Retractile lysyl-tRNA synthetase-AIMP2 assembly in the human multi-aminoacyl-tRNA synthetase complex

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Running title: Retractile LysRS-AIMP2 assembly in human MSC

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ABSTRACT

Multi-aminoacyl-tRNA synthetase complex (MSC) is the second largest machinery for protein synthesis in human cells and also regulates multiple nontranslational functions through its components. Previous studies have shown that the MSC can respond to external signals by releasing its components to function outside it. The internal assembly is fundamental to MSC regulation. Here, using crystal structural analyses (at 1.88 Å resolution) along with molecular modeling, gel filtration chromatography, and co-immunoprecipitation, we report that human lysyl-tRNA synthetase (LysRS) forms a tighter assembly with the scaffold protein aminoacyl-tRNA synthetase complex–interacting multifunctional protein 2 (AIMP2) than previously observed. We found that two AIMP2 N-terminal peptides form an antiparallel scaffold and hold two LysRS dimers through four binding motifs and additional interactions. Of note, the four catalytic subunits of LysRS in the tightly assembled complex were all accessible for tRNA recognition. We further noted that two recently reported human disease-associated mutations conflict with this tighter assembly, cause LysRS release from the MSC, and inactivate the enzyme. These findings reveal a previously unknown dimension of MSC subcomplex assembly and suggest that the retractility of this complex may be critical for its physiological functions.

Aminoacyl-tRNA synthetases (aaRSs) are essential enzymes, that catalyze the attachment of amino acids to their cognate tRNAs, generating aminoacyl-tRNAs that are used by the ribosome for translation (1,2). Higher eukaryotes have uniquely evolved a high-molecular mass multi-aminoacyl-tRNA synthetase complex (MSC). Human MSC consists of 9 aaRSs, including LysRS, ArgRS, GlnRS, AspRS, MetRS, IleRS, LeuRS, and GluProRS, and 3 scaffold proteins (p43/AIMP1, p38/AIMP2, and p18/AIMP3) (3,4). The MSC has been proposed to have a functional dualism: facilitating protein synthesis and serving as a reservoir of nontranslational functions associated with its synthetase and scaffold components (5,6). Assembly or disassembly of the complex is essential to regulate the functions of involved MSC components in cellular homeostasis. For example, GluProRS was reported to have roles in INF-γ related inflammation induced gene-specific silencing of translation and antiviral immunity when released from MSC (7,8). IFN-γ induces sequential phosphorylation of S886 and S999 in the noncatalytic linker of GluProRS, which triggers GluProRS release from the MSC. Phosphorylation of GluProRS is also required for the interaction with NSAP1, ribosomal protein L13a, and GAPDH
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The GAIT complex binds GAIT element in specific mRNA, and eventually represses translation (10). Upon DNA damage response, the GCN2 kinase phosphorylates MetRS at S662. The phosphorylation induces a conformational change of MetRS and releases the scaffold protein AIMP3, resulting in AIMP3 translocation to nucleus to activate ATM/ ATR pathways (11). Another scaffold protein AIMP1 has several proteolytic forms, including EMAP II and p43ARF (12-15). EMAP II, p43ARF, and the full length AIMP1 can all be secreted out of cells to play cytokine functions, including procoagulant, proinflammatory, proapoptotic, and angiogenic activities (12,16-18). Outside of MSC, AIMP2 binds and regulates the protein stability of FB P1 through the central region of AIMP2. AIMP2 promotes FBP1 degradation by Smurf 2-dependent posttranslational ubiquitination, and decreases the transcription level of c-myc (19).

Human LysRS plays noncanonical functions in several cellular processes, including HIV reverse transcription, viral packaging, neuropathy, immune response, cancer metastasis, etc. (20). Upon HIV infection, human LysRS and its cognate tRNA could be selectively packed into the HIV-1 virion. LysRS, tRNA_Lys, viral precursor proteins Gag and GagPol form a cytoplasmic nucleoprotein complex to facilitate the HIV viral reverse transcription after infecting new host cells (21-23). In mast cells, IgE–antigen stimulation activates specific phosphorylation of LysRS on a S207 residue (24). The S207 phosphorylation causes a structural opening of LysRS that disrupts its interaction with AIMP2, and leads to its dissociation from the MSC. The phosphorylated LysRS further enters into the nuclear, binds a transcription factor MITF, and eventually activates the MITF-targeted genes transcription (25). Human LysRS also has a cytokine activity (26). When secreted from macrophage-like differentiated THP-1 cells, LysRS mediates the transduction of inflammatory signals in the Shiga toxin-producing E. coli infected host cell (27). Besides, through a different structural mechanism, LysRS binds to cell membrane through the laminin receptor 67LR to promote epithelial cell migration (28,29).

