

# Cytokine activity of the non-catalytic EMAP-2-like domain of mammalian tyrosyl-tRNA synthetase

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*Cytokine activity of the isolated recombinant C-terminal domain of mammalian tyrosyl-tRNA synthetase (TyrRS), which is homologous to a tumor-derived cytokine, endothelial and monocyte activating polypeptide (EMAP-2) has been studied. It was shown that C-domain induced a ~ 2-fold increase of monocyte chemotaxis. This effect is comparable with the values of chemotaxis induction by EMAP-2 cytokine and proEMAP-2. The truncated catalytic form of bovine TyrRS (2 × 39 kDa) has no effect on monocyte chemotaxis. C-domain of TyrRS also induced a ~ 3-fold increase in tissue factor activity in cultured human endothelial cells. A hypothesis is forwarded that the isolated C-domain of mammalian TyrRS may be released at proteolytic cleavage of TyrRS by some protease, activated at stress conditions, and functions as a mediator via signal transduction through interaction with a putative EMAP-2 receptor.*

**Introduction.** Aminoacyl-tRNA synthetases (ARSases) of higher eukaryotes usually possess amino- and/or carboxy-terminal polypeptide extensions of catalytic enzyme core which are dispensable for their catalytic activities [1–4]. The functions of these extensions are related to the complex organization of protein synthesis apparatus of multicellular organisms. For example, some mammalian ARSases have abilities to form a multi-tRNA synthetase complex which includes 9 ARSases and 3 other auxiliary protein components [1–3]. On other hand, these appended domains could be responsible for the polyanion binding properties of eukaryotic ARSases and compartmentalization of components of protein synthesis machinery on the ribosomes [4, 5].

Recently the amino acid sequences of bovine and human TyrRS were determined [6, 7], revealing a high sequence homology (51 % identity) between the C-terminal, non-catalytic domain of TyrRS and the novel cytokine-like molecule endothelial-monocyte-activating polypeptide (EMAP-2) [6–8]. Previously, we have shown, that bovine tyrosyl-tRNA synthetase ( $\alpha_2$ -dimer, 2 × 59 kDa) could be isolated from bovine

liver both as the full-length protein and as a proteolytically-modified active enzyme form (2 × 39 kDa), which lacks its C-terminal polypeptide extension [9, 10]. Both distinct molecular forms of TyrRS displayed the similar catalytic constants in tRNA aminoacylation reaction [9, 10]. Moreover, the dispensable C-terminal polypeptide extension of bovine TyrRS revealed a significant contribution to the non-specific affinity of this enzyme for RNA [11].

A novel putative cytokine, EMAP-2, modulates a variety of properties of endothelial cells, monocytes and leukocytes *in vitro*, and induces an acute inflammatory reaction *in vivo* [12, 16]. Based on the sequence similarity of mammalian TyrRS C-domain and EMAP-2, we have forwarded the hypothesis that the isolated C-domain may also display the cytokine activity, similar to EMAP-2 cytokine [8]. In order to verify this hypothesis we have cloned and expressed the isolated non-catalytic C-terminal domain of bovine TyrRS [17].

In this work we have discovered several cytokine-like activities of the isolated C-domain of TyrRS *in vitro* which are compared with the properties of recombinant EMAP-2 cytokine.

**Materials and Methods.** *Proteins isolation.* Clo-

ning and bacterial expression of the C-terminal domain of bovine TyrRS was performed as described earlier [17]. The *Bam*HI cDNA fragment encoding residues D322-S528 of bovine TyrRS was cloned into the *pET15b* vector for bacterial expression and recombinant protein was expressed in BL21(DE3) *Escherichia coli* cells harboring the *pEYCD2* plasmid. The supernatant was loaded onto Ni-NTA column and 6His-tagged recombinant protein was eluted with 300 mM imidazole.

Recombinant human EMAP-2 and proEMAP-2 were isolated and characterized as previously described [18].

