

CHAPTER V

CONCLUSION

Conclusion

The formation of hCgA has also been described in association with fibrinolysis system. Recent studies show a new mechanism by which plasminogen activation is enhanced by the recombinant CgA. In order to determine structural region of the recombinant hCgA responsible for the enhancement of plasminogen activation, hCgA gene was reconstructed by PCR. The full-length and truncated hCgA genes were successfully cloned into the pET21b(+) expression plasmid without signal peptide. The truncated hCgA clones encode for proteins of 448, 419, 377, 345, 315, 278, 249, 214, 195, 126 amino acid residues. We found proteins at the expected sizes approximately 70 (full-length), 67, 60, 55, 49, 44, 40, 34, 31, 26 and 20 kDa. All rechCgA fragments were recognized by mouse Anti-Histidine(6X) antibody, but mouse anti-chromogranin A monoclonal antibody recognized the C-terminal region of the hCgA protein. The recombinant proteins were purified by Ni^{2+} affinity column chromatography in one step elution of 250 mM imidazole, and the last step to improve the purity of purified recombinant proteins, full length and truncated hCgA protein was by electroelution.

Plasminogen activation function of the full-length and all truncated hCgA variants were assessed and evaluated. Structural region of the recombinant hCgA showing the most impact on the enhancement of plasminogen activation is the hCgA₁₋₂₄₉. It increased the activation rate of plasminogen via tPA, increased the rates of catalytic efficiency and decreased K_m of plasminogen/plasmin activation. The results suggest that the CgA demonstrated a far better enhancer property for plasminogen/plasmin activation process via tPA than the control at the neutral pH approximately one orders of magnitude. The hCgA₁₋₂₄₉ may dissolve blood clot for thrombosis patients, however, observed side effect and toxicity test in animal model are yet to be further studied.

The truncated protein of hCgA may benefit in the study of several biological pathway in cardiovascular and other system, which is the proposed functions of chromogranin A.

