Journal of Structural Biology 190 (2015) 38–46

Contents lists available at ScienceDirect

Journal of Structural Biology

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

The insertion domain 1 of class IIA dimeric glycyl-tRNA synthetase is a rubredoxin-like zinc ribbon

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ARTICLE INFO

Article history:
Received 10 December 2014
Received in revised form 11 February 2015
Accepted 12 February 2015
Available online 23 February 2015

Keywords:
Glycyl-tRNA synthetase
Ap4A
Zinc finger
Insertion domain 1
Aminoacyl tRNA synthetase
Hereditary motor neuropathy
Charcot–Marie–Tooth disease

ABSTRACT

The insertion domain 1 (ID1) of class IIA dimeric glycyl-tRNA synthetase (α_2GRS) is an appended domain in the core catalytic region of the enzyme. ID1 has been shown to play a role in tRNA aminoacylation, mediating interaction with the acceptor arm of tRNA and diadenosine tetraphosphate (Ap4A) synthesis. Mutations in α_2GRS, including those in the ID1 region, have been implicated in distal hereditary motor neuropathy-V (dHMN-V) and Charcot–Marie–Tooth (CMT) disease. Through sequence and structure based evolutionary analysis, we show that ID1 of α_2GRS is a rubredoxin-like zinc ribbon domain. The zinc-chelating cysteines of ID1 are well conserved in all archaeal versions of the enzyme and also in several eukaryotes, which most likely have acquired them via horizontal gene transfer from bacteria; but in all other eukaryotes, the zinc-chelating residues are not preserved. ID1 from bacteria display a selective preservation of zinc-binding residues, ranging from complete conservation to complete loss. The ID1 from different organisms harbor variable-sized non-conserved insertions between the two zinc-binding half-sites of the zinc ribbon. Three of the previously identified CMT-associated mutations in α_2GRS, viz., human D146N, mouse C157R and human S211F, are located in the zinc ribbon region of ID1. Interestingly, human Asp146 which is implicated in the synthesis of Ap4A, a molecule known to act during neuronal transmission, has also been reported to be mutated in dHMN-V, suggesting a possible link between hereditary motor neuropathy and Ap4A synthesis.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) are enzymes that help in the translation of genetic information (Söll and Schimmel, 1974). Glycyl-tRNA synthetase (GRS) is an interesting enzyme in that two phylogenetically and structurally different forms are known (O'Donoghue and Luthey-Schulten, 2003; Shiba, 2005; Woese et al., 2000). A tetrameric form ($(\alpha\beta)_2$) of GRS is exclusively found in bacteria and a dimeric (α_2) form is present in eukaryotes, archaea and some bacteria (Fig. 1A) (O'Donoghue and Luthey-Schulten, 2003; Woese et al., 2000). The $(\alpha\beta)_2$GRS is related to the class IIC aaRSs (Phe and $(\alpha\beta)_2$GRS) and the α_2GRS belongs to class IIA (Ser, Pro, Thr, His and α_2GRS) (O'Donoghue and Luthey-Schulten, 2003). In the $(\alpha\beta)_2$GRS, the α-subunit harbors the catalytic domain and the β-subunit is the anticodon-binding domain, whereas α_2GRS displays a modular arrangement with both the catalytic and anticodon-binding domains present in a single polypeptide chain (Cader et al., 2007; Logan et al., 1995; Mazauric et al., 1998; O'Donoghue and Luthey-Schulten, 2003) (Fig. 1A).

The catalytic domain of α_2GRS has a typical class II aaRS fold (SCOP identifier 55680) made up of a seven-stranded antiparallel β-sheet (Logan et al., 1995) (Fig. 1B). Three consensus sequence motifs define class II aaRSs; wherein motif 1 (Gϕxxϕxx<u>P</u>ϕϕ) mediates dimerization while motif 2 (F<u>R</u>xE-loop-(H/R)xxxFxxx(D/E)) and motif 3 (GϕGϕGϕ(D/E)<u>R</u>ϕϕϕϕ) (where x is any aminoacid, ϕ is a hydrophobic aminoacid, underlined residues are absolutely conserved) contribute crucial active site residues (Cusack, 1995; Eriani et al., 1990) (Fig. 1A). Motif 2 and motif 3 are preserved in α_2GRS, but motif 1 is atypical, in that the invariant proline residue can be substituted by other aminoacids (Logan et al., 1995; Mazauric et al., 1998). The structure of the anticodon binding domain consists of a mixed β-sheet flanked by α-helices (SCOP fold identifier 52953) (Cader et al., 2007; Logan et al., 1995). In some α_2GRS, N- and C-terminal extensions and additional appended domains are also observed (Logan et al., 1995; Mazauric et al., 1998; Shiba, 2005).

