Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 327 (2005) 382–392
BBRC
www.elsevier.com/locate/ybbrc

Characterization of *Aquifex aeolicus* RNase E/G

Vladimir R. Kaberdin[a,*], Thierry Bizebard[b,1]

[a] *Max F. Perutz Laboratories, Department of Microbiology and Genetics, University Departments at the Vienna Biocenter, Dr. Bohrgasse 9/4, A-1030 Vienna, Austria*
[b] *Laboratoire de Biochimie et Enzymologie Structurales, CNRS UPR 9063, Bat. 34, CNRS, 91198 Gif-sur-Yvette, Cedex, France*

Received 30 November 2004
Available online 18 December 2004

Abstract

The RNase E/G homologue from the thermophilic eubacterium *Aquifex aeolicus* has been overexpressed in *Escherichia coli*, purified, and characterized in vitro. We show that *A. aeolicus* RNase E/G has a temperature-dependent, endoribonucleolytic activity. The enzyme site-specifically cleaves oligonucleotides and structured RNAs at locations that are partly overlapping or completely different when compared to the positions of *E. coli* RNase E and RNase G cleavage sites. The efficiency of cleavage by *A. aeolicus* RNase E/G is dependent on the 5′-phosphorylation status of RNA suggesting differential susceptibility of primary transcripts and their degradative intermediates to the nuclease activity of this enzyme in vivo. Similar to *E. coli* RNase E, *A. aeolicus* RNase E/G is able to selectively cleave internucleotide bonds in the 3′–5′ direction, and to cut in intercistronic regions of putative tRNA precursors, thus suggesting a common function for RNase E/G homologues in eubacteria.
© 2004 Elsevier Inc. All rights reserved.

Keywords: *Aquifex aeolicus*; Thermophilic RNase; RNA processing

The RNase E/G family of endoribonucleases is believed to play the central role in RNA processing and decay in *Escherichia coli*, and presumably in many other bacterial species (for reviews, see [1–3]). The best-characterized members of this family are *E. coli* RNase G and RNase E, polypeptides of 489 and 1061 amino acids, respectively.

Escherichia coli RNase G (previously known as Caf A) was originally characterized as a protein whose overproduction in *E. coli* causes significant morphological changes, including the formation of singular intracellular structures, which are referred to as cytoplasmic axial filaments [4,5]. However, based on its sequence similarity to the nearly entire N-terminal catalytic domain of RNase E (residues 1–498, [6]) and on in vivo analysis, CafA was later renamed and assigned to the family of RNase E-like ribonucleases [7,8].

Similar to *E. coli* RNase E (for a review, see [1]), *E. coli* RNase G site-specifically cleaves in single-stranded regions of structured RNAs [9] and shows a preference for RNA transcripts that bear monophosphate groups at their 5′ ends [9–11]. Although previous work has shown that the substrate specificities of *E. coli* RNase E and RNase G are overlapping [9], little is known about specific sequence determinants recognized by RNase G and their evolutionary conservation.

In contrast to RNase E, which plays a prominent role in ribosomal and tRNA processing in *E. coli* as well in degradation and stability control of mRNA transcripts and small regulatory RNAs [12–16], only a limited number of targets for *E. coli* RNase G have been identified [17–20], from which the best characterized is the precursor of 16S rRNA [7,8].

* Corresponding author. Fax: +43 1 4277 9546.
E-mail address: vladimir.kaberdin@univie.ac.at (V.R. Kaberdin).
[1] Present address: Unité de Régulation de l'Expression Génétique chez les Microorganismes, CNRS UPR9073, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France.

