Classical Swine Fever Virus Glycoprotein E^{rns} Is an Endoribonuclease with an Unusual Base Specificity

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Received 2 October 2003/Accepted 22 January 2004

The glycoprotein E^{rns} of pestiviruses is a virion-associated and -secreted RNase that is involved in virulence. The requirements at the cleavage site in heteropolymeric RNA substrates were studied for E^{rns}. Limited digestion of heteropolymeric RNA substrates indicated a cleavage 5' of uridine residues irrespective of the preceding nucleotide (Np/U). To further study specificity radiolabeled RNA, molecules of 45 to 56 nucleotides in length were synthesized that contained no or a single Np/U cleavage site. Cleavage was only observed in substrates containing an ApU, CpU, GpU, or UpU dinucleotide and occurred in two steps, an initial NpU-specific and a consecutive unspecific degradation. The NpU-specific cleavage was resistant to 7 M urea while the second-order cleavage was sensitive to denaturation. Kinetic analyses revealed that E^{rns} is a highly active endoribonuclease ($k_{cat}/K_m = 2 \times 10^6$ to 10×10^6 M⁻¹ s⁻¹) with a strong affinity to NpU containing single-stranded RNA substrates ($K_m = 85$ to 260 nM).

Pestiviruses are small enveloped viruses with a single positive-stranded RNA genome and belong to the *Flaviviridae* (40). Presently the *Pestivirus* genus contains five species (3), four of which are important pathogens in farm animals, namely, classical swine fever virus (CSFV), bovine viral diarrhea virus types 1 and 2 (BVDV-1 and BVDV-2), and border disease virus (BDV) (40). One of the most remarkable features of pestiviruses is the virus-encoded glycoprotein E^{rns}, which has ribonucleolytic activity. CSFV E^{rns} was shown to be an essential structural component of the virion (41), and the RNase activity has been shown to be a determinant of the virulence of CSFV and BVDV-2 (25, 26). In addition to being a virus-associated protein, E^{rns} is secreted from CSFV-infected cells as soluble protein (32). E^{rns} is a highly N-glycosylated dimeric protein of 227 amino acids, which was typed as a member of the RNase T2 family based on a narrow sequence homology in the active-site domains [cassette 1, XXXHGL(I)WP; cassette 2, F(L)XXH(YD)EXXK(TR)HGT(AW)C] (12, 14, 15, 33, 42). The RNase T2 family encompasses a variety of secreted endoribonucleases with a distribution in all kingdoms (14, 15). RNases of the T2 type are usually larger than 200 amino acids and have no absolute base specificity but a preferred cleavage site. Most fungal T2 RNases cleave at Ap/N bonds, which is in accordance with the finding that in most ribonucleases the base preference is due to interactions with the base located at the 5'-terminal end (B1 site) (36). RNase MC1, which was isolated from bitter melon (*Momordica charantia*) and which only encompasses 191 amino acids, also belongs to the T2 RNase family. It can be distinguished from fungal T2 RNases as well as from smaller RNases in RNase A and RNase T1 families because its base specificity is based on interactions with uridine residues at the 3'-terminal site (B2 site) (16, 29, 38).

Thus far E^{rns} has been shown to degrade polynucleic acid

(42), rRNA (12), and viral genomic RNA (12, 42) in vitro. It has been shown that E^{rns} binds to a variety of cells (13), and a C-terminal transport peptide was identified which translocates the protein through the plasma membrane (20). In order to define potential substrates of E^{rns} we have determined the requirements at the cleavage site in heteropolymeric substrates and the kinetic parameters of the RNase.

