TRANSMEMBRANE HELIX 5 AND LOOP E ARE CRITICAL FOR THE HIGH WATER PERMEABILITY OF AQUAPORIN-2

Michio Kuwahara,[1] Kiyohide Fushimi,[1] and Kenichi Ishibashi,[1]
[1]Second Department of Internal Medicine, Tokyo Medical and Dental University, and [2]Department of Pharmacology, Jichi Medical School

Aquaporin-2 (AQP2), a vasopressin-regulated water channel, and the major intrinsic protein (MIP) of lens fiber are highly homologous (58% amino acid identity) and share a topology of six transmembrane helices connected by five loops (loops A ~ E). However, the water channel function of AQP2 is much higher than that of MIP. To determine the site responsible for this difference, several parts of MIP were replaced by corresponding parts of AQP2. When expressed in *Xenopus* oocytes, the osmotic water permeability (Pf) of MIP and AQP2 was 48 and 245 x 10^{-4} cm/s, respectively. Substitutions of loops B, C, and D failed to increase Pf, whereas substitution of loop E significantly increased Pf 1.5-fold. A similar increase of Pf was observed with the substitution of the front half of loop E. However, Pf values of these loop E chimeras were only ~30% of that of AQP2. Simultaneous exchanges of loop E and a distal half of transmembrane helix 5 just proximal to loop E increased Pf to the level of AQP2. Moreover, replacement of helix 5 alone stimulated Pf 2.7-fold. Pf measurements in a yeast vesicle expression system also confirmed that loop E had a complementary effect, whereas loops B, C, and D did not. Our findings suggested that the distal half of helix 5 and loop E are necessary for maximum water channel function in AQP2. We speculate that this portion contributes to the formation of aqueous pore and determination of the flux rate.