Biochimica et Biophysica Acta 1794 (2009) 347–354

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Two non-redundant fragments in the N-terminal peptide of human cytosolic methionyl-tRNA synthetase were indispensable for the multi-synthetase complex incorporation and enzyme activity

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ARTICLE INFO

Article history:
Received 8 September 2008
Received in revised form 3 November 2008
Accepted 4 November 2008
Available online 19 November 2008

Keywords:
Methionyl-tRNA synthetase
Multi-synthetase complex
Human cytoplasm
Incorporation
Activity

ABSTRACT

In human cytoplasm, nine aminoacyl-tRNA synthetases (aaRSs) and three protein factors form a multi-synthetase complex (MSC). Human cytosolic methionyl-tRNA synthetase (hcMetRS) is a component of the MSC. Sequence alignment revealed that hcMetRS has an N-terminal extension of 267 amino acid residues. This extension can be divided into three sub-domains: GST-like, GN, and GC sub-domains. The effect of each sub-domain in the N-terminal extension of hcMetRS on enzymatic activity and incorporation into the MSC was studied. The results of cellular assay showed that the GST-like sub-domain was responsible for the incorporation of hcMetRS into the MSC. The entire N-terminal extension of hcMetRS is indispensable for the enzymatic activity. Deletion mutagenesis revealed that a seven-amino acid motif within the sub-domain GC was important for the activity of amino acid activation. A conserved proline residue within the seven-amino acid motif was crucial, while the other six residues were moderately important for the amino acid activation activity. Thus, the last 15 residues of previously defined N-terminal extension of hcMetRS was a part of the catalytic domain; whereas the first 252 residues of hcMetRS constitute the N-terminal extended domain of hcMetRS. The formerly defined N-terminal extension of hcMetRS possesses two functions of two different domains.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) catalyze specific aminoacylation of their cognate tRNAs to produce aminoacyl-tRNA. The aminoacyl-tRNAs serve as starting materials for the biosynthesis of proteins. The accurate recognition by aaRSs on their cognate amino acids and tRNAs ensures the fidelity of translation from mRNA to protein [1,2]. Usually, the aminoacylation reaction occurs in two steps: (1) amino acid activation and (2) aminoacylation of tRNA [1,2]. Based on the structural features of the active site, aaRSs can be divided into two classes, namely, I and II, which comprise 10 members each; the two aaRS classes can further be divided into three subclasses [2,3].

In higher eukaryotes from arthropods to mammals, a multi-synthetase complex (MSC) was discovered; this complex is composed of eleven polypeptides including one bi-functional glutamyl-prolyl-tRNA synthetase (GluProRS), seven monospecific aspartyl-, arginyl-, glutaminyl-, lysyl-, methionyl-, leucyl- and isoleucyl-tRNA synthetases (AsnRS, ArgRS, GlnRS, LysRS, MetRS, LeuRS, and IleRS, respectively); and three nonsynthetase protein factors, namely, p18, p38, and p43 [4,5]. The aaRSs in the MSC possess extension peptides at their N- or C-terminus, which are absent in their prokaryotic counterparts [6]. These extended peptides were first thought to be the key players of the MSC assembly. The protein–protein interactions among the MSC components have been studied. An immunoprecipitation (IP) assay showed that the C-terminal extensions of LeuRS interact with the N-terminal extensions of ArgRS [7]. Yeast two-hybrid assays between the extended domains revealed that many pairs of the MSC components interact with each other [5,8]. In another study, cells transfected with N-terminal extension truncated GlnRS showed partial dissociation of the MSC [9]. In addition to their involvement in protein–protein interactions, the extended domains of some MSC components play a role on the catalytic activity of these components: N-terminal extension of LysRS [10,11] and C-terminal extension of MetRS [12] function as tRNA-sequestering factors and N-terminal extension of GlnRS is crucial for its aminoacylation activity [9]. Some functional motifs in these extended domains and in auxiliary factors of the MSC were identified by sequence analysis; these include the helix-turn-helix (HTH) motif in the C-terminus of MetRS and in the linker region of GluProRS, the helical tRNA-binding motif in the N-terminus of both LysRS and AspRS, and the glutathione S-transferase (GST)-homology domain in p18, p38, and the N-terminus of both MetRS and GluProRS

