

Molecular structure of a new chloride ion pump

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An ATP-dependent chloride extrusion system, a Cl^- pump, was found in the rat brain as a candidate for an active chloride transporting system that produces an inwardly directed chloride gradients to enable inhibitory hyperpolarizing responses to occur when transmitter operated chloride channels are facilitated. In this study, we isolated 520 kDa protein with a Cl^- pump activity, and analyzed its subunit structure.

(1) A 520 kDa protein with Cl^- -ATPase/pump activity was isolated from plasma membrane fractions of the rat brain. The protein consisted of at least 4 kinds of peptides with molecular mass of 64, 60, 55 and 51 kDa, and an antibody raised against the latter peptide inhibited both Cl^- -ATPase and $^{36}\text{Cl}^-$ transport activities. A 55 kDa subunit of the Cl^- pump protein also was separated and analyzed for N-terminal amino acid sequence. Using a synthetic peptide corresponding to 18 amino acid residues in the sequence, antibodies were raised against 55 kDa subunit. An antibody obtained reacted with the 55 kDa subunit, and another one reacted with 55 kDa subunit and slightly with 51 kDa subunit. The latter antibody slightly inhibited Cl^- -ATPase activity. (2) Using antisense primers for N-terminal amino acid sequences of 55 kDa subunit and the rat brain cDNA library, cDNA with a sequence for the N-terminal amino acids and 173 bp of 5' upstream region was cloned. On the homology search, no reported sequences were found to show homology over 50% with the cloned cDNA fragment for 55 kDa subunit.

It is suggested that Cl^- pump has multisubunit structure with 51 kDa subunit having Cl^- -ATPase activity.