LysRS’s recruitment to fulfill noncanonical functions involves its dissociation from MSC and association with new binding partners. Characterization of LysRS assembly within the MSC helps to understand these cellular mechanisms. Our previous studies showed one N terminus of the scaffold protein AIMP2 was able to hold one LysRS dimer in MSC (25,30), so that two LysRS dimers and two AIMP2 molecules per MSC exist in redundancy (25,30,31). The two LysRS dimers and the two copies of AIMP2 are packed with a “V” shape in solution (V-form) (Figure S1). In this form, each of the two LysRS dimers is connected by the N-terminal 32 residues from one AIMP2, so that two LysRS dimers do not directly contact. In this loose form, each LysRS dimer could move flexibly in solution, and function for aminoacylation, and diffuse from AIMP2 for functions outside of MSC (30). Here we report a new crystal structure of LysRS-AIMP2 subcomplex, in which the two LysRS dimers are retracted by AIMP2 into a tighter assembly. Two human disease related mutations, disturbed LysRS’s incorporation into MSC in cells, and are conflicted with this tight assembly. This finding reveals a previously unknown dimension of MSC subcomplex assembly, and suggests that the retractility of the complex may be critical for its diverse physiological functions.

Results

Overall structure of the LysRS-AIMP2 subcomplex in a tight assembly

We had previously determined a LysRS-AIMP2 complex at a resolution of 2.86 Å, and discovered that one AIMP2 N terminus was able to bind one LysRS dimer through two LysRS binding motifs (25,30). Recently, we solved a second LysRS-AIMP2 complex structure with 1.88 Å resolution in a different condition (Table 1, Figure S2). In the new crystal, only motif 1 (MYQVKPYH) of AIMP2 interacts with LysRS in the same asymmetric unit (ASU1) (Figure 1A), and motif 2 (MYRLPNVH) extents to a nearby asymmetric unit (ASU2), where it interacts with another LysRS dimer (Figure 1B, 1C). Symmetrically, motif 2 from the AIMP2 in the ASU2 extends back to ASU1 and interacts with the other AIMP2 binding pocket on LysRS dimer 1. Thus, two nearby ASUs form one biological unit that each of the AIMP2s concatenates two LysRS dimers (Figure 1B, 1C). This new crystal structure reveals a tightly packed “X” shape assembly of the LysRS-AIMP2 subcomplex (X-form) (Figure 1C), which is
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... different from the previously discovered V-form assembly (Figure S1). Four tRNA molecules can be docked on the X-form complex at the same time without any clash with other protein or tRNA atoms (Figure 1D), indicating all four LysRS catalytic subunits are functional in this form. It implies that the X-form complex might not only exist in crystal but also reflect physiological assembly.

**LysRS-AIMP2 interactions in the X-form complex**

Consistent with previous reports, the motif 1 and motif 2 of AIMP2 contribute to the major interactions between AIMP2 and LysRS (Figure 2A, 2B, and Figure S3). As a result, among the whole AIMP2 N terminal peptides, the temperature factors (b-factors) of motifs 1 and 2 regions are significantly lower than other parts (Figure 2D). Additional interactions from the linker region of AIMP2 were also discovered (Figure 2C, and Figure S3C). Residues L16, V18, L20, and P21 formed hydrophobic interactions with H354, Y253, F257, and I353 of LysRS dimer 1, the guanidino-group of R17 has stack interaction with Y201 of LysRS dimer 2, and T22 makes an interaction with H100 of LysRS dimer 2 (Figure 2C). The involvement of the L16-T22 linker region in the X-form complex formation is consistent with a recent cross-linking-MS analysis where full-length LysRS and AIMP2 proteins were analyzed (32).