Insertion domain 1 (ID1) is an ~85 residue domain in the structurally characterized α_2GRS (residues 144–228 in human, PDB

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http://dx.doi.org/10.1016/j.jsb.2015.02.004
1047-8477/

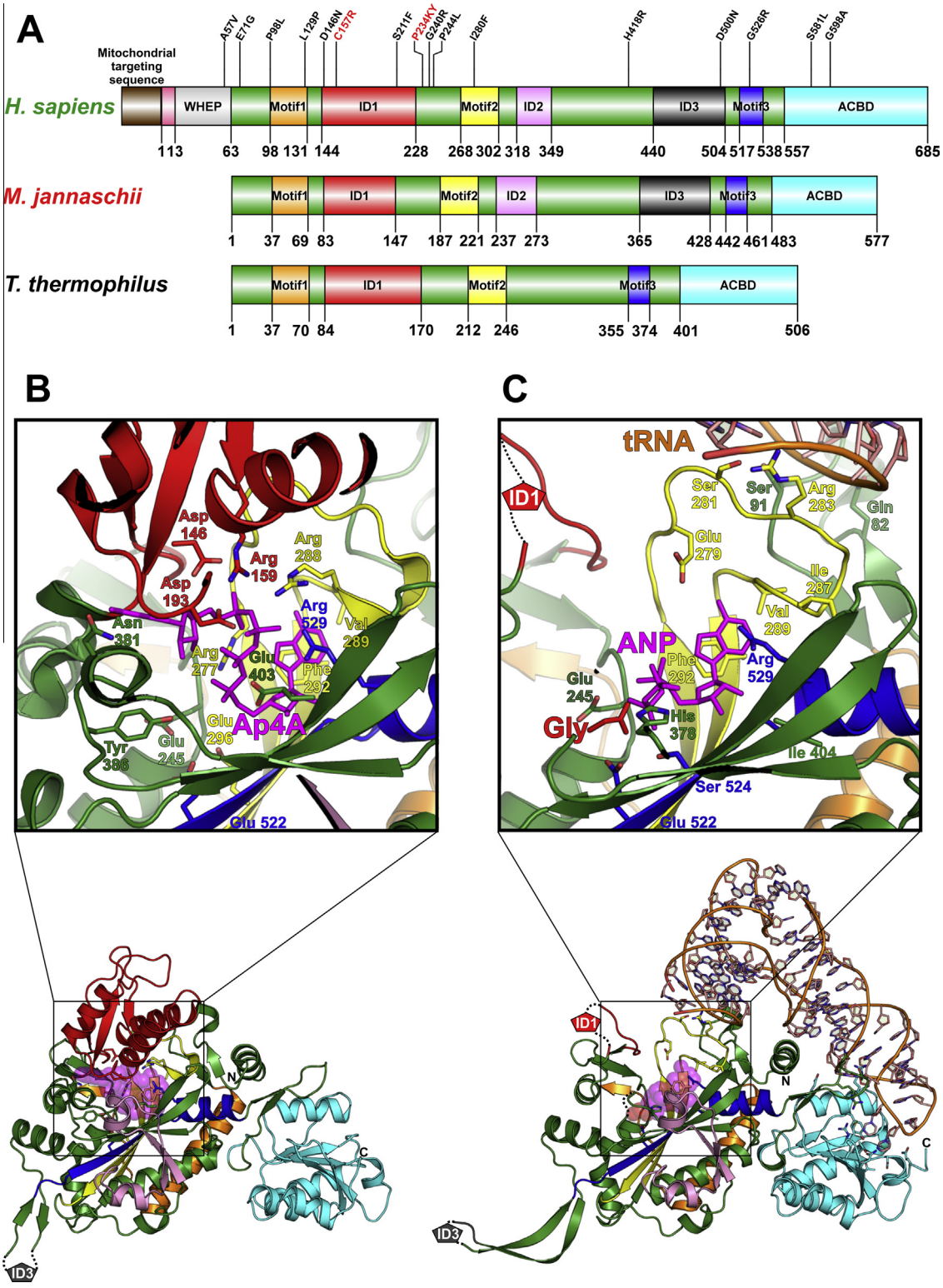

Fig.1. Domain organization of α_2GRS. (A) The domain architectures of representative members of the class IIA GRS are shown. Catalytic domain is colored green and various inserted domains and motifs are indicated. Sixteen known mutations in GRS which lead to CMT and related neuropathies are marked on the human α_2GRS. The mutations that have been identified in humans are labeled black and those identified in mouse are labeled red. (B) Ribbon diagram of human α_2GRS with Ap4A bound at the active site (PDB identifier 2ZT5). (C) Ribbon diagram of human α_2GRS with tRNA, ANP and glycine bound at the active site (PDB identifier 4KR3). Insets reveal a detailed view of the active site pockets and interactions of various residues with the bound moieties. Ap4A and ANP are colored in magenta and glycine at the active site is colored red and shown as spheres and sticks in the full structure and in the inset, respectively. The side chains of various interacting residues are shown as sticks. ANP, Ap4A, tRNA and Gly at the active site and various key residues are labeled. The coloring scheme of the various regions of GRS structures in (B, C) follows that used in (A). Abbreviations: ID (insertion domain), ACBD (anticodon binding domain), ANP (phosphoaminophosphonic acid-adenylate ester), *H. sapiens* (*Homo sapiens*), *M. jannaschii* (*Methanococcus jannaschii*), *T. thermophilus* (*Thermus thermophilus*).