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# Dissociating Quaternary Structure Regulates Cell-signaling Functions of a Secreted Human tRNA Synthetase*[S]

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**Many tRNA synthetases are homodimers that are catalytically inactive as monomers. An example is the 528-amino acid human tyrosyl-tRNA synthetase, which is made up of an N-terminal catalytic unit (TyrRS$^{Mini}$) and a 164-amino acid C-domain. Although native TyrRS has no known cytokine functions, natural proteolysis of secreted TyrRS releases TyrRS$^{Mini}$, which not only has the same aminoacylation activity as native TyrRS but also has strong activity for stimulating migration of polymorphonuclear leukocytes. The migration-stimulating activity is dependent on an ELR tripeptide motif, similar to that in CXC cytokines like IL-8, and also has the familiar bell-shaped concentration dependence seen for CXC cytokines. Here we show that in contrast to IL-8, where the bell-shaped dependence arises from the effects of CXCR1/2 receptor internalization, TyrRS$^{Mini}$ does not induce internalization of CXCR1/2. A rationally designed *non-associating* monomer and a *non-dissociating* dimer were constructed. With these constructs, the bell-shaped concentration dependence of leukocyte migration was shown to arise from the agonist (for migration) activity of the *catalytically inactive* monomer and the antagonist activity of the *catalytically active* dimer. Thus, the dissociating quaternary structure of TyrRS$^{Mini}$ regulates two opposing cytokine activities and suggests the possibility of dissociating quaternary structures regulating novel functions of other tRNA synthetases.**

Aminoacyl-tRNA synthetases are a family of ancient enzymes essential for decoding genetic information in translation (1). Surprisingly, many tRNA synthetases and synthetase-binding proteins have been expropriated for alternative functions in biological pathways not directly connected to translation. These include roles in angiogenesis (2–5), the inflammatory response (6–8), and immunomodulation (9), involve interactions with cell surface receptors, mRNAs, and nuclear partners (10), and are conferred or regulated by novel domains and sequence motifs added progressively during the evolution of eukaryotes (11). Mechanisms for activation of these expanded functions include post-translational modification, *e.g.* phosphorylation (7, 10), alternative splicing, or natural proteolysis (4, 5, 12). The variety of disease-associations with tRNA synthetases that have been annotated (13–15), including the identification of casual mutations (16, 17), is thought to reflect, at least in part, the connections of tRNA synthetases to these alternative functions (11).

Tyrosyl-tRNA synthetase (TyrRS)[3] is a homodimer throughout evolution. Although the catalytic site for attachment of activated tyrosine to the 3′-end of tRNA$^{Tyr}$ is entirely contained within the monomer unit, the dimeric state is required for amino acid activation (and therefore for aminoacylation activity) (18, 19). This requirement is due partially to a conformational change across the dimer interface (20). The overall design of TyrRS is different as the tree of life is ascended (21). For example, human TyrRS has a C-terminal domain not found in orthologs of lower eukaryotes, archaebacteria, or prokaryotes (see Fig. 1) (11, 22). Previous work showed that although native human TyrRS is inactive as a cytokine, under inflammatory conditions, it can be secreted and split by leukocyte elastase into two fragments having distinct cytokine activities (4). The C-terminal domain, which is the structural homolog of the mature form of human endothelial monocyte-activating polypeptide II (EMAP II), has potent leukocyte and monocyte chemotaxis activity and stimulates production of myeloperoxidase, tumor necrosis factor-$\alpha$, and tissue factor (4). On the other hand, the N-terminal catalytic domain TyrRS$^{Mini}$ functions in part as an interleukin-8 (IL-8)-like cytokine that stimulates migration of polymorphonuclear (PMN) cells through a mechanism that requires an ELR tripeptide motif like that found in CXC cytokines (4, 5, 23). This tripeptide motif is masked in TyrRS but is exposed when the C-domain is removed to release TyrRS$^{Mini}$. The stimulating effect of TyrRS$^{Mini}$ on PMN cell migration has a bell-shaped concentration dependence (4, 24, 25), similar to that seen with IL-8 family members. In the case of IL-8, increasing concentrations are associated with desensitization through internalization of its CXCR1/2 receptors (26–28).

