Analysis of Structure and Function of Water channel Proteins

Sei Sasaki, Kiyohide Fushimi, Shinichi Uchida
Second Internal Medicine, School of Medicine, Tokyo Medical and Dental University

Summary

Receiving 1992 Research Grant from the Salt Science Research Foundation, we have succeded in cloning of the ADH-regulated collecting duct water channel and named it AQP-CD. The aime of the study this year was to get insight into the relationship of protein structure and function of this protein. Two approaches were adopted; 1) site-directed mutagenesis of AQP-CD and its functional assay in the Xenopus oocytes, and 2) cloning of another water channel protein and comparisson of sequences and functions with AQP-CD.

From site-directed mutagenesis study, several observations were made. 1) Removal of upto 42 amino acids of C-terminal did not affect its water channel function, implying this portion does not constitute water channel pore. 2) Mutation of 256-Ser inhibited the response of AQP-CD to exogenous cAMP, implying direct regulation of water channel function by phosphorylation by A-kinase. 3) 181-Cys was the site where HgCl2 affect its inhibitory action, implying this area may compose water channel pore.

From cloning study, we obtaind another water channel protein, and named it AQP3. AQP3 is 279-amino acid protein which belong to MIP family with homology to AQP-CD 34%, E.coli glycerol facilitator 42%. Its localization distributes kidney collecting duct basolateral membrane, GI tracts, bladder, choloid plexsas. Functional assay confirmed its water channel function, but it also showed that AQP3 transports glycerol and urea. This functional characteristics is distinct from other water channels, and may offer a key clue for the identification of the filter of water channel.