Besides, the two LysRS dimers in the X-from complex are mainly held by the two AIMP2s. There are only a few direct interactions between the two LysRS dimers, such as a hydrophobic interaction between P390 and H348, a Van der Waals interaction between T388 and S478 (Figure S4).

**Presence of X-form complex in human cells**

To confirm the X-form complex exists in human cells, we designed an MSC incorporation assay based on gel filtration chromatography. In this assay, we fused the LysRS binding region of AIMP2 (aa 1-36) to the N terminal end of eGFP, and expressed the fusion protein in HEK293T cells. Distinct from the V-form, only the X-form assembly allows two AIMP2 N terminal peptides to bind corporately across two LysRS dimers. If the X-form indeed exists in cells, the AIMP2N-eGFP protein can be incorporated into the MSC by forming a heterogeneous X-form complex together with one endogenous AIMP2 and two LysRS dimers (Figure 3A). While in the V-form complex, one AIMP2 N terminal peptide binds one LysRS dimer. Competitive binding of AIMP2N-eGFP with the endogenous AIMP2 will lead to release of LysRS from the complex (Figure 3B). As a result, when we loaded the AIMP2N-eGFP expressing cell lysate onto a Superose 6 gel filtration column, the AIMP2N-fused protein was detected in both low molecular weight fractions and high molecular weight fractions (>1 MDa). While the control eGFP only existed in low molecular weight fractions (Figure 3C). Therefore, the presence of AIMP2N-eGFP in the high molecular weight fractions implies the existence of X-form complex in human cells. In addition, more LysRS in low molecular weight fractions was found from the AIMP2N-eGFP expressing cells comparing to the eGFP control cells (Figure D), suggesting that X-form and V-from may co-exist in HEK293T cells.

**Two human disease related mutations conflict with the X-form complex**

A 14-year-old-girl patient harboring two novel biallelic mutations in LysRS (L350H, P390R) was reported recently (33). The L350H mutation was inherited from her mother, while the P390R mutation was a de novo mutation. The patient manifests a severe form of cardiomyopathy associated with lactic acidosis, mild myopathy and intellectual disability (33). The pathogenesis is unclear. We therefore analyzed the two mutations on the X-form complex. L350 locates on the bottom side of LysRS, which is opposite to the tRNA binding side, and is about 6.5 Å away from the M3 residue of AIMP2 (Figure 4A). Side chain of L350 makes hydrophobic interactions with LysRS residues F340, M342, A345, and A545 (Figure 4A). Among these residues, M342 and A345 have direct hydrophobic interaction with M3 residue of AIMP2 (Figure 4A). When L350 is mutated to a bigger histidine residue, the M342 and A345 residues are slightly pushed away for about 2.0 Å and 0.6 Å, which is negative for AIMP2 binding (Figure 4B, and Figure S4). The P390 residue locates 13 Å away from AIMP2 binding (Figure 4B, and Figure S4). The P390 residue locates 13 Å away from AIMP2 binding (Figure 4C), thus P390R mutation should not affect the AIMP2 binding directly. However, P390 closely faces to the other LysRS dimer in the X-form complex form, and makes hydrophobic interaction with H348 from the other LysRS dimer (Figure 4C). The P390R mutation causes clashes with the LysRS dimer 2 at residues such as Y347, H348, M351, and R393 (Figure 4D).
Due to the crystallographic symmetry, the P390R mutation from LysRS dimer 2 causes same clash with LysRS dimer 1. This indicates that the P390R mutation disturbs the X-form complex. On the other side, the two LysRS dimers in the previous V-form complex are distant from each other, thus the P390R mutation might not affect the V-form complex assembly.

