CCA tRNA nucleotidyltransferase 2.7.7.72

1 Nomenclature

EC number
2.7.7.72

Systematic name
CTP,CTP,ATP:tRNA cytidylyl,cytidylyl,adenylyltransferase

Recommended name
CCA tRNA nucleotidyltransferase

Synonyms
A-adding tRNA nucleotidyltransferase <6> [1]
C-adding tRNA nucleotidyltransferase <6> [1]
CCA transferase <1> [2]
CCA-adding enzyme <7,8> [1]
CCA-adding enzyme/poly(A) polymerase <7,8> [1]
CCase <1> [2]
NTSFII <6> [1]
NTSFIII <6> [1]
TNT <6> [1]

2 Source Organism

<1> *Bacillus subtilis* [2]
<2> *Escherichia coli* [3]
<3> *Homo sapiens* [3]
<4> *Geobacillus stearothermophilus* [3]
<5> *Archaeoglobus fulgidus* [4]
<6> *Geobacter sulfurreducens* [1]
<7> *Bacillus clausii* (UNIPROT accession number: Q5WGA1) [1]
<8> *Thermus thermophilus* (UNIPROT accession number: Q72K91) [1]

3 Reaction and Specificity

Catalyzed reaction
a tRNA precursor + 2 CTP + ATP = a tRNA with a 3' CCA end + 3 diphosphate
a tRNA precursor + CTP = a tRNA with a 3' cytidine end + diphosphate
a tRNA with a 3' cytidine + CTP = a tRNA with a 3' CC end + diphosphate
a tRNA with a 3' CC end + ATP = a tRNA with a 3' CCA end + diphosphate

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Springer Handbook of Enzymes S9, DOI 10.1007/978-3-642-36240-8_97,

Substrates and products

S ATP + tRNA-C-C <6,7,8> (Reversibility: ?) [1]
P tRNA-C-C-A + diphosphate
S CTP + tRNA-C <6> (Reversibility: ?) [1]
P tRNA-C-C + diphosphate
S $tRNA^{Cys}$ + 2 CTP + ATP <1> (<1> insertional editing of substrate is not required for addition of the CCA sequence by CCase [2]) (Reversibility: ?) [2]
P $tRNA^{Cys}$ with 3'-CCA end + 3 diphosphate
S yeast $tRNA^{Phe}$ + 2 CTP + ATP <2,3,4> (<2,3,4> preparation of substrate lacking the CCA-terminus or ending with a partial CCA-end [3]) (Reversibility: ?) [3]
P yeast $tRNA^{Phe}$ with 3'-CCA end + 3 diphosphate
S Additional information <6> (<6> NTSFIII is inactive with CTP and $tRNA^{Asp}$-C as substrates [1]) (Reversibility: ?) [1]
P ?

4 Enzyme Structure

Molecular weight

45590 <6> (<6> NTSFII [1]) [1]
98390 <6> (<6> NTSFIII [1]) [1]

Subunits

Additional information <3> (<3> class II enzymes found in bacteria and eukaryotes carry a flexible loop in their catalytic core required for switching the specificity of the nucleotide binding pocket from CTP- to ATP-recognition, with the existence of conserved loop families. Loop replacements within families do not interfere with enzymatic activity. Modeling experiments suggest specific interactions of loop positions with important elements of the protein, forming a lever-like structure [3]) [3]

5 Isolation/Preparation/Mutation/Application

Purification

<6> (purified by a combination of ammonium sulfate fractionation, gel filtration, and hydrophobic interaction chromatography (NTSFII)) [1]
<6> (purified by a combination of anion-exchange chromatography and hydrophobic interaction chromatography) [1]

Crystallization

<3> (modeling of a loop sequence inserted into the structure of human CCA-adding enzyme based on PDB-entry 1OU5. The conserved loop residue R105 forms a salt bridge to the first residue E164 of the amino acid template EDxxR in motif D) [3]