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.12.017

Given that it is not always easy to distinguish whether a homologue in an organism distantly related to *E. coli* is an RNase E or RNase G, the term RNase E/G has recently been adopted [21–23]. Based on the position and sequence of their putative catalytic domains and on the presence or absence of auxiliary regions, RNase E/G-like proteins have been classified into four groups [23]. According to this classification, *E. coli* RNase E and RNase G belong to type I and type IV enzymes, respectively [23]. Despite its apparently redundant role in *E. coli*, RNase G-like enzymes (type IV, [23]) may have essential functions in other bacteria, particularly in species lacking RNase E/G homologues of type I, II or III, such as the thermophilic bacterium *Aquifex aeolicus* [24]. This consideration prompted us to purify and characterize *A. aeolicus* RNase E/G. The biochemical analysis of this enzyme revealed significant differences in the substrate specificity of the *A. aeolicus* and *E. coli* RNase E/G homologues, and demonstrated the 5′-end dependency and 3′–5′ directionality of *A. aeolicus* RNase E/G cleavages as well as differential susceptibility of RNase E/G cleavage sites under different temperature conditions. Moreover, our data suggest a role of RNase E/G cleavages in the processing of *A. aeolicus* tRNA.

Materials and methods

Plasmid construction. The entire sequence of *A. aeolicus rng* gene (Accession No. AAC07269) was generated by PCR amplification of the corresponding region of genomic DNA (a generous gift from Prof. Karl O. Stetter) using oligonucleotide primers 5′-GGGAATTCCATATGCTGGGCGACGTAGAAATC-3′ and 5′-CGGGATCCTTATCAGTAGTAGT-CCGAGTAGG-3′. The PCR product was cleaved with *Nde*I and *Bam*HI, gel purified, and ligated into pET16-b (Novagen) upon cleavage with *Nde*I and *Bam*HI.

In vitro transcription of RNA. *Hae*III-linearized plasmid pTH90 [25] was used as the template for in vitro synthesis of 9S RNA; whereas the template for in vitro synthesis of L1 transcript, corresponding in sequence to the 5′-end single-stranded region of RNAI and its first stem–loop, was prepared by annealing of two oligonucleotides 5′-GGTACCTAATACGACTCACTATAGGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACC-3′ and 5′-GGTACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCCTATAGTGAGTCGTATTAGGTACC-3′. The templates for in vitro synthesis of GGGRNAI and the *A. aeolicus* tRNA precursor were generated by PCR amplifications of pBR322 and *A. aeolicus* genomic DNA, respectively. The primers used are 5′-GGTACCTAATACGACTCACTATAGGGACAGTATTTGGTATCTGCG-3′ and 5′-AACAAAAAAACCACCGCTACC-3′, and 5′-CGAATTCTAATACGACTCACTATAGGGATATTACTGAGCGGGCGTAG-3′ and 5′-TGGAGCCCAGGACCGGACTCGAACCGG-3′, respectively. The corresponding transcripts were transcribed with T7 RNA polymerase using an in vitro transcription kit from Ambion, dephosphorylated with bacterial alkaline phosphatase (MBI), and 5′-labelled using T4 polynucleotide kinase (MBI) and an excess of [γ-^{32}P]ATP (Amersham). 5′-end-labelled 9S RNA, L1 RNA, tRNA precursor, and RNAI species were then fractionated in a 6% (w/v) polyacrylamide sequencing gel and individually purified as described [26].

Synthetic oligonucleotide substrates. Oligonucleotides 5′-GGGACAGUAUUUG-3′ (BR13), 5′-ACAGAAUUUG-3′ (9SA) 5′-GAAGGAUUUAA-3′ (Omp11), 5′-UUUUUUUUUUUUUUUUUUUUU (U$_{27}$) as well as oligonucleotides 5′-pGGGACAGUAUUUG (pBR13-Fluor) and 5′-HOGGGACAGUAUUUG (HO-BR13-Fluor) with a fluorescein tag at their 3′ ends were purchased from VBC Genomics (Vienna).