In exploratory experiments (see below), we found that although receptor internalization could be demonstrated for CXCR1- or CXCR2-expressing HEK 293 cells treated with IL-8, no such internalization was seen when the same cells were treated with TyrRS$^{Mini}$. To understand this observation, we considered the possibility that TyrRS$^{Mini}$ was a monomer at concentrations (10 nM) where it acted as an agonist for PMN

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[3] The abbreviations used are: TyrRS, tyrosyl-tRNA synthetase; EMAP II, endothelial monocyte-activating polypeptide II; PMN, polymorphonuclear.

cell migration. Considering that the $K_d$ for the monomer-dimer equilibrium of *Neurospora crassa* mitochondrial TyrRS (which is orthologous to $TyrRS^{Mini}$) is ~100 nM (29) and considering the smaller dimer interface seen in our high-resolution (1.18 Å) structure of human $TyrRS^{Mini}$ (22) as compared with that of *N. crassa* mitochondrial TyrRS (30), we inferred that the $K_d$ of $TyrRS^{Mini}$ would be ≥100 nM. These considerations led us to hypothesize that monomeric $TyrRS^{Mini}$ is the active cytokine. However, this hypothesis alone does not explain the bell-shaped concentration dependence, and for that reason, we were also interested in exploring the role of the dimer that is formed at higher concentrations. For this purpose, we set out to determine the $K_d$ for the monomer-dimer equilibrium and also to investigate the activities of rationally designed *non-associating* monomers and *non-dissociating* dimers. The results of these investigations support the view that the monomer-dimer equilibrium is a critical regulator of the cytokine function of human TyrRS.

## EXPERIMENTAL PROCEDURES

Details of experimental protocols are given in the supplemental material. The receptor internalization assay was done with HEK 293 cells that stably express CXCR1 or CXCR2 (a gift from Dr. Adit Ben-Baruch at Tel Aviv University). The plasmid encoding wild-type (WT) human $TyrRS^{Mini}$ was been cloned previously (4) and was used to generate $TyrRS^{Mini}$ variants by site-directed mutagenesis using the QuikChange™ mutagenesis kit from Stratagene (La Jolla, CA). Circular dichroism (CD) spectra were obtained with an Aviv model 400 CD spectrometer (Aviv Biomedical, Inc. Lakewood, NJ). Analytical gel chromatography was done by injecting each protein sample (200 μl of 10 μM) onto a Superdex 200 chromatography column (GE Healthcare, 10/300GL) in PBS containing 5 mM β-mercaptoethanol. Amino acid activation assays were performed at 25 °C as described previously (31), with some modifications. Receptor binding assays were done with CXCR1- and CXCR2-transfected HEK 293 cells that were incubated with purified $His_6$-tagged WT $TyrRS^{Mini}$ or $TyrRS^{Mini\text{-}Mono}$. The Transwell cell migration assay was done with human PMN cells that were prepared from heparin-treated whole blood obtained from healthy volunteers using the RosetteSep human granulocyte enrichment kit (StemCell Technologies, Vancouver, BC, Canada).

