ as a function of $1/r_m$ allowed calculation of single channel ammonium permeability (Fig. 5, inset):

$$p_{NH₃} = p_{NH₃,c}A/n = p_{NH₃,c}br_mM_p/2M_L$$ (11)

where $p_{NH₃,c}$ is the incremental NH₃ permeability introduced by AQP8 reconstitution. Assuming 50 % reconstitution efficiency $p_{NH₃}$ was equal to $(2.7 \pm 0.2) \times 10^{-14}$ cm s⁻¹ and, thus, twofold higher than the respective single channel coefficient for water. The number of neutral ammonia molecules, $T_{NH₃}$, transported by AQP8 per second can be assessed by calculation of the turnover number per channel:

$$T_{NH₃} = N_Ap_{NH₃}/V_{NH₃} = 7.7 \times 10^5 s^{-1}$$ (12)

where $N_A$ and $V_{NH₃}$ are the Avogadro number and the molecular volume of NH₃, respectively.

**DISCUSSION**

We have shown that AQP8 transports both water and ammonia very efficiently. Reconstitution of the purified protein increased the respective permeabilities of model membranes mimicking apical membranes of epithelial cells up to three or six fold. As most members of the aquaporin family (25;26), AQP8 prevents ions from passing the channel, i.e. it allows exclusive transport of the neutral NH₃ molecule. This observation contrasts with the proposed NH₄⁺ permeability of AQP8 made in the Xenopus oocyte expression system (7). However, the ion fluxes detected in the oocyte system are not necessarily NH₄⁺ fluxes. They may also represent endogenous pH-sensitive currents of the oocyte. Because NH₃ acidifies the aqueous solution it leaves behind, and augments the pH in the solution it enters (20), a transmembrane proton gradient builds up which gives rise to proton and counterion currents provided that the membrane is permeable to these charged species. Subtracting the membrane permeability of non-transfected oocytes may be misleading because the expression of integral membrane proteins may modify ion channels endogenous to Xenopus oocytes (27). Since reconstituted planar bilayers lack these disadvantages, and because their extremely low intrinsic proton (24) and ion permeabilities (15;17) are not altered by AQP8, it is concluded that the protein excludes NH₄⁺.

AQP8 transports ammonia very efficiently. With about $8 \times 10^5$ substrate molecules per second per channel (Equation 12), $T_{NH₃}$ of AQP8 is comparable to the respective number of $2 \times 10^6$ estimated for the human rhesus-associated glycoprotein (28). Since the physiological NH₃ concentration is orders of magnitude smaller than the concentration of H₂O, the NH₃ conduction rate need not be as high as that of H₂O to match diffusion limited rates for arriving at the pore (29). In agreement with this prediction, we have obtained a 500-fold higher $T_W$ (Equation 7).

Comparison of the actual single channel permeability coefficients reveals, in contrast, a preference for NH₃ over H₂O:

$$p_{f}/p_{NH₃} = 1.2/2.7 \approx 1/2$$ (13)

Due their larger molecular volume ($V_{NH₃} >> V_W$), the channel accommodates less gas than water molecules. Thus, albeit their higher transport velocity, AQP8 transports less NH₃ molecules than water molecules. It should be noted that the actual velocity of water transport may be underestimated since it was derived assuming that the pores are densely packed with water. In single file transport, the density of water inside the channel may be lower than in bulk (30;31). Molecular dynamics simulations support the view that liquid – vapour oscillations occur in the channel (32;33).

Equation 13 reveals a preference for ammonia over water. This result conflicts with a cell culture study, in which the fluorescence of the pH-sensing yellow fluorescent protein was used to assess rat or mouse AQP8 ammonia permeability. The reason for the reported extremely low AQP8 single channel NH₃-to-water permeability only 0.03 (9) is not clear.
However, it is likely that the kinetics of pH changes are, at least in part, determined by compensatory transport events aimed to maintain cellular pH. Calculating $P_{\text{NH}_3}$ from the time course of pH changes alone (9) should be hampered (i) by passive fluxes of CO$_2$ and H$^+$ through the plasma membrane as well as (ii) by different types of pH-regulating transporters, like cation-H$^+$ exchangers, HCO$_3^-$ transporters, and H$^+$-ATPases, and (iii) proton exchange with intracellular compartments, such as mitochondria and lysosomes. Lacking the entire list of uncertainties, reconstituted bilayers offer the opportunity to measure NH$_3$ flux directly. The only uncertainty of the bilayer system is that the reconstituted protein may adopt a slightly different quaternary structure. At least for the aqueous pore of the AQP8 monomer, which is the channel path for water and ammonia, this can be ruled out by the perfect match between the $p_f$ values of the reconstituted protein and the one in an expression system (23). Theoretically, there is still a possibility that transport of charged species occurs through a putative fifth pore in the centre of the aquaporin teramer. It is rather unlikely in AQP8 because (i) so far ion channel activity has been ascribed only to AQP1 (34), (ii) nonphysiologically high cGMP concentrations (1 mM) are required (35) and (iii) ion channel activity was not reproduced by a variety of laboratories (36;37).