Mutations related to human disease intervene in MSC assembly and enzyme activity

To confirm the effect of the mutations upon MSC assembly in cell, we performed co-immunoprecipitation (co-IP) experiment to examine the ability of MSC association of LysRS wild type and mutant proteins. L350H mutant protein co-precipitated with AIMP2 and MetRS as wild type protein (Figure 5A, and Figure S5), indicating single L350H mutation did not significantly affect the MSC association, in consistence with the subtle change in the L350H crystal structure and the normal phenotype of the patient’s mother. The P390R mutation was also able to interact with AIMP2 and MetRS as normal, while the L350H/P390R double mutant significantly lost the ability to interact with AIMP2 and MetRS in the co-IP experiment (Figure 5A, and Figure S5). These results indicate that the two mutations L350H or P390R might each weakly affect the association of LysRS within MSC. However, the dual mutations can aggravate the interruption of MSC assembly.

We then examined how the two mutants might affect the enzyme activity for supporting essential protein translation using a functional replacement assay in *Saccharomyces cerevisiae* yeast (25). Wild type LysRS could substitute for the yeast cytoplasmic LysRS (which is controlled by a tetracycline-induced promoter and can be suppressed by doxycycline) and sustain normal cell growth (Figure 5B). The inactive LysRS S207D mutant was used as a negative control (25). Both L350H and P390R single mutants were as active as wild type in supporting cell growth. However the L350H/P390R double mutant was completely inactive (Figure 5B). There results show the two mutations have a synergetic effect in disrupting both the MSC assembly and the enzyme activity.

Notably, the L350 locates at the alpha helix (aa 346-366) right below the 7-strand central β-sheet of the active center. And the P390 locates at a long loop connecting the same helix to the β3 of the 7-strand β-sheet (Figure S7). The double mutation might have a synergistic affect in altering the local conformation of the helix (346-366) and the central β-sheet of the enzyme, which disturbed both X-form complex assembly and enzyme activity. Either of the dual defects or both might play a role in the pathogenesis.

Discussion

A pseudo-disulfide bond stabilizes the X-form complex

The two AIMP2 N terminal peptides bind anti-parallelly across the two LysRS dimers (Figure 6A). Interestingly, C23 next to the motif 2 formed a disulfide bond in the crystal structure, which stabilizes the compact X-form complex (Figure 6B, Figure S8). Following the LysRS binding sequence, AIMP2 has a leucine-zipper region at residues 48-81, and a GST domain at the C terminus. The leucine-zipper region dimerizes AIMP2 and interacts with AIMP1, ArgRS, and GlnRS, while the GST domain interacts with AspRS, GluProRS (34,35). The distance between the two H31 residues of AIMP2 in the X-form complex is about 41 Å (Figure 6C), agreeable to the dimerization of the C terminal part of the protein for further assembly of the whole MSC complex (Figure 6D). If the disulfide bond formed in *vivo*, it would stabilize not only the LysRS-AIMP2 subcomplex but also the whole MSC.

Interestingly, the C23S mutant of the AIMP2N-eGFP protein was still capable of forming X-form complex with endogenous AIMP2 and LysRS (Figure 3C), suggesting the disulfide bond is not a prerequisite of the X-form assembly. On the other side, the C23 together with residues involved in the X-form complex formation (such as L16-T22) are strictly conserved from zebra fish to human (Figure S9). In addition, LysRS from the cells expressing AIMP2N-eGFP C23S mutant showed slightly different distribution comparing to the cells expressing wild type fusion protein (Figure 3D). These results imply the C23 might play a role in controlling the MSC assembly at certain physiological conditions, for example, when intracellular redox potential fails under oxidative stress conditions (Figure 6E).

Potential advantage for the retractile assembly of MSC subcomplex
From our previously solved LysRS-AIMP2 crystal structure, one LysRS dimer forms two symmetric AIMP2 binding pocket by both N terminal anticodon binding domain and C terminal catalytic domain (25). Phosphorylation of S207 on the N-C domain interface triggers significant conformational change to LysRS and disrupts the AIMP2 binding pocket, thus releases LysRS from MSC for nontranslational function in mast cell activation (25). In the current crystal structure, we show LysRS could also form tight assembly with AIMP2 (Figure 1). In this assembly, the major interactions between LysRS and AIMP2 are consistent with the previous result (Figure 2), which double ensures the molecular mechanisms for the phosphorylation triggered LysRS release from MSC (25). These two complex forms could both represent the assembly of two LysRS dimers and two AIMP2 proteins in the human MSC. The two forms might co-exist in an equilibrium in cells.