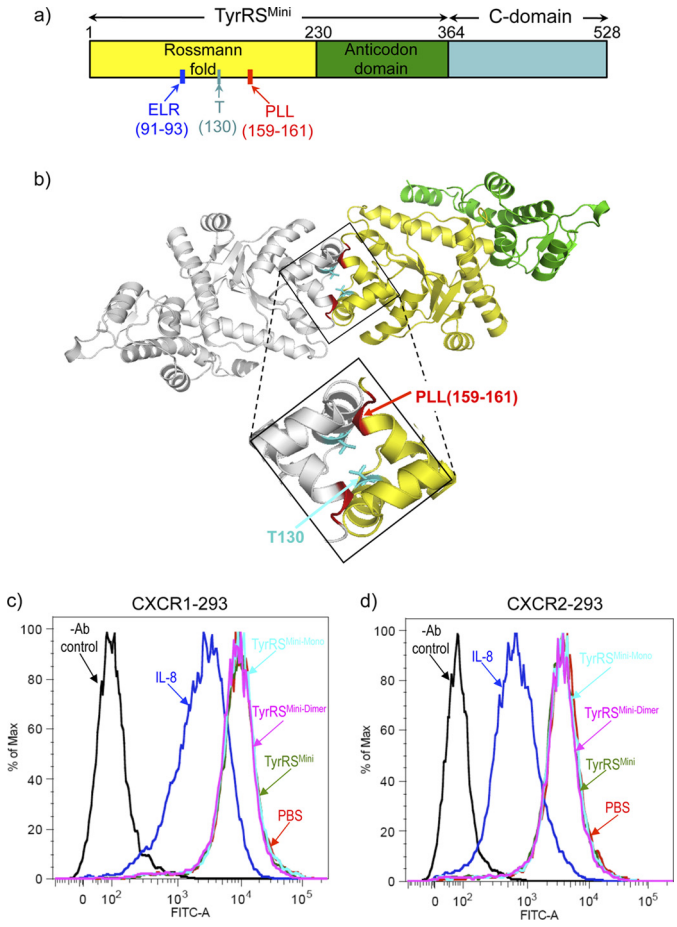


FIGURE 1. *a*, schematic of the structural organization of human TyrRS, showing the N-terminal $TyrRS^{Mini}$ domain that is orthologous to TyrRSs of lower eukaryotes and bacteria, the C-terminal EMAP II-like domain, and specific landmarks of the structure such as the critical (for cytokine signaling) ELR motif and the engineered residues used in this study. *b*, a rationally designed non-associating monomeric $TyrRS^{Mini}$ and a disulfide-linked non-dissociating dimeric $TyrRS^{Mini}$. The structure of $TyrRS^{Mini}$ dimer with individual monomers is shown in *yellow* and *light gray* (Protein Data Bank (PDB) 1q11), and the structure of the dimer interface with the location of a three-residue deletion (Pro-159, Leu-160, Leu-161) that disrupts $TyrRS^{Mini}$ dimerization is highlighted in *red*. The Thr-130 that was replaced with Cys to create a disulfide-linked dimer is highlighted in *turquoise*. *c* and *d*, FACS analysis to probe CXCR1 (*c*) or CXCR2 (*d*) internalization upon treatment with $TyrRS^{Mini}$ or with IL-8. CXCR1- and CXCR2-transfected HEK 293 cells were incubated with 100 nM $TyrRS^{Mini}$ or IL-8 at 37 °C for 2 h. The cells were washed and stained with fluorescein-conjugated anti-α-CXCR1 or anti-α-CXCR2 antibodies and subjected to FACS analysis. The untreated cells were stained with either α-CXCR1 or α-CXCR1 (without $TyrRS^{Mini}$ or IL-8 (labeled as *PBS*)). Control cells were not stained with α-CXCR1 or α-CXCR2 (−*Ab control*).

## RESULTS

*Testing for Receptor Internalization*—Several laboratories provided evidence that internalization of CXCR1 and CXCR2 receptors upon treatment of CXCR1- or CXCR2-expressing HEK 293 cells with IL-8 accounts for the bell-shaped response of the migration of these cells to the concentration of exogenously added IL-8 (26, 27). We explored receptor internalization by using the same HEK 293 cells transfected with genes expressing either CXCR1 or CXCR2. Using FACS analysis with both the CXCR1 and the CXCR2 cell lines, we observed a strong reduction of the fluorescent signal from fluorescein-labeled α-CXCR1 or α-CXCR2 antibodies bound to cells treated with IL-8 (Fig. 1, *c* and d). This reduction corresponds to the change associated with receptor internalization. In contrast, treatment of the same cells with $TyrRS^{Mini}$ induced no change, suggesting that $TyrRS^{Mini}$ does not promote receptor internalization.

*Rationally Designed Trapped Monomeric and Dimeric $TyrRS^{Mini}$*—The Rossmann fold of all class I tRNA synthetases is split by an insertion known as connective polypeptide 1 (CP1) (32). This insertion makes important contacts for formation of the dimer interface of human $TyrRS^{Mini}$. In particular, our three-dimensional structure showed that Pro-159, Leu-160, and Leu-161 in CP1 are involved in backbone hydrogen-bonding interactions that stabilize the two subunits (22) (Fig. 1*b*). Previously, monomeric $TyrRS^{Mini}$ (designated here as $TyrRS^{Mini\text{-}Mono}$) was generated by a Δ159–161 deletion, which resulted in a stable recombinant protein that could be

expressed and purified in *Escherichia coli* (24). To generate a non-dissociating dimer, a disulfide trap strategy was employed. This strategy has been successfully used to create a non-dissociating IL-8 dimer (33–35). With this method, an exposed cysteine is introduced at the dimer interface of each individual monomer so that the -SH groups are spatially proximal in the dimer. Upon oxidation, a single disulfide link can form across the dimer interface.