Recombinant CSFV E^{rns}, which was expressed in insect cells and purified by immunoaffinity chromatography, was used for limited digestion of a ³²P-labeled RNA substrate (4, 34). As substrate the polylinker sequence of pBS SKII was transcribed with T7 RNA polymerase and was radiolabeled at the 5' end with [γ -³²P]ATP and T4 polynucleotide kinase. Digests using 20 ng of E^{rns}, 2 U of RNase T₁, or 2 U of RNase PhyM and 10⁵ cpm (5'-³²P) of RNA substrate were performed under denaturing conditions (7 M urea, 1 mM EDTA, and 20 mM sodium-citrate, pH 6.0) for 15 min at 55°C in order to avoid secondary structure formation of the RNA substrate. The RNase digestions were subjected to denaturing gel electrophoresis in an analytical polyacrylamide gel (8% polyacrylamide [PAA], 7 M urea) side by side with a radiolabeled sequencing reaction mixture of the analogous cDNA region (Fig. 1). Comparison of the patterns generated by E^{rns} and the reference endoribonucleases RNase T₁ (Gp/N) and PhyM (Up/N, Ap/N) as well as the sequencing reaction revealed that cleavage by E^{rns} occurred 5' of uridine residues. Cleavage products were identified at all Np/U dinucleotide sequences (ApU, CpU, GpU, and UpU). E^{rns} retains activity in the presence of 7 M urea even at elevated temperatures (55°C), thus the enzyme is remarkably stable. To further determine the requirements at the NpU cleavage site, experiments with synthetic RNA oligonucleotides (5'-GCCGACUUC3') were undertaken. Unfortunately these molecules were not cleaved by E^{rns}, because they were either too small or were uncleavable for other reasons. Therefore, larger single-stranded RNA substrates were designed as runoff transcripts from cDNA. To avoid the formation of secondary structures the substrate molecules consisted mainly of homopolymeric A. For this purpose oligo(dT/dA) adaptors

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FIG. 2. E^{rns} endoribonuclease requires uridine residues in position B2. (A) [32 P]UTP labeled Sub-ApU (5×10^4 cpm) was incubated without (lane 1) or with 100 ng (lane 2), 50 ng (lane 3), or 25 ng (lane 4) of purified E^{rns} in 40 mM Tris-acetate, 0.5 mM EDTA (pH 6.0) at 37°C for 20 min. The reactions were subjected to high-voltage polyacrylamide gel electrophoresis in a denaturing 8% PAA sequencing gel. Sub-ApU appears as a doublet of 57 and 58 nucleotides due to purification of the RNA transcript. The predominant cleavage results in 32 P-labeled products (PI) that are at least 14 nucleotides shorter than Sub-ApU, which corresponds to the position of the uridine residue. Cleavage products are unstable and are further degraded to oligonucleotides (PII). (B) 32 P-labeled Sub-ΔU (5×10^4 cpm) was incubated without (lane 1) or with 100 ng (lane 2) or 50 ng (lane 3) of purified E^{rns} as described above and was analyzed on the same gel. RNA without a uridine residue does not serve as substrate for E^{rns} . (C) 32 P-labeled Sub-ApU (5×10^4 cpm) was incubated for 5, 15, 30, and 60 min without (lane 1) or with Triton X-100 lysates (1%) of 10^4 PK15 cells that were not infected (panel 3) or that were infected with CSFV (panel 2) or recombinant CSFV- E^{rns} H₇₉D (panel 4) at a multiplicity of infection of 1. nt, nucleotides.

it is conceivable that E^{rns} remains attached to PI after completion of the first cleavage and continues to cleave PI at random positions. This model could explain the stabilizing effect of urea on PI, because it likely reduces the affinity of E^{rns} to the NpU-free substrate more efficiently than the affinity to the NpU site. Determination of the precise mechanism of the second cleavage is the subject of future analysis.

For the functional characterization of an enzyme and its substrate it is important to know the kinetic properties in-

involved. Thus far, kinetic data for E^{rns} are only available for an UpU dinucleotide as substrate (42). To analyze whether the ribonucleolytic activity of E^{rns} in a heteropolymeric substrate depends on the nucleotide in the 5' position of NpU, the cleavage kinetics for the radiolabeled Sub-ApU, Sub-CpU, Sub-GpU, and Sub-UpU were determined. An E^{rns} degradation assay was developed in which 20, 40, 60, 80, and 100 ng of the unlabeled RNA substrates containing trace amounts (0.2%) of the same 32 P-labeled molecule were incubated with