Complementary to the previously solved V-form complex, the X-form setup may have a merit of orderliness. The more organized status can avoid potential clash of tRNAs, and ensure efficient aminoacylation catalysis (Figure 7A). While the V-form assembly has an advantage in release one LysRS dimer from the scaffold for nontranslational functions upon cellular stimuli, while retain the other LysRS dimer for fundamental protein translation (Figure 7B, 7C). The two forms of assembly may reflect different stages of LysRS function (Figure 7). The mechanisms for controlling the switch of the two forms yet need to be explored through further research.

In summary, this work solved a tighter LysRS-AIMP2 subcomplex in a compact X-form. The study reveals a previously unknown dimension of MSC subcomplex assembly, and suggests that the retractility of the complex may be critical for its diverse physiological functions.

**Experimental Procedures**

**Protein Preparation**

Untagged Human LysRS (70-584) was constructed in vector PET20b. The N-terminal human AIMP2 sequence (1-36) was cloned into vector pET28a with a C-terminal 6xHis tag. The two protein/peptide were co-expressed in BL21 (DE3) strain with a “-LEHHHHHHH” tag at its C-terminus. The protein was induced to express with 0.2 mM IPTG for 20 h at 16 °C. The cell pellet (from 4-8 liters) was lysed in lysis buffer (500 mM NaCl, 20 mM Tris-HCl pH 8.0, and 15 mM imidazole), loaded onto a Ni- HiTrap column and washed with lysis buffer. Protein was eluted with elution buffer (500 mM NaCl, 20 mM Tris-HCl pH 8.0, and 250 mM imidazole). The Ni-HiTrap purified LysRS-L350H proteins were concentrated and further purified by gel filtration Superdex 200 column (GE Healthcare, 10/300 GL). The peak fractions were then concentrated in 20mM Tris-HCl pH 8.0, 150mM NaCl for crystallization.

**Crystallization**

The LysRS-AIMP2 complex crystallization was done by the sitting drop method. 30 mg/ml LysRS-AIMP2 was pre-mixed with 5 mM L-lysine, 5 mM AMPcPP, 15 mM MgCl2 at 4 °C, and was crystallized by mixing 0.2 µl of protein solution with 0.2 µl of precipitant solution, containing 8 % PEG 20000, 0.1 M Tris pH8.0, and 0.1 M sodium chloride. After incubation at 18 °C for 3-7 days, crystals were flash-frozen in liquid nitrogen for data collection with the cryo solution containing 6 % PEG 20000, 0.075 M Tris pH8.0, 0.075 M sodium chloride, and 25 % glycerol.

The LysRS L350H protein was crystallized by the sitting drop method. 30 mg/ml LysRS L350H was pre-mixed with 2 mM Lys-SA at 4 °C, and was crystallized by mixing 0.2 µl of protein solution with 0.2 µl of precipitant solution, containing 13 % PEG 2000 MME, 0.4 M Ammonium acetate pH8.0. After incubation at 18 °C for 3-7 days, crystals were
flash-frozen in liquid nitrogen for data collection with the cryo solution containing 9.8 % PEG 2000 MME, 0.3 M Ammonium acetate pH8.0, and 25 % glycerol.

**Structure Determination**

The LysRS-AIMP21-36 complex crystal diffraction data was obtained from beamline LS-CAT at Advanced Photon Source of Argonne National Laboratory. The LysRS L350H crystals diffraction data were obtained from beamline 17U1 at Shanghai Synchrotron Radiation Facility (36). All datasets were processed with HKL2000 (37). The structures were solved by molecular replacement using human LysRS structure (pdb: 3BJU) with the program Molrep (38). Iterative model building and refinement were performed using Coot and Phenix (39,40). Data collection and refinement statistics are given in Table 1.

**Modeling LysRS-AIMP2-tRNA complex structure**

Crystal structure of AspRS-tRNA<sup>asp</sup> complex (1IL2) was used to generate the LysRS-AIMP2-tRNA structure model used in Figure 1D, since no LysRS-tRNA crystal structure is currently available. The chain A (AspRS) and chain C (tRNA<sup>asp</sup>) were extracted from 1IL2, and aligned onto the LysRS-AIMP2 complex structure based on the protein Cα atoms in pymol (root mean square deviation: 2.317 Å). The tRNA molecules could then be merged into the LysRS-AIMP2 structure with reasonable coordinates. The LysRS-AIMP2-tRNA model is generated by repeating this operation for all four LysRS subunits in the X-form complex.