Inspection of the structure of $TyrRS^{Mini}$ suggested that the Thr-130 side chain OHs of each subunit were separated by only 3.5 Å and therefore could be tried as sites to introduce cysteine replacements (Fig. 1*b*). Recombinant T130C $TyrRS^{Mini}$ was created and expressed in and purified from *E. coli*. Subsequent $I_2$ oxidation led to the formation of a disulfide bond between the two subunits to give a stable dimer designated as $TyrRS^{Mini\text{-}Dimer}$ (supplemental Fig. 1*a*).

As expected (18, 19, 21), $TyrRS^{Mini\text{-}Mono}$ was inactive for aminoacylation. $TyrRS^{Mini\text{-}Dimer}$ was also inactive. However, the uncross-linked T130C $TyrRS^{Mini}$ was fully active, suggesting that flexibility of the dimer interface was needed for catalysis (supplemental Fig. 1*b*). Far-UV CD measurements (to monitor secondary structure) of $TyrRS^{Mini\text{-}Mono}$, $TyrRS^{Mini\text{-}Dimer}$, and $TyrRS^{Mini}$ were similar (supplemental Fig. 1*c*), and the thermal melting profile showed no significant difference in the thermal stability of $TyrRS^{Mini\text{-}Mono}$ as compared with $TyrRS^{Mini}$ (supplemental Fig. 1*d*). Interestingly, $TyrRS^{Mini\text{-}Dimer}$ had a melting curve with the same shape as those of $TyrRS^{Mini\text{-}Mono}$ as compared with $TyrRS^{Mini}$ but shifted 7.5 °C to higher temperatures, as expected for the extra stabilization provided by the covalent intersubunit linkage (supplemental Fig. 1*d*). These results collectively suggested that all three proteins were properly folded.

*Investigation of Monomer-Dimer Equilibrium by Gel Filtration*—Analytical gel filtration chromatography, at a concentration of 10 $\mu$M, showed that as expected, $TyrRS^{Mini}$ and $TyrRS^{Mini\text{-}Dimer}$ eluted as dimers, whereas $TyrRS^{Mini\text{-}Mono}$ migrated mostly as a monomer (Fig. 2*a*). By varying the protein concentration, the apparent dissociation constant for the dimer-monomer equilibrium of native $TyrRS^{Mini}$ was estimated as a $K_d$ value of ~100 nM (Fig. 2*b*) In contrast, the apparent dissociation constant for $TyrRS^{Mini\text{-}Mono}$ was estimated as ~100 $\mu$M (Fig. 2*c*), ~1000-fold higher than that of $TyrRS^{Mini}$.

*$TyrRS^{Mini\text{-}Mono}$ and $TyrRS^{Mini\text{-}Dimer}$ Bind to CXCR1 and CXCR2 Receptors*—CXCR1 was initially identified as a receptor for $TyrRS^{Mini}$ in PMN cells (4). Subsequent work established CXCR2 as a second receptor. For this purpose, we used confocal microscopy with HeLa cells transiently expressing CXCR1 or CXCR2. We chose HeLa cells because their morphology made them particularly amenable to visualizations by confocal microscopy. We generated HeLa cell lines transiently expressing CXCR1 or CXCR2 receptors. Twenty-four hours after cells were transfected, they were treated with purified $His_6$-$TyrRS^{Mini}$ for 1 h at 4 °C. After treatment, cells were washed twice with PBS and then fixed for immunofluorescence staining using anti-$His_6$- and anti-V5-antibodies for $TyrRS^{Mini}$ and CXCR1/2 receptors, respectively. Fig. 2*d* shows that cells expressing CXCR1 or CXCR2 (*red staining*) have a much higher density of binding $TyrRS^{Mini}$ (*green staining*) than do parental HeLa cells. In further work, we investigated stably transfected HEK 293 cells that were stably transfected with each receptor. (The morphology on plates of HEK 293 cells makes them less amenable to visualization techniques using confocal microscopy.) HEK 293/CXCR1 or HEK 293/CXCR2 cells were incubated with 100 nM of purified $His_6$-tagged $TyrRS^{Mini}$ or $TyrRS^{Mini\text{-}Mono}$. The binding of exogenously added TyrRSs was detected with $\alpha$-$His_6$ antibodies. Fig. 2*e* shows that after treatment with 100 nM of either $TyrRS^{Mini}$ or $TyrRS^{Mini\text{-}Mono}$ and using Western blots (with $\alpha$-$His_6$ antibodies) of PAGE gels that resolved proteins bound to cells expressing either CXCR1 or CXCR2, $TyrRS^{Mini}$ and $TyrRS^{Mini\text{-}Mono}$ each bound to cells expressing either receptor. (Note that in these experiments, the input of $TyrRS^{Mini}$ is less than that of $TyrRS^{Mini\text{-}Mono}$. After normalization to the input, the binding of $TyrRS^{Mini}$ and $TyrRS^{Mini\text{-}Mono}$ was closely similar.) In contrast, no binding was observed to the non-receptor-expressing parental HEK 293 cell line (Fig. 2*e*).