**Monocyte chemotaxis:** Freshly-isolated monocytes were then suspended in RPMI-1640 medium with 10 % foetal calf serum, at  $2 \times 10^6$  cells per ml. Solutions of proteins (TyrRS C-domain, 39 kDa TyrRS and EMAP-2) or chemotactic peptide, formyl-methionyl-leucyl-phenylalanine (fMLP) (31  $\mu$ l) at the indicated concentrations were placed in the bottom wells of ChemTx micro-chemotaxis plates (Neuro Probe, Inc.) in triplicate. The filter was placed on top of the solution in such a way as to provide fluid continuity between the upper and lower chambers. An aliquot of monocyte suspension (27  $\mu$ l) was then placed on top of the filter above each well, and the plates incubated with lids on for 1.5 hr at 37 °C in air/5 % CO<sub>2</sub>. After incubation, cells binding to the membrane were fixed by the addition of 15  $\mu$ l of ice-cold 20 % formaldehyde in phosphate-buffered saline, and those that had migrated to the underside of the membrane were counted with a haemocytometer.

**Procoagulant activity assay:** Human umbilical vein endothelial cells (HUVEC) were isolated essentially by the method of Jaffe et al. [19]. Confluent endothelial monolayers at passages 2 to 3 were used to assess tissue factor-dependent procoagulant activity [20]. Coagulation was initiated by the addition of 100  $\mu$ l of 30 mM CaCl<sub>2</sub> solution at 37 °C and the time for visible fibrin strand/gel formation was determined. Procoagulant activity of endothelial monolayers was expressed as tissue factor equivalents (TFE, pg/10<sup>6</sup> cells) [20].

**Results and Discussion.** Purification of recombinant C-domain of TyrRS, expressed in *E. coli* cells after induction by IPTG and containing a 6His-tag, has been performed by metal-chelation chromatography. According to gel-electrophoresis data, the homogeneity of recombinant protein was about 95 % [17].

Since EMAP-2 cytokine has been shown to induce migration of monocytes and polymorphonuclear leukocytes *in vitro* [12, 18], we therefore examined

the effects of TyrRS C-domain on monocyte migration, using a micro-chemotaxis chamber assay. The addition of TyrRS C-domain to the lower chamber led to a ~2-fold enhancement of monocyte migration (Fig. 1). Monocyte migration was induced by C-domain in the range 1 pM–10 nM (between 1 pM and 100 pM this increase was significant,  $p < 0.05$ ) at levels slightly greater than that induced by recombinant EMAP-2 over a similar concentration range, but not as high as those achieved with 10 nM control chemotactic peptide fMLP. In contrast to isolated C-domain, the truncated 39K form of mammalian TyrRS, which lacks this domain, did not affect monocyte migration (Fig. 1).

A defining biological activity of EMAP-2 is its ability to induce tissue-factor-dependent procoagulant activity on the surface of endothelial cells *in vitro*, and furthermore to potentiate procoagulant activity induced by tumour necrosis factor (TNF) *in vitro* [12]. Therefore we studied the abilities of TyrRS C-domain and EMAP-2 polypeptide to induce tissue factor-mediated procoagulant activity on the surface of cultured HUVEC.

The exposure of endothelial cells to isolated C-domain for 4 hr at concentrations of 1–100 pM led to a dose-dependent increase in cell surface tissue factor between 0.36 and 0.77 pg TFE/10<sup>6</sup> cells on endothelial cells (Fig. 2, *a*). EMAP-2 also induced the enhancement of tissue factor activity in a dose-dependent manner (Fig. 2, *b*), but maximum activity was observed at lower mediator concentration at about 1 pM.

Our data suggest that the isolated C-domain of mammalian TyrRS reveals cytokine-like activities both as a chemotactic factor for monocytes and as an inducer of tissue factor expression on human endothelial cells similar to EMAP-2 cytokine. We propose, therefore, that the C-domain of TyrRS could potentially mimic the action of EMAP-2 cytokine through the interactions with complementary sites on the specific receptor.

Moreover, the cellular effects of TyrRS C-domain that we have observed, in particular the induction of tissue factor activity, cannot be fully explained by an interaction of its N-terminal region with a receptor. As indicated earlier, the chemotactic and tissue factor-inducing activity of EMAP-2 are believed to reside within different regions of the molecule [14]. Since we have also observed tissue factor induction in response to the TyrRS C-domain, it is possible that other functional domains of this C-terminal module, except its N-terminal region, could be involved in its cytokine activities.