**Immunoprecipitation**

LysRS WT, LysRS L350H, LysRS P390R, LysRS L350H/P390R, with a 3×Flag tag at the N terminus, were expressed in the HEK293T cells with DMEM+10%FBS culture media. After a 48-h transfection, cells were washed with cold PBS and then lysed with 1 ml lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.1% SDS, 1 mM PMSF) supplemented with 1× protease-inhibitor cocktail (Bimake, Cat# B14012). Supernatants of HEK293T cells were incubated with Flag beads overnight at 4°C. The beads were then washed four times with 1 ml of cold PBS with 1× protease-inhibitor cocktail (Bimake, Cat# B14012) and 1 mM PMSF. The beads-bound proteins were eluted and denatured with 5× SDS loading buffer and subjected to SDS-PAGE and immunoblotted with primary antibodies, including anti-Flag (Proteintech, Cat# 20543-I-AP, 1:2000 dilution), anti-AIMP2 (Proteintech, Cat# 10424-I-AP, 1:2000 dilution), anti-MetRS (Abcam, Cat# ab180497, 1:5000 dilution) and anti-β-tubulin (Proteintech, Cat# 66240-I-lg, 1:5000 dilution), and second antibodies, HRP-conjugate anti-Rabbit IgG (BBI Life Sciences, Cat# D110058-0100, 1:5000 dilution) or anti-Mouse IgG (BBI Life Sciences, Cat# D110087-0100, 1:5000 dilution).

**Yeast Viability Assay**

The cDNA sequences encoding full length human LysRS and its mutations were constructed in the p413GPD vector multi-cloning site. The plasmids were then transformed into the yTHC mutant strain from Open Biosystems (Dharmacon). The endogenous promoter of yeast cytoplasmic LysRS gene (krs1) has been replaced with a TET-titratable promoter in the yTHC genome. Thus the expression of the gene can be switched off by the addition of doxycycline to the growth medium. Tenfold serial dilutions of freshly grown yeast cells were spotted onto selective media SCM-HIS with or without doxycycline. Plates were incubated at 30°C for 3 days and then photographed.

**MSC incorporation assay**

Human AIMP2/p38 (1-36) and the C23S mutant was fused to the N-terminus of eGFP, and expressed in HEK293T cells using a pMSCV-puro vector. EGF alone was also constructed in the same vector and expressed in HEK293T cells as control. After 48h transfection, cells were collected and lysed in lysis buffer (150 mM NaCl, 20 mM Tris-HCl pH 8.0, 1 mM EDTA, and 1% NP-40), and then loaded onto a Superose 6 gel filtration column (GE Healthcare, 10/300 GL) with 20 mM Tris-HCl pH 8.0, 150 mM NaCl. The fractions were collected for immunoblot analysis with anti-GFP antibody (Proteintech, Cat# 50430-2-AP, 1:2000 dilution) or anti-LysRS (Abcam, Cat# ab134912, 1:2000 dilution).
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Conflict of interest:
The authors declare that they have no conflicts of interest with the contents of this article.
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**Abbreviations:**
The abbreviations used are: aaRS, aminoacyl-tRNA synthetase; MSC, multi aminoacyl-tRNA synthetase complex; LysRS, lysyl-tRNA synthetase; ArgRS, arginyl-tRNA synthetase; GlnRS, glutaminyl-tRNA synthetase; AspRS, aspartyl-tRNA synthetase; MetRS, methionyl-tRNA synthetase; IleRS, isoleucyl-tRNA synthetase; LeuRS, leucyl-tRNA synthetase; GluProRS, bifunctional glutamyl prolyl-tRNA synthetase; NSAP1, NS1-associated protein-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAIT, gamma interferon-activated inhibitor of translation; GCN2, general control nonrepressed-2; ATM, ataxia telangiectasia mutated; ATR, ATM- and Rad3-related; FBP1, far upstream element binding protein 1; *E. coli*, *Escherichia coli*; AMPcPP, diphosphomethylphosphonic acid adenosyl ester; Lys-SA, 5′-O-[(L-lysylamino)sulfonyl]adenosine.
Table 1. Data collection and refinement statistics.