Abbreviations: aaRS, aminoacyl-tRNA synthetase; hctRNA$_i^{Met}$, human cytosolic initiator tRNA^{Met}(ATG); WB, western blot; NTA, nitrilotriacetic acid; GST, glutathione S-transferase; hcMetRS/MRS, human cytosolic methionyl-tRNA synthetase (other aminoacyl-tRNA synthetases are abbreviated by the three-letter code of the appropriate amino acid followed by "RS"); MSC, multi-synthetase complex

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doi:10.1016/j.bbapap.2008.11.003

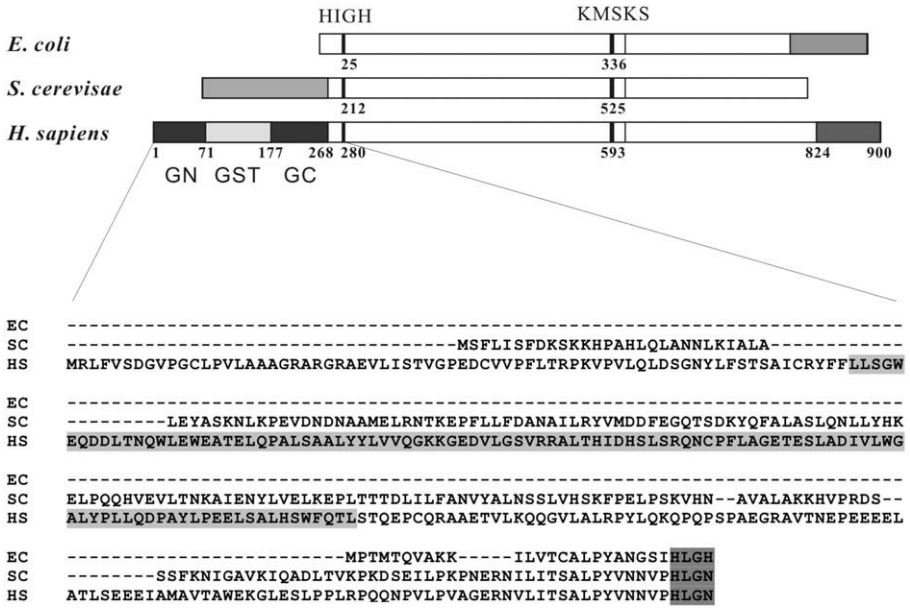

Fig. 1. Alignment of the N-terminal extension of MetRSs from different species. EC, SC, and HS, the N-terminal appended amino acid sequence of MetRSs from the cytoplasm of *E. coli*., *Saccharomyces cerevisiae*, and *Homo sapiens*, respectively; the GST-like sub-domain of *H. sapiens* cytosolic MetRS (hcMetRS) is shown in gray; the GN and GC sub-domains are shown as black boxes; the consensus HIGH(HLGN) motif is shown in dark gray.

[13]. These motifs were thought to be important for assembling the MSC and recruiting tRNA for aaRSs [8,13]. The HTH motif in GluProRS was proved to interact with the C-terminus of IleRS [8]. However, the functions of the GST-homology domains, which are present in several proteins of the MSC, have not been elucidated [13,14].