If the EMAP-2-like domain of TyrRS is released

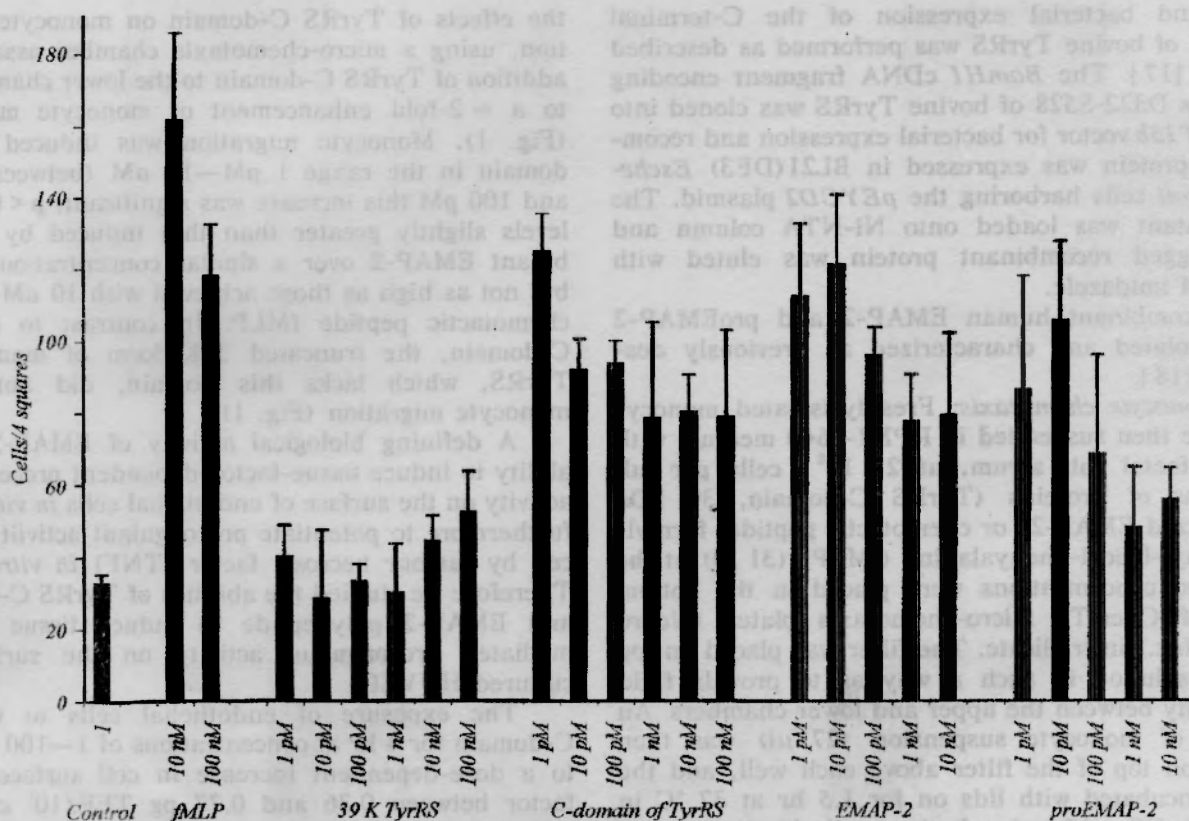


Fig. 1. Induction of monocytes migration by recombinant C-domain of TyrRS and EMAP-2 proteins as studied by micro-chemotaxis chamber assay. Cell migration assays were performed as described under «Materials and Methods». Data shown the standard deviations estimated with medium alone control. Chemotactic peptide fMLP was used as a positive control

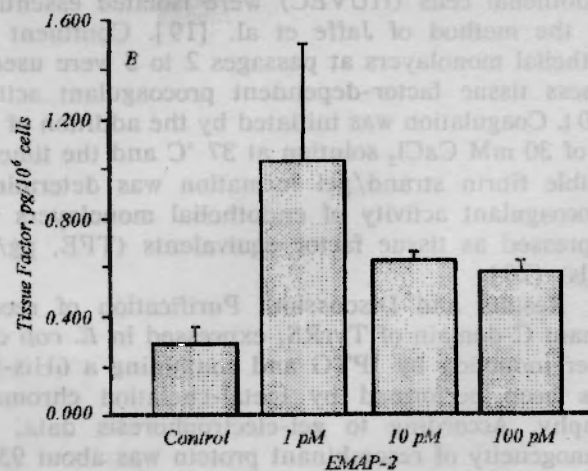
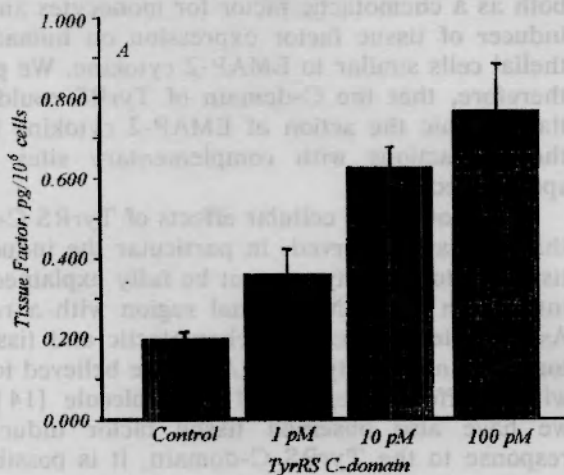