|                      | LysRS-AIMP2 complex X-form | LysRS L350H mutant |
|----------------------|---------------------------|-------------------|
| **PDB code**         | 6ILD                      | 6ILH              |
| **Data collection**   |                           |                   |
| Space group          | C222₁                     | P3₂21             |
| Cell dimensions      | a, b, c (Å) 96.51, 100.07, 270.98 | 152.18, 152.18, 106.32 |
|                      | α, β, γ (°) 90.00, 90.00, 90.00 | 90.00, 90.00, 120.00 |
| Resolution (Å)       | 50.00-1.88(1.95-1.88)      | 50.00-2.50(2.57-2.50) |
| Rsym or Rmerge (%)   | 6.8(57.1)                  | 15.8(67.3)        |
| Mean I/σ(I)          | 29.3(3.5)                  | 14.5(4.0)         |
| Completeness (%)     | 98.5(97.6)                 | 89.8(99.5)        |
| Redundancy           | 7.9(7.5)                   | 19.1(18.7)        |
| **Refinement**       |                           |                   |
| Resolution (Å)       | 50.00-1.88(1.95-1.88)      | 50.00-2.50(2.59-2.50) |
| Total reflections    | 101191(7696)               | 44302(4869)       |
| Rwork / Rfree (%)    | 15.4/18.4                  | 19.4/22.6         |
| **No. atoms**        |                           |                   |
| Protein              | 8218                      | 7974              |
| Ligand               | 45                        | 64                |
| Solvent              | 940                       | 53                |
| **B-factors**        |                           |                   |
| Protein              | 25.98                     | 56.97             |
| Ligand               | 30.57                     | 47.73             |
| Solvent              | 36.34                     | 50.29             |
| **R.m.s. deviations**|                           |                   |
| Bond length (Å)      | 0.010                     | 0.005             |
| Bond angle (°)       | 1.30                      | 1.04              |
| Ramachandran plot    |                           |                   |
| Most favored [%]     | 98.92                     | 99.00             |
| Additional allowed [%]| 1.08                      | 1.00              |

Statistics for the highest-resolution shell are shown in parentheses.
Figure legends:

Figure 1. The LysRS-AIMP2<sub>1-36</sub> crystal structure in a tight form assembly.
(A) Crystal structure of LysRS-AIMP2<sub>1-36</sub> in one asymmetric unit (ASU). The LysRS protein is shown as green and cyan cartoon. The AIMP2 N terminal peptides is shown as sticks. The LysRS catalytic domain (CD), anti-codon binding domain (ABD), LysRS binding motifs 1, and 2 are indicated.
(B) Crystal structure of one biological unit of LysRS-AIMP2 complex. LysRS protein from ASU1 is shown as (A). LysRS protein from ASU2 is shown as light cyan surface. AIMP2 from ASU1 is shown as line. AIMP2 from ASU2 is shown as pick surface.
(C) A cartoon drawing of the X-form assembly of LysRS-AIMP2 complex.
(D) Four tRNA molecules can be modeled on the X-form complex without any clashes.

Figure 2. Zoom-in view of the LysRS-AIMP2 interaction in the X-form complex.
(A) LysRS-AIMP2 interactions at motif 1. LysRS protein is shown as green and cyan surface. LysRS binding motif 1 of AIMP2 is shown as black sticks.
(B) LysRS-AIMP2 interactions at motif 2. LysRS protein is shown as green and cyan surface. LysRS binding motif 2 of AIMP2 is shown as blue sticks.
(C) Additional LysRS-AIMP2 interactions. LysRS protein is shown as green (from ASU1) and light blue (from ASU2). AIMP2 is shown as pink sticks. The distances of the interacting residues are indicated.
(D) The b-factors of each residue of the AIMP2 N terminal peptide in the X-form complex.

