N-domain angiotensin-I converting enzyme is expressed in immortalized mesangial, proximal tubule and collecting duct cells

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Abstract

Somatic ACE (sACE) is found in glomerulus, proximal tubule and excreted in urine. We hypothesized that N-domain ACE can also be found at these sites. ACE profile was analyzed in mesangial (IMC), proximal (LLC-PK1), distal tubule (MDCK) and collecting duct (IMCD) cells. Cell lysate and culture medium were submitted to gel filtration chromatography, which separated two peaks with ACE activity from cells and medium, except from distal tubule. The first had a high molecular weight and the second, a lower one (65 kDa; N-domain ACE). We focused on N-domain ACE purification and characterization from LLC-PK1. Total LLC-PK1 N-domain ACE purification was achieved by ion-exchange chromatography, which presented only one peak with ACE activity, denominated ACEint2A. ACEint2A activity was influenced by pH, NaCl and temperature. The purified enzyme was inhibited by Captopril and hydrolyzed AngI, Ang1-7 and AcSDKP. Its ability to hydrolyze AcSDKP characterized it as an N-domain ACE. ACEint2A also presented high amino acid sequence homology with the N-terminal part of sACE from mouse, rat, human and rabbit. The presence of secreted and intracellular N-domain ACE and sACE in IMC, LLC-PK1 and IMCD cells confirmed our studies along the nephron. We identified, purified and characterized N-domain ACE from LLC-PK1.

Keywords

Angiotensin-I converting enzyme, N-domain ACE, Proximal tubule cells, LLC-PK1.