FIG. 3. Separation of primary and secondary cleavage by urea. ³²P-labeled Sub-ApU (5×10^4 cpm) was incubated with or without (w/o) E^{rns} (100, 30, 10, 3, or 1 ng) for 15 min in nondenaturing (40 mM Tris-acetate, 0.5 mM EDTA [pH 6.0]) (A) or denaturing buffer (7 M urea, 1 mM EDTA, 20 mM sodium-citrate [pH 6.0]) (B) at 37°C. The reactions were electrophoretically separated in small denaturing polyacryamide gels. Due to the mini-gel format, the cleavage fragments PI and PII form discrete bands. The overall activity of E^{rns} is threefold lower in urea than in Tris-acetate buffer. While in panel A the substrate is rapidly degraded into PII, panel B shows that the presence of 7 M urea stabilizes PI. Also shown is steady-state analysis of the endoribonucleolytic activity of E^{rns} .

10 ng of purified recombinant E^{rns} at 37°C for 10 to 60 s in 40 mM Tris-acetate, 0.5 mM EDTA (pH 6.0). The degradation reactions were stopped by addition of an equal volume of formamide, heated to 95°C and separated by electrophoresis in 8% PAA gels containing 7 M urea in a Mini Protean II apparatus (Bio-Rad). Each analysis was repeated at least three times. The amount of radioactivity per band was analyzed in a Fuji BAS1000 PhosphorImager. As an example, the degradation of 40 ng of Sub-GpU is shown in Fig. 4A. Because the

TABLE 1. Steady-state kinetic parameters for cleavage of Sub-ApU, Sub-CpU, Sub-GpU, and Sub-UpU by E^{rns}

Substrate	Kinetics parameter ^a			
	K_m (10^{-9} M)	V_{max} (fmol/s)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1} s^{-1}$)
Sub-ApU	258.7	151.2	1.33	5×10^6
Sub-UpU	83.8	104.8	0.9	10×10^6
Sub-GpU	103.3	95.2	0.84	8×10^6
Sub-CpU	220.1	53.7	0.47	2×10^6

^a Kinetic parameters were derived from degradation experiments (example shown in Fig. 4A) and calculated from Lineweaver-Burk plots (Fig. 4B). All parameter values are the average values of at least three independent experiments.

amount of radioactivity (in counts per minute per nanogram) was known, the turnover of RNA substrate could be measured at all time points (T_0 to T_{60}). The kinetic parameters K_m and V_{max} of Sub-ApU, Sub-CpU, Sub-GpU, and Sub-UpU were calculated from the Lineweaver-Burk plots: $1/v$ versus $1/[S]$ (22), by using Sigmablot (SPSS-Science) (shown for GpU in Fig. 4B).

Determinations of the K_m values indicate that E^{rns} has a very high affinity (K_m , 84×10^{-9} to 259×10^{-9} M) for the tested substrates in the order UpU>GpU>CpU>ApU (Table 1). For Sub-UpU the affinity of E^{rns} is 1,000-fold higher than that for the dinucleotide UpU (K_m , $872.5 \times 10^{-6} \pm 125.9 \times 10^{-6}$ M) (42). In contrast, the turnover of the substrates was fastest for Sub-ApU (151.2 fmol/s), which is three times faster than that determined for Sub-CpU. For UpU the results are ambiguous, because the cleavage site (ApUpU) actually allows two cleavages to occur. The catalytic activity of E^{rns} indicated by the k_{cat}/K_m values is considerably high (2×10^6 to $1 \times 10^7 M^{-1} s^{-1}$) and operates near the limits of diffusion (10^{-8} to $10^{-9} M^{-1} s^{-1}$) (8).

E^{rns} is a highly active RNase of the T2 RNase family with an unusual cleavage site preference. Interestingly, the same cleavage preference (Np/U) was recently determined for RNase MC1, although there is very little sequence homology outside

FIG. 4. (A) Forty nanograms of Sub-GpU, including trace amounts of ³²P-labeled Sub-CpU, was incubated with 10 ng of E^{rns} for 10, 20, 30, or 60 s at 37°C and was analyzed by electrophoresis in a small denaturing 8% PAA gel. The radioactivities of substrate and cleavage products were determined by phosphorimaging, and the counts per minute at $t = 0$ were equated with the amount of RNA substrate which allowed calculation of the turnover of RNA substrate at $t = 10, 20, 30$, and 60 s. The same experimental procedure was applied for 20, 60, 80, and 100 ng of Sub-GpU and the other substrates: Sub-ApU, -GpU, and -UpU. (B) Lineweaver-Burk analysis of values determined for the degradation of different concentrations of Sub-GpU E^{rns} .