Figure 3. Incorporation of exogenous AIMP2 peptides into MSC as X-form complex.
(A) Schematic diagram showing that X-form assembly allows incorporation of AIMP2N-eGFP into MSC.
(B) Schematic diagram showing that AIMP2N-eGFP competes with endogenous AIMP2 and releases LysRS from MSC.
(C) AIMP2-eGFP and eGFP expressing cell lysates are fractionized with Superose 6 gel filtration and immunoblotted by anti-eGFP antibody.
(D) AIMP2-eGFP and eGFP expressing cell lysates are fractionized with Superose 6 gel filtration and immunoblotted by anti-LysRS antibody.

Figure 4. Structure analysis of human disease related mutations.
(A) L350 region of wild type LysRS-AIMP2 complex. LysRS protein is shown as cyan. AIMP2 is shown as pink and black sticks.
(B) L350H mutant crystal structure (light brown) is superimposed on the LysRS-AIMP2 complex (cyan).
(C) Location of P390 on the X-form complex.
(D) P390R mutation causes clash with the second LysRS dimer in the X-form complex.

Figure 5. Human disease related mutation affects MSC assembly and enzyme activity.
(A) Flag tagged LysRS wild type (WT), L350H (LH), P390R (PR) co-precipitated with AIMP2 and MetRS similarly. While L350H/P390R (LH/PR) double mutant lost the ability to associate with AIMP2 and MetRS.
(B) Functional replacement assays in yeast show that the LysRS wild type (WT), L350H (LH), P390R (PR) are active in cells while the L350H/P390R (LH/PR) dual mutant is defective in essential translational functions in cells.

Figure 6. AIMP2 C23 might form a potential disulfide bond to stabilize MSC.
(A) AIMP2 forms an anti-parallel scaffold in the X-form complex. The two AIMP2 peptides are shown as sticks. The LysRS binding motifs 1 and 2 are indicated.
(B) Zoom-in view of the disulfide bond formed by residue C23 in crystal structure. An alternative conformation of the disulfide bond is colored in black.
(C) Side view of the X-form complex. The LysRS proteins are shown as transparent yellow and green surface. The two AIMP2 peptides are shown as spheres.
(D) The model of the AIMP2 interaction within whole MSC. ArgRS, AIMP1, GlnRS interacts with AIMP2 through the leucine zipper region of AIMP2. AIMP2 further associated to the rest of MSC through its GST domain.
(E) AIMP2 C23 might form disulfide bond to stabilize MSC assembly when intracellular redox potential fails under oxidative stress conditions.

Figure 7. Different forms of LysRS-AIMP2 assembly model within MSC.
(A) The compact and ordered X-form complex ensures efficient tRNA aminoacylation.
(B) Two LysRS dimers are held by the two AIMP2 N terminus separately in a V-form assembly, which is ready to release one LysRS dimer for nontranslational functions.
(C) A potential third assembly form of LysRS-AIMP2 complex, in which one LysRS dimer was released for nontranslational function, and one LysRS dimer was retained for fundamental translational function.
Figure 1
Figure 2
Figure 3

AIMP2N-eGFP leads to release of LysRS from the V-form complex.
Figure 4
Figure 5

A

- **IP:** flag
  - **WB:**
    - α-AIMP2
    - α-MetRS
    - α-flag
  - Sizes: 45 KDa - 35 KDa, 180 KDa - 140 KDa, 100 KDa - 75 KDa, 140 KDa - 100 KDa, 75 KDa - 60 KDa

- **WCL:**
  - α-AIMP2
  - α-MetRS
  - α-flag
  - α-tubulin
  - Sizes: 45 KDa, 35 KDa, 180 KDa - 140 KDa, 100 KDa - 75 KDa, 140 KDa - 100 KDa, 75 KDa - 60 KDa

B

- **+ Dox**
  - 10-fold dilution
  - LysRS WT (positive control)
  - LysRS LH
  - LysRS PR
  - LysRS LH/PR
  - LysRS S207D (negative control)

- **No Dox**
  - 10-fold dilution
Figure 6
Retractile lysyl-tRNA synthetase-AIMP2 assembly in the human multi-aminocetyl-tRNA synthetase complex
Zhoufei Hei, Siqi Wu, Zaizhou Liu, Jing Wang and Pengfei Fang

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