The $\alpha$-$His_6$ antibodies used in these studies only weakly reacted with $TyrRS^{Mini\text{-}Dimer}$, and for that reason, reliable data at 100 nM were not obtained and are omitted. As a way to study binding of $TyrRS^{Mini\text{-}Dimer}$, we investigated whether $TyrRS^{Mini\text{-}Dimer}$ can compete with $TyrRS^{Mini}$ for binding to the two receptors. We found that increasing concentrations (above 100 nM) of $TyrRS^{Mini\text{-}Dimer}$ displaced binding of $TyrRS^{Mini}$ (Fig. 2*f*).

*Monomer Is an Agonist and Dimer Is an Antagonist of Induced PMN Cell Migration*—$TyrRS^{Mini}$ has potent activity for inducing the migration of human PMN leukocytes (4, 24, 25). We investigated the PMN cell migration activity of $TyrRS^{Mini\text{-}Mono}$ and $TyrRS^{Mini\text{-}Dimer}$. At low concentrations, $TyrRS^{Mini\text{-}Mono}$ and $TyrRS^{Mini}$ stimulated migration to roughly the same degree (compare Fig. 3*a* at 1 and at 10 nM). (Because the apparent $K_d$ for the dimer-monomer equilibrium of $TyrRS^{Mini}$ is ~100 nM, $TyrRS^{Mini}$ is predominantly a monomer at low nanomolar concentrations.) At higher concentrations, $TyrRS^{Mini}$ showed the characteristic bell-shaped dependence of migration on concentration. In contrast, $TyrRS^{Mini\text{-}Mono}$, at concentrations up to 1 $\mu$M, did not show a large diminution in stimulation of cell migration. In contrast, no PMN cell migration activity was observed when $TyrRS^{Mini\text{-}Dimer}$ was used. Collectively, these results showed that the monomer of $TyrRS^{Mini}$ is the active and the dimer is the inactive ligand for PMN cell migration.

The decrease in cell migration activity of $TyrRS^{Mini}$ as the concentration was raised coincides with the concomitant increase in the dimer-to-monomer ratio of $TyrRS^{Mini}$. Because $TyrRS^{Mini\text{-}Dimer}$ is not active for stimulation of cell migration and because it competes with $TyrRS^{Mini}$ for binding to CXCR1 or CXCR2, we speculated that the dimer is a non-functional receptor-binding form that, at high concentrations, blocks binding of monomeric $TyrRS^{Mini}$. Because it can block monomeric $TyrRS^{Mini}$, the cell migration activity is reduced and, at the limit of high concentrations, there should be little or no migration. To test this hypothesis, we performed competitive cell migration assays in which we pretreated PMN cells with progressive increases in the concentration of $TyrRS^{Mini\text{-}Mono}$ or $TyrRS^{Mini\text{-}Dimer}$. Consistent with the bell-shaped response of PMN cell migration to the concentration of $TyrRS^{Mini}$ being caused by the antagonist activity of the dimer, $TyrRS^{Mini}$, but