<5> (cocrystal structures of the enzyme complexed with both a tRNA mimic and nucleoside triphosphates under catalytically active conditions. The structures suggest that adenosine 5'-monophosphate is incorporated onto the A76 position of the tRNA via a carboxylate-assisted, one-metal-ion mechanism with aspartate 110 functioning as a general base. The discrimination against incorporation of cytidine 5'-triphosphate at position 76 arises from improper placement of the a phosphate of the incoming CTP, which results from the interaction of C with arginine 224 and prevents the nucleophilic attack by the 3' hydroxyl group of cytidine75) [4]

Cloning

<2> (expression in Escherichia coli) [3]
<3> (expression in Escherichia coli) [3]
<4> (expression in Escherichia coli) [3]
<6> (expressed in Escherichia coli BL21(DE3)/pLysS Rosetta cells) [1]
<7> (expressed in Escherichia coli) [1]
<8> (expressed in Escherichia coli) [1]

Engineering

Additional information <1,2,3,4> (<1> construction of a conditional CCA transferase mutant that exhibits a 20% increase in doubling time when grown in the absence of inducer IPTG, and a growth rate identical to that of the wild-type strain when grown with IPTG. The cca mutation in combination with either pnpA, encoding PNPase, an enzyme with exonuclease and poly(A) polymerase activities, or rnr, encoding RNase R, an enzyme that degrades strong stem-loop structures, affects growth more than either mutation alone [2]; <2> replacement of residues 100-117 in the human enzyme by the corresponding part of the Escherichia coli enzyme, positions 66-87, leading to the chimera HEH with human enzyme N-terminus, Escherichia coli flexible loop, human enzyme C-terminus. Replacement of the region in the Escherichia coli enzyme by either the human loop element, representing the reciprocal experiment, chimera EHE, or by the Bacillus stearothermophilus part, resulting in chimera EBE. Whereas the wild-type enzymes incorporate the complete CCA sequence, the chimeric enzymes EHE, HEH and EBE show a reduced activity and add only 2 C residues to the tRNA substrate. The chimeras EHE, HEH show a 45-to 145fold reduced k_{cat} for A-incorporation. The corresponding K_M values are consistent with the K_M values of the loop donor enzymes [3]; <3> replacement of residues 100117 in the human enzyme by the corresponding part of the Escherichia coli enzyme, positions 6687, leading to the chimera HEH with human enzyme N-terminus, Escherichia coli flexible loop, human enzyme C-terminus. Replacement of the region in the Escherichia coli enzyme by the human loop element, representing the reciprocal experiment, chimera EHE. Whereas the wild-type enzymes incorporate the complete CCA sequence, the chimeric enzymes EHE, HEH show a reduced activity and add only 2 C residues to the tRNA substrate. The chimeras EHE, HEH show a 45- to 145fold reduced k_{cat} for A-incorporation. The corresponding K_M values are consistent with the K_M values of the loop donor enzymes [3]; <4> replacement of residues 66-87 in the Escherichia coli en-

zyme by the Bacillus stearothermophilus loop element, resulting in chimera EBE. Whereas the wild-type enzymes incorporate the complete CCA sequence, the chimeric enzyme EBE shows a reduced activity and adds only 2 C residues to the tRNA substrate. The chimera EBE shows a reduced k_{cat} for A-incorporation. The corresponding K_M value is consistent with the K_M values of the loop donor enzymes [3]) [2,3]

References

[1] Bralley, P.; Cozad, M.; Jones, G.: Geobacter sulfurreducens contains separate C- and A-adding tRNA nucleotidyltransferases and a poly(A) polymerase. J. Bacteriol., **91**, 109-114 (2009)
[2] Campos-Guillen, J.; Arvizu-Gomez, J.L.; Jones, G.H.; Olmedo-Alvarez, G.: Characterization of tRNA(Cys) processing in a conditional Bacillus subtilis CCase mutant reveals the participation of RNase R in its quality control. Microbiology, **156**, 2102-2111 (2010)
[3] Hoffmeier, A.; βt, H.; Bluschke, A.; Gunther, R.; Junghanns, S.; Hofmann, H.J.; Morl, M.: Unusual evolution of a catalytic core element in CCA-adding enzymes. Nucleic Acids Res., **38**, 4436-4447 (2010)
[4] Pan, B.; Xiong, Y.; Steitz, T.A.: How the CCA-adding enzyme selects adenine over cytosine at position 76 of tRNA. Science, **330**, 937-940 (2010)