AQP8 is the first ammonia transporting channel for which the exclusion of NH$_4^+$ has been shown. The net transport of NH$_3$ across the plasma membrane by kidney Rh glycoproteins RhBG and RhCG results from an exchange of NH$_4^+$ for H$^+$ (38). A similar mechanism for AQP8 can be ruled out because the estimated upper limit of NH$_4^+$ or H$^+$ fluxes of $1 \times 10^{15}$ mol cm$^{-2}$ s$^{-1}$ is negligible compared to the ammonia flux of about 0.4 nmol cm$^{-2}$ s$^{-1}$ (Equation 8). NH$_3$/H$^+$ cotransport or NH$_4^+$ transport as proposed for AMT-1 from the hyperthermophilic archaeon *Archaeglobus fulgidus* (39) or the plant ammonium transporters LeAMT1 (40) and LeAMT2 (41) can be excluded for the same reason. Exclusion of NH$_3$ as well as saturable transport kinetics found for LeAMT1;1 (42) indicate a transporting mechanism that contrasts with AQP8.

A transport mechanism much closer to AQP8 has been proposed for AmtB from *E.coli*. Mainly based on structural considerations (43;44) and on molecular dynamics simulations (45), NH$_3$ selectivity was suggested. However, occasional passage of NH$_4^+$ or H$^+$ was not excluded. It would be stabilized by ring currents of the rich aromatic environment at the constriction zone using the acid/base properties of the imidazole nitrogens to assist in proton transfer (43). The authors concluded that a stoichiometric measure of conductance of neutral and charged species is required to establish whether ion conductance takes place – a study we now have carried out for AQP8.

Summarizing, AQP8 exhibits a preference for neutral NH$_3$ molecules over water suggesting a physiological role in maintenance of acid base equilibrium. In physiological concentrations AQP8 may augment the basal NH$_3$ conductivity three to fivefold.

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**FOOTNOTES**

*We thank Quentin Beatty for critically reading the manuscript. Financial support from the Austrian Science Fund (FWF W1201-N13) is gratefully acknowledged.*

**FIGURE LEGENDS**

**Fig. 1** Water transport by AQP8. Reconstitution of AQP8 (protein:lipid=1:100) augmented water permeability of bare lipid bilayers (cholesterol : *E.coli* lipid extract : sphingomyelin = 3 : 2 : 1) from 11 µm/s to 27 µm/s. Water permeability was calculated from the dilution of Na⁺ ions shown as a function of the distance to the membrane. Osmotic water flux was induced by 1 M urea. The buffer contained 20 mM MES, 100 mM NaCl, 1 mM NH₄Cl (pH 6.0).

**Fig. 2** Exclusion of NH₄⁺ transport by AQP8. The current voltage characteristics of bare lipid bilayers and bilayers reconstituted with AQP8 are not altered by the hundredfold augmentation of the NH₄Cl bulk concentration. A voltage ramp (duration: 3 minutes) was applied in the interval from -90 to +90 mV. The current was measured with a frequency of 100 Hz and than filtered at 0.3 Hz (compare Materials and Methods). The resulting data cloud is plotted. The spline lines were obtained by applying a local smoothing technique (100 intervals) using polynomial regression and weights computed from the Gaussian density function (SigmaPlot). The similarity of their slopes indicates that AQP8 excludes other ions as well. Membrane and buffer composition were as in Fig. 1.

**Fig. 3** NH₃ flux J as a function of the transmembrane NH₄Cl concentration gradient. The NH₄⁺ concentration in the trans compartment was measured by scanning microelectrodes as a function of the distance to the membrane. (A) Representative concentration profiles visualizing NH₃ diffusion through a bare lipid bilayer made of a lipid mixture (cholesterol : *E.coli* lipid extract : sphingomyelin = 3 : 2 : 1). (B) Representative concentration profiles showing NH₃ diffusion through reconstituted AQP8. For membrane formation the same lipid composition as in A was used. The protein : lipid ratio was 1/50. (C) NH₃ flux at different NH₄⁺ bulk concentrations in the cis compartment. J_{NH₃} and P_{NH₃} were calculated using the analytical model of weak base diffusion (Equations 1-5). The regression lines correspond to NH₃ permeabilities of 16 and 105 µm/s for bare and AQP8 containing bilayers, respectively. The buffer solution contained 20 mM MES, 100 mM NaCl (pH 6.0). The trans compartment contained 1 mM NH₄Cl.

**Fig. 4** Inhibition of AQP8 mediated NH₃ transport by Hg²⁺. Reconstitution of AQP8 in a protein : lipid ratio of 1/120 resulted in a NH₃ permeability of 50 µm/s. 1 mM Hg²⁺ reduced the permeability to that of a bare bilayer (here to 15 µm/s). The corresponding transmembrane NH₃ fluxes were 0.2 nmol cm⁻² s⁻¹ and
0.06 nmol cm\(^{-2}\) s\(^{-1}\) in the absence and presence of Hg\(^{2+}\), respectively. The trans and cis NH\(_4\)Cl concentrations in the bulk were equal to 1 and 80 mM, respectively. Buffer composition was as in Fig. 1.

**Fig. 5** NH\(_3\) membrane permeability as a function of AQP8 membrane abundance. The trans and cis NH\(_4\)Cl concentrations in the bulk were equal to 1 and 80 mM, respectively. In the absence of the protein (spline line), a modest increase in the cis NH\(_4^+\) concentration was observed in the immediate membrane vicinity. Upon reconstitution of AQP8, the augmentation of NH\(_4^+\) concentration adjacent to the membrane became more pronounced. The more protein was reconstituted, the higher was the concentration polarization. The gray lines represent concentration profiles generated by the theoretical model of weak base diffusion (Equations 1-5). The \(P_{NH3}^M\) values used to calculate the theoretical curves are plotted in the inset as a function of the protein:lipid ratio. Buffer composition was as in Fig. 1.
Fig. 1
Fig. 2
Fig. 3
Fig. 4, Saparov et al.
Fig. 5
Fast and selective ammonia transport by aquaporin-8
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J. Biol. Chem. published online December 21, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M609343200

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