Title

Large Conformational Changes of Insertion 3 in Human Glycyl-tRNA Synthetase (hGlyRS) during Catalysis*

Journal

Journal of Biological Chemistry, 291(11)

ISSN

0021-9258

Authors

Deng, Xiangyu
Qin, Xiangjing
Chen, Lei
et al.

Publication Date

2016-03-01

DOI

10.1074/jbc.m115.679126

Peer reviewed

Names:
Dongsheng Lei; Gang Ren
Affiliate:
The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA,

Results:

Negative-staining electron microscopic images of Glycyl-tRNA-Synthetase and its bound to tRNA

The samples of Glycyl-tRNA-Synthetase (the molecular mass ~144 kDa) and its bound to tRNA were prepared by the optimized negative-staining (OpNS) [1,2], and examined by TEM at room temperature. The reason we chose OpNS rather than cryo-electron microscopy (cryo-EM) was because that: although cryo-EM is often the method of choice for studying protein structure under physiological conditions (because it avoids the artifacts induced by fixatives and stains), small molecules (< 200 kDa) are difficult to be visualized by cryo-EM due to their low image contrast. Our OpNS protocol, refined from the conventional NS protocol, eliminates rouleaux-artifact of lipoprotein particles, and has been statistically validated by cryo-EM images. As a method, OpNS has been used to image the structure-known proteins, such as 53kDa cholesteryl ester transfer protein (CETP), GroEL and proteasome[2,3].

The survey OpNS-EM image (**Fig. xxxA**) and selected particles (**Fig. xxxxB**) of Glycyl-tRNA-Synthetase sample show a globular shape with a dimension of ~60–90 Å. The selected reference-free class averages (calculated from 3157 particles) confirmed the globular shape of the Glycyl-tRNA-Synthetase (**Fig. xxxxC**). The survey OpNS image **(Fig. xxxxD)** and selected particles (**Fig. xxxE)** of tRNA bounded Glycyl-tRNA-Synthetase also show a globular shaped structure, but with two different features. One is the particle surfaces were adhered with the fiber-shaped densities; another is the particles seems larger than that without tRNA binding. Reference-free class averaging from 6248 particles showed the fiber-shaped densities were absent from the surface of the particles **(Fig. xxxxF)**, suggesting the fiber-shaped densities were structural flexible. To confirm the particle size changes, 1000 particles of Glycyl-tRNA-Synthetase and 1000 particles of Glycyl-tRNA-Synthetase bound to tRNA were selected and submitted for geometric dimensional measurement based on each particle's longest diameter and its perpendicular diameter. Since the geometric average is the square root of the product of these two diameters, it reflects the in-plane area of a particle on the supporting film. This 2D dimensional measurement (in-plane area) should more accurately reflect the 3D particle volume than any one-dimensional measurement. The distribution of geometric averages of Glycyl-tRNA-Synthetase showed that mean size is 76.2 ± 9.8 Å and the peak size is 75.9 Å with a peak population of ~20.6% **(black solid line in Fig. xxxxG)**. In comparison, the distribution of geometric averages of Glycyl-tRNA-Synthetase bound to tRNA showed that mean size is 96.4 ± 11.4 Å and the peak size is 96.5 Å with a peak population of ~18.7% **(blue dash line in Fig. xxxxG)**. The statistical analysis showed the tRNA caused the Glycyl-tRNA-Synthetase particle diameter increased ~27%.

Figure legend:

Figure xxxx | Negative-staining electron microscope images of Glycyl-tRNA-Synthetase and its bound to tRNA. (A) EM micrograph, **(B)** twelves representative raw particles and **(C)** eight representative reference-free class averages (from a total of 3157 particles) of Glycyl-tRNA-Synthetase. **(D)** EM micrograph, (**E)** sixteen representative raw particles and **(F)** eight

representative reference-free class averages (from a total of 6248 particles) of Glycyl-tRNA-Synthetase bound to tRNA. **(G)** Histograms of the diameter of Glycyl-tRNA-Synthetase (black squares) compared to that of protein bound to tRNA (blue circles). Distribution of the particle diameters fitted by a Gaussian curve show that the largest population of protein without tRNA has a diameter at 75.9 Å (~20.6%) while the protein bound to tRNA has a diameter of 96.5 Å (~18.7%). Bars: 500 Å.

Method:

EM specimen preparation by optimized negative-staining (OpNS) protocol. Specimens were prepared for EM by OpNS protocol as described[1,4]. In brief, an aliquot (□4 □l) of sample (at concentration of ~0.015 mg/mL) was placed on an ultrathin-carbon-coated 200 mesh copper grid (CF200-Cu-UL, Electron Microscopy Sciences, Hatfield, PA) that had been glow-discharged. After □1 min incubation, the excess solution was blotted with filter paper. The grid was then submitted for water washing and 1□ uranyl formate (UF) staining on Parafilm before being air-dried [1,4].

TEM data acquisition and image processing. The OpNS EM samples were examined at room temperature with a Zeiss Libra 120 Plus TEM (Carl Zeiss NTS), operating at 120 kV high-tension with 20 eV in-column energy filtering. The micrographs were acquired on a Gatan UltraScan 4Kx4K CCD under low defocus condition (< ~1 um) and a magnification of 125 kx (each pixel of the micrographs corresponded to 0.94 Å in specimens) under dose of ~40 - ~90 $e^-/Å^2$. The defocus and astigmatism of each micrograph was examined by EMAN *CTFfit* software [5]. X-ray speckles were removed. A total number of 9406 particles were selected (3157 for tRNA free and 6248 for tRNA bound Glycyl-tRNA-Synthetase sample) with a box size of 192 x 192 or 256 x 256 pixels. All particles were masked by a round mask (diameter ~180 Å) with Gaussian boundary generated by SPIDER [6]. Particles were divided into 620 classes and averaged images were shown in **Fig. xxxc and xxxF**.

Statistical analyses of the particle size.

For statistical analysis of particle size distribution, 1000 particle images from the micrographs of each condition were used. Domain size was determined by measuring diameters in two orthogonal directions [4], one of which was the longest dimension of the particle. The geometric mean (the square root of the product) of the perpendicular diameters was used to represent the domain size/diameter. Histograms for the size were generated with a sampling step of 5.0 Å. Each histogram was fitted with a Gaussian function in MATLAB for data analysis.

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