Fig. 2. Induction of procoagulant activity of tissue factor in human endothelial cells by recombinant C-domain of TyrRS (A) or EMAP-2 cytokine (B). Results are the means of 6 replicates pooled from 2 separate experiments

after proteolytic cleavage at the loop, connecting the catalytic 39 kDa enzyme core and this C-domain, it could be involved as a mediator in signal transduction process through the interactions with a putative EMAP-2 receptor. The nature of the EMAP-2 receptor is not known, although cross-linking studies have demonstrated binding of EMAP-2-derived peptides to a 73K protein associated with the monocyte cell surface [21], suggesting the existence of a distinct receptor.

Recently, it was shown that in cultured cells post-translational processing of proEMAP-2 into mature cytokine EMAP-2 occurred coincidentally with apoptosis programmed cell death [22]. It is well known that during apoptosis process some proteases, e. g. interleukin-1 converting enzyme (ICE, caspase) are activated [22]. It is possible to propose, that mammalian TyrRS could be cleaved during apoptotic proteolytic cascade, or other protease activation process.

It is interesting to note, that other component of protein biosynthesis machinery, auxiliary p43 protein of multi-synthetase complex, is proposed to be a precursor of EMAP-2 cytokine [23].

Our results suggest a novel non-canonical function of mammalian aminoacyl-tRNA synthetases in higher eukaryotic cells, which may be associated with signal transduction process. Furthermore this function may only be expressed in conditions where cellular proteases are activated.

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Цитокинова активність некаталітичного EMAP-2-подібного домена тирозил-тРНК синтетази ссавців

Резюме

Досліджено цитокинову активність ізольованого рекомбінантного С-кінцевого домена тирозил-тРНК синтетази (тирРС) ссавців, гомологічного EMAP-2 цитокіну. Показано, що С-домен індукує збільшення хемотаксису моноцитів у 2 рази. Цей ефект близький до такого, спричиненого EMAP-2 та проEMAP-2. Протеолітично модифікована каталітична форма тирРС (2 × 39 кДа) не впливала на хемотаксис моноцитів. С-домен тирРС також індукує зростання активності тканинного фактора ендотеліальних клітин людини в 3 рази. Пропонується гіпотеза стосовно того, що ізольований С-домен може вивільнятися при протеолітичному розщепленні тирРС певною протеазою, яка активується в стресових умовах, і функціонувати як медіатор шляхом передачі сигналу при взаємодії з рецептором EMAP-2 цитокіну.

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Цитокиновая активность некаталитического EMAP-2-подобного домена тирозил-тРНК синтетазы млекопитающих

Резюме

Изучена цитокиновая активность изолированного рекомбинантного С-концевого домена тирозил-тРНК синтетазы (тирРС) млекопитающих, гомологичного EMAP-2 цитокину. Показано, что С-домен индуцирует увеличение хемотаксиса моноцитов в 2 раза. Этот эффект близок к таковому, вызываемому EMAP-2 и проEMAP-2. Протеолитически модифицированная каталитическая форма тирРС (2 × 39 кДа) не влияет на хемотаксис моноцитов. С-домен тирРС также индуцирует рост активности тканевого фактора эндотелиальных клеток человека в 3 раза. Предложена гипотеза относительно того, что изолированный С-домен может высвободиться при протеолитическом расщеплении тирРС определенной протеазой, активируемой в стрессовых условиях, и функционировать как медиатор путем передачи сигнала при взаимодействии с рецептором цитокина EMAP-2.

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