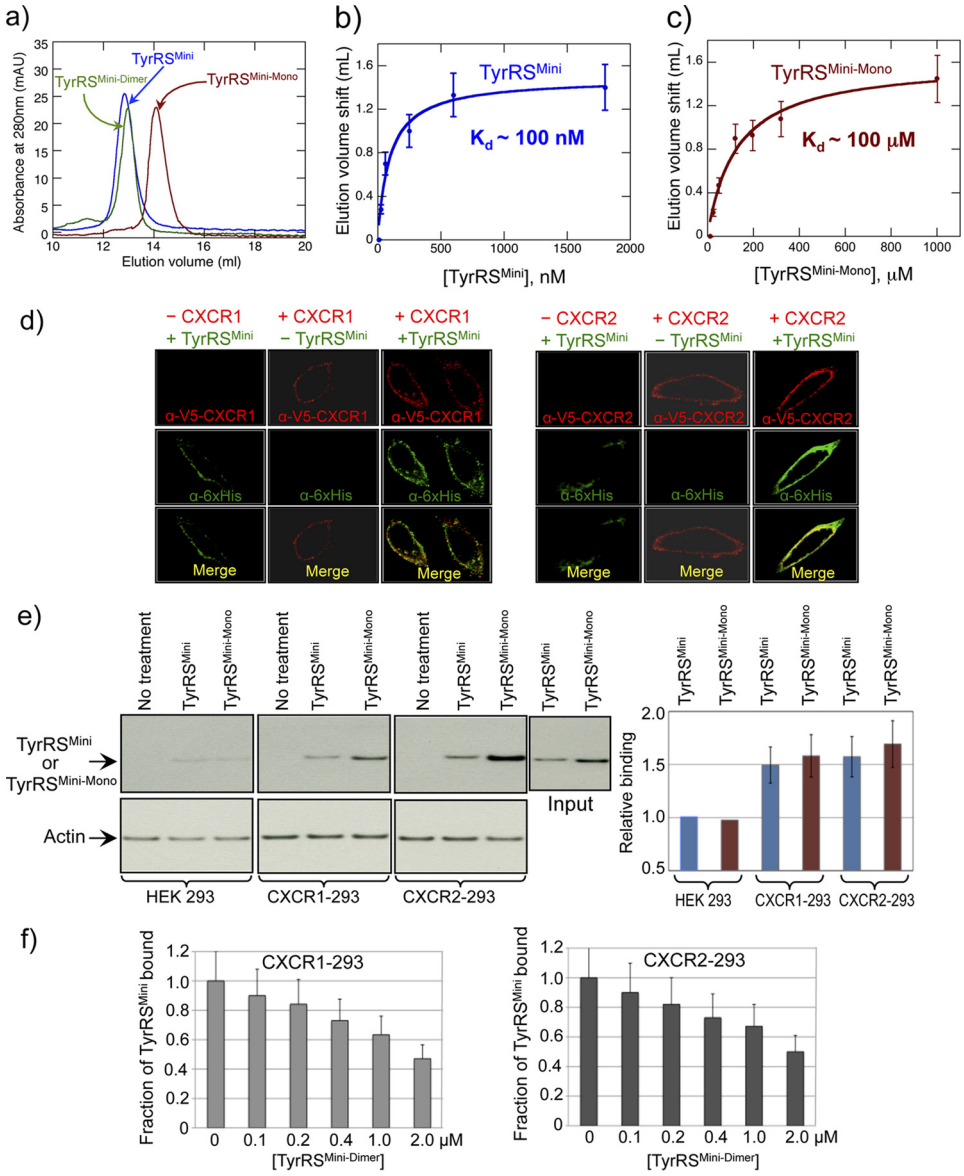


FIGURE 2. **Determination of dimer-monomer equilibrium dissociation constants for WT and Δ159–161 TyrRS$^{Mini}$.** *a*, analytical gel chromatography of WT, Δ159–161, and T130C$^{OX}$ His$_6$-tagged TyrRS$^{Mini}$. WT and T130C$^{OX}$ peaks correspond to dimeric TyrRS$^{Mini}$ (named TyrRS$^{Mini-Dimer}$), and Δ159–161 peak corresponds to monomeric TyrRS$^{Mini}$ (named TyrRS$^{Mini-Mono}$). *mAU*, milliabsorbance units. *b* and *c*, estimation of dimer-monomer equilibrium dissociation constant for WT TyrRS$^{Mini}$ (*b*) or TyrRS$^{Mini-Mono}$ (*c*) by gel filtration chromatography in combination with immunoblotting. *Error bars* correspond to mean ± S.D. from three independent experiments. *d*, TyrRS$^{Mini}$ binds to CXCR1 and CXCR2 receptors transiently expressed in HeLa cells with V5-tagged CXCR1/2. Cells were fixed and doubly stained with anti-His$_6$ (*green*) and anti-V5 (*red*) antibodies to detect TyrRS$^{Mini}$ and CXCR1/2 receptors, respectively. *e*, TyrRS$^{Mini}$ and TyrRS$^{Mini-Mono}$ bind to CXCR1 and CXCR2 receptors expressed in HEK 293 cells. The bar graph was generated by normalizing binding to the input of TyrRSs and amount of actin (as a measure of cell concentration) and is shown as mean ± S.D. from three independent experiments. *f*, TyrRS$^{Mini-Dimer}$ competes with WT TyrRS$^{Mini}$ for binding to CXCR1 and CXCR2 receptors. CXCR1-293 and CXCR2-293 cells were incubated with 