Structure and function of aquaporin water channel

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Water channel function of all aquaporins (AQPs) but AQP4 can be inhibited by mercury reagents. To compare mercury sensitivity, AQP2 and AQP3 were expressed in Xenopus oocytes, and osmotic water permeability (Pf) of oocyte membrane was measured. Preincubation with 0.3 mM $HgCl_2$ decreased Pf by ~50% in AQP2 and ~30% in AQP3, suggesting that AQP3 is more resistant to mercury than AQP2. Mercurial reagents are believed to bind specifically to cysteine residue and block the aqueous pore of AQPs. Because of the low homology of AQP3 to other AQPs, it is not certain whether the pore structure of AQP3 is similar to that of the others. Determination of mercury-sensitive cysteine residues in AQP3 and comparison with those in other AQPs will help to resolve this question. To identify the mercury-sensitive site, 6 individual cysteine residues in human AQP3 (at positions 11, 29, 40, 91, 174, 267) were altered by site-directed mutagenesis. Mutant of C11S or C11A had a similar basal P_f as wild-type but acquired mercury resistance. Replacement of Cys-11 with Trp, which possesses a large side chain, did not decrease P_f. Mercurial inhibition of P_f was still observed in 5 other Ser-to-Cys mutants. These results suggest that Cys-11 is the mercury-sensitive residue in AQP3, and that this residue might be independent of the aqueous pore formation. Mutation of Tyr-212, a position corresponding to the mercury-sensitive residues in AQP1 and AQP2, to cysteine enhanced the mercurial inhibition of P_f. T212W had no water channel activity. Expression of AQP3 increased glycerol permeability (P_{gly}) 3.1-fold, whereas P_{gly} of Y212W-expressing oocytes was similar to P_{gly} of control oocytes. Cysteine mutation at Tyr-212 increased the inhibitory effect of mercury on P_{gly}. The activation energy for P_{gly} was 4.5 kcal/mol. These results suggest that the structure of the aqueous pore of AQP3 resembles to those of AQP1 and AQP2, and support the hypothesis that water and small molecules share a common pore in AQP3.