

# **RESEARCH FINAL REPORT**

**on**

## **Production and Characterization of Chimeric Transthyretin Scavenger for Amyloidosis and Alzheimer's Disease Related Proteins**

**by**

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**Granted by**

**Thai Government Budget 2007-2008**

## ABSTRACT

Transthyretin (TTR) is one of the three major thyroid hormone (TH) binding proteins found in serum and cerebrospinal fluid of human. It is one of the most abundant protein components of amyloid fibrils that lead to amyloidosis. Currently, the cryptic proteolytic activity of TTR was revealed and found associates with the occurrence of amyloidosis. However, the catalytic site and the mechanism underlying are still unclear. Several evidences on evolution of TTR structure and functions led us to a hypothesis that the catalytic site of TTR locates at or nearby the C-terminal region of TTR and the N-terminal region of TTR also play an influence on this activity of the protein. To elucidate the influence of the primary structure of the N- and C-terminal regions on this biological function of TTR, the recombinant TTRs and their chimeras were synthesized using the heterologous protein expression of *P. pastoris*. These included the recombinant human TTR (recomb. huTTR), the *C. porosus* wild type TTR (crocTTR), the huTTR that the N-terminal region was changed to that of *C. porosus* TTR (croc/huTTRTTR), the *C. porosus* TTR that the N-terminus was changes to that of human TTR (hu/crocTTR), the *C. porosus* TTR that the C-terminus was replaced by the C-terminal region of pig TTR (pigC/crocTTR), the *C. porosus* TTR that lacked the N-terminus (truncated crocTTR), and the *C. porosus* TTR that the N-terminal region was replaced by the N-terminus of *Xenopus laevis* TTR (xeno/crocTTR).

The cDNAs coding for all recombinant TTRs were successfully constructed in the pPIC3.5 (for recombinant huTTR) and in pPIC9 (for all other TTRs). The cDNAs were expressed; the proteins were synthesized and extracellularly secreted into culture medium outside yeast cell. The subunit mass, molecular weight of TTR tetramer and reactivity to the specific antibodies against TTR were determined. It showed that all recombinant TTRs produced by *Pichia* had all physicochemical properties similar to those vertebrate TTRs found in nature.

The influence of N- and C-terminal regions on the proteolysis property of TTR was studied using casein and apoA-I as substrates. The results showed that the chimeric TTRs those N- or C-terminal region was altered had different activity in comparing to the wild type TTRs. In the presence of the specific substrate, apoA-I, the croc/huTTR showed to lower activity ( $V_0$  was  $3.24 \pm 0.28$  ng/min) in comparing to that of the human wild type TTR ( $V_0$  was  $9.56 \pm 0.36$  ng/min). The catalytic activity of hu/crocTTR was 0.78 fold of that crocTTR ( $V_0$  of crocTTR and hu/crocTTR was  $7.15 \pm 1.15$  ng/min and  $5.55 \pm 0.62$  ng/min, respectively). The xeno/crocTTR and pigC/crocTTR showed high catalytic

activity with  $V_0$  of  $17.07 \pm 2.21$  ng/min and  $26.17 \pm 0.91$  ng/min, respectively. These were 2.39 folds (xeno/crocTTR) to 3.66 folds (pigC/crocTTR) greater than crocTTR. In addition, the truncated crocTTR showed very low activity ( $V_0$  was  $2.16 \pm 0.91$  ng/min) in comparing to crocTTR. The results obtained could demonstrate to the influence of N- and C-terminal regions on the catalytic activity of TTR. Length and hydrophathy of the N- or C-terminus may affect on conformation of the TTR subunit and/or molecule, which led to changes in the activity either in promote or inhibit manner.