100 nM WT His$_6$-tagged TyrRS$^{Mini}$ or TyrRS$^{Mini-Mono}$ at 4 °C for 1 h and then analyzed as described in the supplemental material. Data in bar graph format are shown as mean ± S.D. ($n$ = 3 for each concentration of TyrRS$^{Mini-Dimer}$).

not TyrRS$^{Mini-Mono}$, inhibited cell migration in a dose-dependent manner (Fig. 3*b*).

Lastly, the lack of receptor internalization seen with TyrRS$^{Mini}$ was also seen with TyrRS$^{Mini-Mono}$ and TyrRS$^{Mini-Dimer}$ (Fig. 1, *c* and *d*). Thus, other than the difference of agonist *versus* antagonist activity, the rationally designed TyrRS$^{Mini-Mono}$ and TyrRS$^{Mini-Dimer}$ behave like TyrRS$^{Mini}$.

## DISCUSSION

Fig. 3*c* summarizes our results showing that dimer dissociation switches secreted TyrRS$^{Mini}$ between two opposing activities for cell signaling. Thus, the present work supports an emerging theme of a role for alternative quaternary structures in the novel functions of human tRNA synthetases. For example, mutations in homodimeric glycyl-tRNA synthetase (GlyRS) are casually linked to Charcot-Marie-Tooth disease, the most common peripheral neuropathy (16). A detailed analysis in the light of a high-resolution structure showed that these mutations are in and around the dimer interface (36). In another example, homodimeric human lysyl-tRNA synthetase (LysRS), which is packaged into the HIV virion with the tRNA$^{Lys3}$ primer (for reverse transcription) and the Gag pro-

