A new approach to obtain the catalytic site of human Angiotensin Converting Enzyme

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Angiotensin-converting enzyme I (ACE) is a key part of the renin-angiotensin system whose main function is to regulate blood pressure. The sACE possesses two domains, N- C-, with catalytic sites which exhibit 60% sequence identity. These domains differ in terms of chloride-ion activation profiles, rates of peptide hydrolysis of angiotensin I, bradykinin, angiotensina (1-7), beta-amyloid peptide and sensitivities to various inhibitors. A more detailed analysis shows that these regions are composed of HEMGH and EAIGD sequences, which are the catalytic sites. Our question is: If the synthesis of catalytic sites with corrects structure and activity could be a good model per si to study new drugs. In our laboratory the catalytic site of the C-domain was obtained with correct structural conformation and with enzymatic activity.

The objective this work is to obtain the Ala361 to Gli468 catalytic site, N-domain, in a structural conformation that resembles its native form.

The 380 pb cDNA to catalytic site was cloned in the pE1 vector (kindly provided by Dr. David Wood), elastin-like-polyptide (ELP) tag sequence was linked with catalytic site, ELP~csACEn recombinant protein, this was expressed in bacteria with Terrificus broth with 0.1 mM IPTG for 20h. Harvested cells were resuspended in TE buffer, after sonication and centrifugation the pellet was resuspended the same buffer. The ELP~csACEn was precipitated with 0.8 M ammonium sulfate and the tag sequence was cleaved by pH change, pH 6.2. All steps were analyzed by SDS-PAGE, Dot and Western blotting.

The catalytic site was synthesized from bacterial system with ELP sequence tag in soluble form. The purification process was done by ammonium sulfate precipitation and cleavage of the ELP sequence by changing to acidic pH. The characterization of catalytic site by SDS-PAGE shows that this is pure and Western blotting immunological assay confirmed the identity of the protein as csACEn.

The strategy used to obtain the Ala361 to Gli468 catalytic site in soluble and pure form was successful. The next steps: we will continue with the Maldi-tof and structural conformation analyzes.

Keywords: ACE, catalytic site, N domain, expression in E. coli.

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