the active center. RNase MC1 cleaves phosphodiester bonds of A, C, G, or UpU, the most favorable substrate being CpU (16). Kinetic parameters of RNase MC1, which have been determined for CpU (K_m , $1.38 \times 10^3 \pm 0.2 \times 10^3$ M; k_{cat} , 746 ± 95 min⁻¹), and the k_{cat}/K_m value ($542 \times 10^{-3} \pm 50 \times 10^{-3}$ M⁻¹ min⁻¹) (29), reveal that E^{rns} has a 10⁷-fold higher activity at least with respect to the oligomeric substrates used in our analysis.

The aim of this study was to determine the substrate specificity of the RNase E^{rns} of CSFV. In particular, the question was addressed whether E^{rns} prefers distinct sequence motifs or substrates (i.e., 28S rRNA) as it is described for several secreted ribonucleases (i.e., onconase [21]; BS-RNase [18]; and α -Sarcin, restrictocin, or mitogillin [24]) or targets a wide range of single-stranded RNA molecules. The kinetic data revealed that the substrate recognition of E^{rns} itself is not very discriminating, because the enzyme degraded the test substrates near its theoretical maximum. In other words, the affinity of E^{rns} for substrates containing the ubiquitous NpU cleavage is already so high that an even higher specificity for other (unknown) substrates is unlikely. Although the substrate specificity of E^{rns} was unaltered by using PK15 cell lysates, it is possible that other factors define the actual targets of E^{rns} as it is reported for vhs RNase of alphaherpesviruses (35). vhs RNase, which is located in the tegument of the virion (19), is released during fusion at the plasma membrane and acts as an unspecific modulator of host cell translation by cleaving mRNAs (7, 37). Degradation of mRNAs by vhs RNase is initiated near the 5' end of target mRNAs (6) and requires a cellular factor (23).

It was recently shown that nonreplicative recombination in poliovirus requires 3' phosphate and 5' hydroxyl modifications of the joining RNA fragments (9, 10). Strikingly, these ends are produced by many ribonucleases, including RNases of the T2 family, and it is also evident that E^{rns} transfers the ³²P-labeled α -phosphate of the uridine residue to the 3' end of the 5' fragment. While it is not shown whether nonreplicative recombination also applies for pestiviruses, recombinations of virus genomes with host cellular mRNAs (e.g., ubiquitin) occur with an unprecedented high frequency (1, 2, 27, 39). It is tempting to speculate that E^{rns} may even be advantageous for the virus survival and evolution by being mechanistically involved in the recombination of pestiviruses, but extensive future analyses are needed to test this hypothesis.

This work was funded by Deutsche Forschungsgemeinschaft, SFB 535. Y.H. was and G.R.-S. is a fellow of the Graduiertenkolleg "Biochemistry of Nucleoprotein-Complexes."

