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Regulation of Glutamatergic Chemical Transduction by Chloride

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Summary

Vesicular glutamate transporters (VGLUTs) mediate glutamate signaling through its ability to uptake L-glutamate into secretory vesicles using electrochemical gradient of H^+ generated by V-ATPase. In spite of physiological importance of VGLUTs, molecular mechanism of glutamate transport is far less understood due to lack of efficient assay system. We established a new system of VGLUT2 that use over expression system in combination of co-reconstitution of F_oF_1-ATPase. VGLUT2 was over expressed in the insect cells by recombinant baculovirus infection and purified through Ni-NTA column chromatography after solubilization of membranes by octyl-β-glucoside. Reconstituted proteoliposome exhibited marked glutamate transport activity driven by membrane potential generated by F_oF_1-ATPase. Kinetic analysis showed that purified VGLUT has similar properties as like synaptic vesicles. Mutagenic analysis was carried out using our new system to identify the essential of VGLUT. Mutations were introduced to charged conserved residues in the transmembrane domain. Mutations on His128 and Arg184 greatly reduced transport activity suggesting essential roles of these residues on glutamate transport. Amino acid replacement of Arg88 and Glu191 also reduced activity whereas Arg322 mutant exhibited wild-type activity. Using the procedure, we found that low concentration of chloride (around 4 mM) strikingly stimulated glutamate transport by purified VGLUT. The chloride dependency is an intrinsic property of purified VGLUT: it activates the transport activity through binding at the specific binding site. Thus, VGLUT is a chloride anion-dependent transporter. Our study raises a possibility that chloride anion regulates glutamatergic signal transmission through regulation of VGLUT moiety.