Aspartyl-aminopeptidase regulates albumin endocytosis by interaction with ClC-5

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ClC-5 is a voltage gated chloride channel that is essential for the constitutive reuptake of urinary albumin in the renal proximal tubule. Studies by our group and others have demonstrated that ClC-5 has a key role in mediating the assembly of the albumin endocytic complex that contains ClC-5, the albumin receptor megalin and several other interacting proteins. In order to identify novel proteins that interact with the endocytic complex, we adopted a proteomics approach to isolate proteins that associated with the C-terminus of ClC-5. Rat kidney lysates were incubated with a glutathione S-transferase (GST) fusion protein expressing the C-terminus of ClC-5 and pull-down assays performed to isolate interacting proteins. Bound proteins were resolved on SDS-PAGE and candidate bands were excised and prepared for mass-spectrometry. One protein that was very prominent in the samples was identified as aspartyl-aminopeptidase (EC 3.4.11.21), a recently characterised cytosolic protein that catalyses the release of N-terminal aspartate/glutamate residues from target peptides. The binding of aspartyl-aminopeptidase to ClC-5 was confirmed both *in vitro* by GST pull-down and *in vivo* by co-immunoprecipitation. Opossum kidney (OK) cells are the standard model for renal albumin endocytosis. Confocal immunofluorescence revealed that exposure of OK cells to albumin resulted in a recruitment of aspartyl-aminopeptidase to the apical domain of the cells where it co-localized with ClC-5. Over-expression of aspartyl-aminopeptidase in OK cells increased albumin uptake (~20% above control), which was accompanied by increased cell-surface levels of ClC-5. In contrast, over-expression of a catalytically-inactive form of aspartyl-aminopeptidase prevented both stimulation of albumin uptake and increase in cell-surface levels of ClC-5. These data show that aspartyl-aminopeptidase is a novel binding partner of ClC-5 and plays a key role in albumin uptake.