REFERENCES

- Baroth, M., M. Orlich, H. J. Thiel, and P. Becher. 2000. Insertion of cellular NEDD8 coding sequences in a pestivirus. *Virology* **278**:456–466.
- Becher, P., G. Meyers, A. D. Shannon, and H. J. Thiel. 1996. Cytopathogenicity of border disease virus is correlated with integration of cellular sequences into the viral genome. *J. Virol.* **70**:2992–2998.
- Becher, P., R. A. Ramirez, M. Orlich, S. C. Rosales, M. König, M. Schweizer, H. Stadler, H. Schirrmeier, and H. J. Thiel. 2003. Genetic and antigenic characterization of novel pestivirus genotypes: implications for classification. *Virology* **311**:96–104.
- Donis-Keller, H., A. M. Maxam, and W. Gilbert. 1977. Mapping adenines, guanines, and pyrimidines in RNA. *Nucleic Acids Res.* **4**:2527–2538.
- Draper, D. E., S. A. White, and J. M. Kean. 1988. Preparation of specific ribosomal RNA fragments. *Methods Enzymol.* **164**:221–237.
- Elgadi, M. M., C. E. Hayes, and J. R. Smiley. 1999. The herpes simplex virus vhs protein induces endoribonucleolytic cleavage of target RNAs in cell extracts. *J. Virol.* **73**:7153–7164.
- Everly, D. N., P. Feng, S. Mian, and G. S. Read. 2002. mRNA degradation by the virion host shutoff (vhs) protein of herpes simplex virus: genetic and biochemical evidence that vhs is a nuclease. *J. Virol.* **76**:8560–8571.
- Fersht, A. 1999. Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding. W. H. Freeman & Co., New York, N.Y.
- Gmyl, A. P., E. V. Belousov, S. V. Maslova, E. V. Khitrina, A. B. Chetverin, and V. I. Agol. 1999. Nonreplicative RNA recombination in poliovirus. *J. Virol.* **73**:8958–8965.
- Gmyl, A. P., S. A. Korshenko, E. V. Belousov, E. V. Khitrina, and V. I. Agol. 2003. Nonreplicative homologous RNA recombination: promiscuous joining of RNA pieces? *RNA* **9**:1221–1231.
- Helm, M., H. Brulé, R. Giegé, and C. Florentz. 1999. More mistakes by T7 RNA polymerase at the 5' ends of in vitro-transcribed RNAs. *RNA* **5**:618–621.
- Hulst, M. M., G. Himes, E. Newbigin, and R. J. Moormann. 1994. Glycoprotein E2 of classical swine fever virus: expression in insect cells and identification as a ribonuclease. *Virology* **200**:558–565.
- Hulst, M. M., and R. J. Moormann. 1997. Inhibition of pestivirus infection in cell culture by envelope proteins E(rns) and E2 of classical swine fever virus: E(rns) and E2 interact with different receptors. *J. Gen. Virol.* **78**:2779–2787.
- Irie, M. 1997. RNase T1/RNase T2 family RNases, p. 102–130. In G. D'Alessio and J. F. Riordan (ed.), *Ribonucleases: structures and functions*. Academic Press, New York, N.Y.
- Irie, M., and K. Ohgi. 2001. Ribonuclease T2. *Methods Enzymol.* **341**:42–56.
- Irie, M., H. Watanabe, K. Ohgi, Y. Minami, H. Yamada, and G. Funatsu. 1993. Base specificity of two plant ribonucleases from *Momordica charantia* and *Luffa cylindrica*. *Biosci. Biotechnol. Biochem.* **57**:497–498.
- Kholod, N., K. Vassilenko, M. Shlyapnikov, V. Ksenzenko, and L. Kisselev. 1998. Preparation of active tRNA gene transcripts devoid of 3'-extended products and dimers. *Nucleic Acids Res.* **26**:2500–2501.
- Kim, J. S., J. Soucek, J. Matousek, and R. T. Raines. 1995. Mechanism of ribonuclease cytotoxicity. *J. Biol. Chem.* **270**:31097–31102.
- Lam, Q., C. A. Smibert, K. E. Koop, C. Lavery, J. P. Capone, S. P. Weinheimer, and J. R. Smiley. 1996. Herpes simplex virus VP16 rescues viral mRNA from destruction by the virion host shutoff function. *EMBO J.* **15**:2575–2581.
- Langedijk, J. P. 2002. Translocation activity of C-terminal domain of pestivirus E(rns) and ribotoxin L3 loop. *J. Biol. Chem.* **277**:5308–5314.
- Leland, P. A., and R. T. Raines. 2001. Review: cancer chemotherapy - ribonucleases to the rescue. *Chem. Biol.* **8**:405–413.
- Lineweaver, H., and D. Burk. 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* **56**:658–666.
- Lu, P., H. A. Saffran, and J. R. Smiley. 2001. The vhs1 mutant form of herpes simplex virus virion host shutoff protein retains significant internal ribosome entry site-directed RNA cleavage activity. *J. Virol.* **75**:1072–1076.
- Makarov, A. A., and O. N. Ilinskaya. 2003. Minireview: cytotoxic ribonucleases: molecular weapons and their targets. *FEBS Lett.* **540**:15–20.
- Meyer, C., M. von Freyburg, K. Elbers, and G. Meyers. 2002. Recovery of virulent and RNase-negative attenuated type 2 bovine viral diarrhea viruses from infectious cDNA clones. *J. Virol.* **76**:8494–8503.
- Meyers, G., A. Saalmüller, and M. Buttner. 1999. Mutations abrogating the RNase activity in glycoprotein E(rns) of the pestivirus classical swine fever virus lead to virus attenuation. *J. Virol.* **73**:10224–10235.
- Meyers, G., N. Tautz, P. Becher, H. J. Thiel, and B. M. Kümmerer. 1996. Recovery of cytopathogenic and noncytopathogenic bovine viral diarrhea viruses from cDNA constructs. *J. Virol.* **70**:8606–8613.
- Milligan, J. F., D. R. Groebe, G. W. Witherell, and O. C. Uhlenbeck. 1987. Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. *Nucleic Acids Res.* **15**:8783–8798.
- Numata, T., A. Suzuki, M. Yao, I. Tanaka, and M. Kimura. 2001. Amino acid residues in ribonuclease MC1 from bitter melon seeds which are essential for uridine specificity. *Biochemistry* **40**:524–530.
- Ohgi, K., M. Iwama, K. Tada, R. Takizawa, and M. Irie. 1995. Role of Lys108 in the enzymatic activity of RNase Rh from *Rhizopus niveus*. *J. Biochem. (Tokyo)* **117**:27–33.
- Pleiss, J. A., M. L. Derrick, and O. C. Uhlenbeck. 1998. T7 RNA polymerase produces 5' end heterogeneity during in vitro transcription from certain templates. *RNA* **4**:1313–1317.
- Rümenapf, T., G. Unger, J. H. Strauss, and H. J. Thiel. 1993. Processing of the envelope glycoproteins of pestiviruses. *J. Virol.* **67**:3288–3294.
- Schneider, R., G. Unger, R. Stark, E. Schneider-Scherzer, and H. J. Thiel. 1993. Identification of a structural glycoprotein of an RNA virus as a ribonuclease. *Science* **261**:1169–1171.
- Simoncsits, A., G. G. Brownlee, R. S. Brown, J. R. Rubin, and H. Guilly. 1977. New rapid gel sequencing method for RNA. *Nature* **269**:833–836.
- Smiley, J. R., M. M. Elgadi, and H. A. Saffran. 2001. Herpes simplex virus vhs protein. *Methods Enzymol.* **342**:440–451.
- Steyaert, J. 1997. A decade of protein engineering on ribonuclease T1-