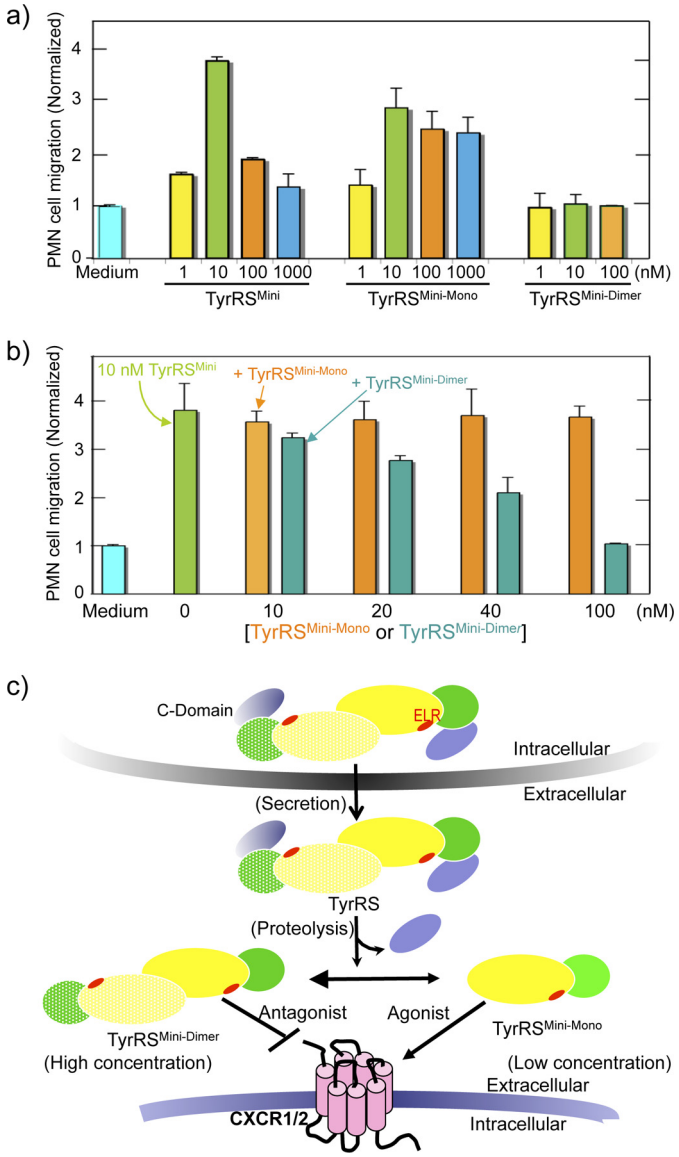


FIGURE 3. **Effects of TyrRS$^{Mini}$, TyrRS$^{Mini-Mono}$, and TyrRS$^{Mini-Dimer}$ on PMN cell migration activity.** *a*, TyrRS$^{Mini-Dimer}$ inhibits WT TyrRS$^{Mini}$-induced PMN cell migration. *b*, human PMN cell migration was performed using a Transwell permeable support with 5-$\mu$m pores as described in the supplemental material. Data from at least four experiments were collected, and mean ± S.D. is shown. *c*, a schematic diagram illustrating a proposed model for how TyrRS$^{Mini}$ mediates and regulates cytokine signaling. In this model, the dissociated monomer form of homodimeric TyrRS$^{Mini}$ is the active cytokine, and the dimer is a non-functional antagonist. Dimeric TyrRS$^{Mini}$ is labeled with individual monomers shown in *lemon* and *banana*, and the ELR motif is labeled in red.

tein, is proposed to dissociate into a monomer that bridges between tRNA$^{Lys}$ and Gag (37). However, in contrast to these examples where the role of the monomer-dimer equilibrium is inferred, we provide here direct evidence for the functional significance of this equilibrium.

The use of a monomer-dimer equilibrium to regulate cytokine function is seen with other CXCR1/2-acting cytokines such as IL-8 and NAP-2 (neutrophil-activating peptide 2). In the case of these cytokines, whereas the monomer-dimer equilibrium affects binding and signaling, an agonist/antagonist relationship has not yet been worked out (34, 38, 39). It will be of interest to investigate whether this agonist/antagonist relationship for two quaternary forms of TyrRS$^{Mini}$ is also mirrored in the alternative forms of IL-8 and NAP-2.

From an evolutionary perspective, because the dimeric form of TyrRS is universal and therefore was present before the emergence of higher eukaryotes, the cytokine function *per se* was not the selective pressure for dimer formation. Instead, a pre-existing monomer-dimer equilibrium was adopted for a regulatory role, at the time of insects, when the critical (for cytokine signaling) ELR motif and EMAP II-like C-domain were simultaneously incorporated into TyrRS(11). As stated above, the dimer interface of human TyrRS is looser than that of a bacterial ortholog. Our calculated buried surface area from formation of the homodimer is 1129 and 1392 Å$^2$ for human (22) and *Bacillus stearothermophilus* TyrRS (40), respectively.[4] Possibly, the development of a looser dimer interface that enabled a more facile monomer-dimer equilibrium may have occurred at the same time as the adoption of the ELR motif and EMAP II-like C-domain.

By analogy with $k_{cat}$ and $K_m$ parameters for catalysis of aminoacylation, the antagonist activity of the TyrRS$^{Mini}$ dimer suggests that the synthetase has $k_{cat}$ (for signaling) and $K_m$ (for binding) parameters for its interaction with the CXCR1/2 receptors. The concentration dependence of the inhibition of the monomer-induced cell migration by the dimer suggests that the apparent $K_m$ values for the monomer and dimer are similar (about 10–50 nM (Fig. 3*b*)). In contrast, to achieve the apparent complete inactivity of the dimer in the cell migration assay (Fig. 3*a*), we estimate that the $k_{cat}$ for signaling by the dimer must be reduced at least 10-fold as compared with that of the monomer to make it an operational antagonist. Possibly, the dimer interface has determinants that block a conformational change needed for activation of signaling after TyrRS$^{Mini}$ is bound to CXCR1/2.

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