Purification of Rne498, EcoRng, and AqaRng. E. coli. BL21(DE3) cells (Novagen) containing pET16b-based plasmids encoding the first 498 amino acids of *E. coli* RNase E [27], *E. coli* RNase G [9] or *A. aeolicus* RNase E/G (this study) were used to produce the Rne498, EcoRng, and AqaRng polypeptides, respectively. These His-tagged polypeptides were purified by immobilized metal affinity chromatography (IMAC) under denaturing conditions as described by the vendor of the chromatography column (Qiagen). The eluates were dialyzed against 50 mM Tris–HCl (pH 8.0), 0.1 mM EDTA, 0.5 M NaCl, and 20% (v/v) glycerol, and dithiothreitol was added to a final concentration of 1 mM. *A. aeolicus* RNase E/G was also purified under native conditions followed by the His-tag removal as described below. The bacterial extract containing 10His-tagged protein in buffer A (0.5 M NaCl, 20 mM sodium/potassium phosphate buffer, pH 7.4) was applied onto a HisTrap (Pharmacia) column equilibrated in the same buffer; the column was extensively washed with buffer A containing 50 mM imidazole and then the protein was eluted with buffer A containing 400 mM imidazole. The 10His-tag was cleaved out by incubating 10His-tagged AqaRng with factor Xa (Novagen) at 20 °C for 12 h and using 30 U of this protease per each milligram of the purified protein. Factor Xa was then removed using Xarrest (Novagen) resin according to the vendor's instructions. The separation of the polypeptide lacking the tag from free 10His-tag and uncleaved protein was performed by a second HisTrap chromatographic step using the column equilibrated in buffer A containing 50 mM imidazole. Finally, the purified protein was dialyzed against its storage buffer (0.5 M NaCl, 50 mM Tris, pH 8, 20% glycerol, 0.1 mM EDTA, and 1 mM DTT) [26] and stored in small aliquots at −80 °C. The purity of the polypeptides in their preparations was greater than 95% as judged by Coomassie blue staining of samples run on SDS/polyacrylamide gels. Mass spectrometry analysis of AqaRng purified under native and denaturing conditions was performed on a Voyager DE-STR (Applied Biosystems) MALDI-TOF mass spectrometer (courtesy of C. Marchand, IBBMC, Université Paris-Sud, Orsay, France).

Limited proteolysis. EcoRng, Rne498 or AqaRng (1.5 μg) was incubated with V8 protease (5% w:w), trypsin (1% w:w), or chymotrypsin (1% w:w) during 1 h at 37 °C in 0.15 M NaCl, 10 mM Hepes, pH 7.5, 0.1 mM EDTA, and 1 mM DTT. The reactions were stopped by heating 4 min at 95 °C in Laemmli sample buffer [28] and analyzed on a 12% SDS/polyacrylamide gel. The N-terminal sequencing of the 45-kDa fragment obtained after V8 protease treatment of EcoRng was performed on a 476A protein sequencer (ABI).

Site-specific RNase E/G cleavage of RNA. RNase E/G cleavage reactions was performed as described previously [26] using Rne498, EcoRng, AqaRng, and 5′-end-labelled substrates. The 1 nt ladders were prepared either by partial alkaline hydrolysis of each 5′-end-labelled substrate in 1 mM EDTA, 50 mM sodium carbonate (pH 9.2) at 85 °C for 20 min or by partial digestion of 5′-end-labelled oligonucleotides with S1 nuclease in buffer provided by the vendor (MBI). RNase T$_1$ digests were prepared by incubation of 5′-end-labelled transcripts in 10 mM Tris–HCl (pH 8.0), 100 mM sodium chloride, and 5 mM magnesium chloride with 1 U of RNase T$_1$ (MBI) at 37 °C for 10 min.

Results

Cloning and purification of A. aeolicus RNase E/G

To assess the evolutionary conservation of RNase E/G homologues and to learn more about RNA processing at high temperature, the *A. aeolicus* RNase E/G

Download English Version:

https://daneshyari.com/en/article/10771866

Download Persian Version:

https://daneshyari.com/article/10771866

Daneshyari.com