- automatic dissection of the enzyme-substrate interactions. *Eur. J. Biochem.* **247**:1–11.
37. **Strom, T., and N. Frenkel.** 1987. Effects of herpes simplex virus on mRNA stability. *J. Virol.* **61**:2198–2207.
38. **Suzuki, A., M. Yao, I. Tanaka, T. Numata, S. Kikukawa, N. Yamasaki, and M. Kimura.** 2000. Crystal structures of the ribonuclease MC1 from bitter melon seeds, complexed with 2'-UMP or 3'-UMP, reveal structural basis for uridine specificity. *Biochem. Biophys. Res. Commun.* **275**:572–576.
39. **Tautz, N., G. Meyers, and H. J. Thiel.** 1993. Processing of poly-ubiquitin in the polyprotein of an RNA virus. *Virology* **197**:74–85.
40. **Thiel, H. J., P. G. W. Plagemann, and V. Moennig.** 1996. Pestiviruses, p. 1059–1073. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), *Fields Virology*, 3rd ed. Lippincott-Raven Publishers, Philadelphia, Pa.
41. **Widjoatmodjo, M. N., H. G. van Gennip, A. Bouma, P. A. van Rijn, and R. J. Moormann.** 2000. Classical swine fever virus E(rns) deletion mutants: trans-complementation and potential use as nontransmissible, modified, live-attenuated marker vaccines. *J. Virol.* **74**:2973–2980.
42. **Windisch, J. M., R. Schneider, R. Stark, E. Weiland, G. Meyers, and H. J. Thiel.** 1996. RNase of classical swine fever virus: biochemical characterization and inhibition by virus-neutralizing monoclonal antibodies. *J. Virol.* **70**:352–358.