Methionyl-tRNA synthetase (MetRS) belongs to class Ia aaRSs [2]. Human cytosolic MetRS (hcMetRS) is a component of the human MSC [4]. In the cDNA encoding hcMetRS, the open reading frame (ORF) encodes a 900-amino acid protein with a predicted molecular mass of 101 kDa [15]. Compared to its prokaryotic counterparts, appended peptides were found at both terminus of the catalytic core of hcMetRS; the N-terminal extension spans the first 267 amino acid residues and the C-terminal extension covers the residues from Thr824 to Lys900. The catalytic domains of MetRS were conserved during evolution. However, the extended domains at both terminuses underwent major variations during evolution. The changes in the C-terminal extension of MetRSs during evolution have been well studied. In prokaryotes, the appended C-domain of MetRS is responsible for its dimerization; this domain also acts as a non-specific tRNA-binding domain to improve tRNA binding affinity [16]. The C-terminal extension in higher eukaryotic MetRSs shows no similarity in the primary structure as compared to its prokaryotic counterparts. In hcMetRS, an HTH motif and a specific C-terminal KGKKKK lysine-rich cluster were found in it's C-terminal extension; these two non-redundant tRNA-sequestering elements ensure an efficient capture of $tRNA^{Met}$ by the enzyme [12], in MetRSs from *Oryzae sativa*, a general RNA binding domain conserved to Trbp111 was found on C-terminus and acts as a cis-acting cofactor for aminoacylation [17]. The N-terminal extended peptide of MetRSs from different species also changed during evolution. In MetRSs from prokaryotes and some eukaryotes such as *Caenorhabditis elegans* and *Oryzae sativa*, there is no N-terminal extension. MetRSs from most eukaryotes have a long N-terminal extension; however, their sequences vary greatly and show no obvious similarity to each other. In hcMetRS, the entire N-terminal extended peptide is divided into three sub-domains: the GST-like sub-domain in the center of the N-terminal extension spans from residues Leu71 to Leu176 [13], the GN sub-domain (N-terminal region of the GST-like sub-domain) with the first 70 amino acid residues, and the GC sub-domain (C-terminal region of the GST-like sub-domain) spanning from residues Ser177 to Leu267; the last two sub-domains were named in the present study (Fig. 1). More than 2 decades ago, it was found that digestion of mammalian MetRS with trypsin produces three peptides; among these peptides, the 77-kDa peptide exhibited full activity as compared to that of MetRS purified from sheep liver; however, the peptide was released from the MSC in the form of a monomer [18,19]. Sequencing of cDNA coding hcMetRS revealed that trypsin digestion removed the first 214 amino acid residues on the N-terminus of hcMetRS, resulting in the generation of the 77-kDa polypeptide without GN, GST-like, and partial GC sub-domains [12]. The free monomeric species of the 77-kDa polypeptide could not be incorporated into the MSC. Thus, the GN and GST-like sub-domains appear to have no effect on the enzyme activity; however, the role of the GC sub-domain in aminoacylation remains unknown. The determinant motif for MSC incorporation of hcMetRS is located in the N-terminal extension; however, it remains unknown which part of this N-terminal extensive peptides is responsible for the association of hcMetRS with the MSC.

Here, we investigated the effect of each sub-domain of the N-terminal extension of hcMetRS on the catalytic activity and incorporation into the MSC by using a series of sub-domain truncated derivatives of hcMetRS. The results showed that the GST-like sub-domain plays an important role in the incorporation of hcMetRS into the MSC. We found that a seven-amino acid motif from Gln253 to Pro259 within the GC sub-domain was very important for its activity in the first step of the aminoacylation reaction and that the conserved residue Pro259 within the motif was crucial. This seven-amino acid motif would be a part of the catalytic core of the enzyme. Thus, the definition of N-terminal extension of hcMetRS should be revised on the sight of function. The formerly defined N-terminal extensive peptide of hcMetRS possesses two functions of two different domains in our new definition.

2. Material and methods

2.1. Materials

All chemicals and reagents were purchased from Sigma (USA), except otherwise noted. Kinase, ligase and restriction endonucleases were obtained from Fermentas (Lithuania). All oligonucleotides for