THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 24, Issue of June 15, pp. 21280–21284, 2001

Printed in U.S.A.

Isolation and Characterization of a U-specific 3′-5′-Exonuclease from Mitochondria of *Leishmania tarentolae**

Received for publication, January 12, 2001, and in revised form, March 21, 2001
Published, JBC Papers in Press, March 28, 2001, DOI 10.1074/jbc.M100297200

Ruslan Aphasizhev‡ and Larry Simpson‡§¶

From the ‡Department of Microbiology, Immunology and Molecular Genetics and the §Howard Hughes Medical Institute, University of California, Los Angeles, California 90095

We have purified a 3′-5′-exoribonuclease from mitochondrial extract of *Leishmania tarentolae* over 4000-fold through six column fractionations. This enzyme digested RNA in a distributive manner, showed a high level of specificity for 3′-terminal Us, and was blocked by a terminal dU; there was slight exonucleolytic activity on a 3′-terminal A or C but no activity on a 3′-terminal G residue. The enzyme preferred single-stranded 3′-oligo(U) overhangs and did not digest duplex RNA. Two other 3′-5′-exoribonuclease activities were also detected in the mitochondrial extract, one of which was stimulated by a 3′-phosphate and the other of which degraded RNAs with a 3′-OH to mononucleotides in a processive manner. The properties of the distributive U-specific 3′-5′-exoribonuclease suggest an involvement in the U-deletion RNA editing reaction that occurs in the mitochondrion of these cells.

3′-5′-Exoribonucleases have been shown to play important roles in the maturation and degradation of RNAs, including the 3′-end maturation of the 5.8 S rRNA (1) and pre-tRNAs (2) and both deadenylation-dependent (3) and deadenylation-independent mRNA decay in eukaryotes (4). An exonuclease activity specific for removal of the post-transcriptionally added (5) 3′-oligo(U) tail of U6 small nuclear RNA has been partially purified and characterized (6). A number of 3′-5′-exoribonucleases have also been described in bacteria and shown to be involved in RNA processing. For example, RNase E from *Escherichia coli* is a site-specific endoribonuclease that also has a 3′-5′-exonuclease activity on poly(A) and poly(U) tails (7). There is conservation of some exoribonucleases between bacteria and eukaryotes. In yeast, four of the five components of the "exosome" complex required for the 3′-end processing of the 5.8 S rRNA are homologous to bacterial 3′-5′-exoribonucleases that have distributive, processive, and phosphorolytic activities (8). In addition, a conserved 3′-5′-exonuclease domain that is similar to the 3′-5′-proofreading domain of DNA polymerases has been identified by hidden Markov model and phylogenetic analysis (9).

The presence of a U-specific 3′-5′-exoribonuclease in mitochondria of kinetoplastid protozoa was predicted by the enzyme cascade model of U-insertion/deletion RNA editing (10). This model involves an initial base-pairing interaction of specific guide RNAs with the pre-edited mRNAs just downstream of the first editing site, and a cleavage occurs at the first mismatched base of the mRNA. Then, in the case of U-deletion editing, a proposed U-specific 3′-5′-exonuclease removes non-base-paired Us from the 3′-end of the 5′-cleavage fragment, which is followed by ligation of the two mRNA cleavage fragments. Evidence from analysis of *in vitro* editing systems from the trypanosomatids *Trypanosoma brucei* (11–13) and *Leishmania tarentolae* (14, 15) has provided strong evidence for this model. All of the proposed enzymatic activities have been detected in mitochondrial extracts, including a U-specific 3′-5′-exoribonuclease activity (16). This activity has also been detected in a partially purified 20 S editing complex that showed *in vitro* U-deletion editing activity (17, 18). The exonuclease activity was proposed to be a reversal of a 3′-terminal uridylyltransferase (TUTase)[1] that adds Us to the 3′-end of the 5′-cleavage fragment at a U-insertion editing site (19), but this was disproved by showing that pyrophosphate inhibits TUTase but does not inhibit the exonuclease activity (16).

As part of a project to isolate the enzymatic and structural components of U-insertion/deletion RNA editing from mitochondria of *L. tarentolae*, we have detected several 3′-5′-exoribonuclease activities in chromatographic fractions. In this paper we report the partial purification and characterization of a U-specific 3′-5′-exonuclease activity from *L. tarentolae* mitochondria, which represents a candidate for the enzyme involved in removal of the Us in U-deletion RNA editing.

EXPERIMENTAL PROCEDURES

Cell Culture and Isolation of Mitochondrial Fractions—L. tarentolae UC strain promastigote cells were cultured in Brain Heart Infusion medium (Difco) with 10 μg/ml hemin (Sigma) at 27 °C in a BioFlow IV Fermenter (New Brunswick) in 15-liter quantities to a cell density of 100–150 × 10^6 cells/ml (late log phase). Cells were harvested by concentration in a transverse filter (Millipore Pellicon) and centrifugation. Mitochondria were isolated by the hypotonic lysis Renografin density gradient method, which yields mitochondrial fractions with little cytosolic contamination (20). Approximately 10–20 g wet weight of mitochondria were obtained from 15 liters of cell culture; ~50–100 g of mitochondria were used for each enzyme isolation experiment.

Synthetic RNAs—RNA substrates were chemically synthesized by Oligos Etc., Inc. The following synthetic RNAs were used as substrates for exonuclease assays: [G]12-U, 5′-GCUAUGUCUGCUAACUUGUUUUUUUUUUU-3′; [G]6-U, 5′-GCUAUGUCUGCUAACUUGUUUUUU-3′; [G]5-G, 5′-GCUAUACAUGCUACAUUGGGGGG-3′; [G]6-C, 5′-GCUAUGUCUGCUAACUUGCCCCCC-3′; [G]6-A, 5′-GCUAUGUCUGCUAACUUGAAAAAA-3′; [C]6-U, 5′-GCUAUGUCUGCUAACUUCUUUUUU-3′; [A]6-U, 5′-GCUAUGUCUGCUAACUUAUUUUUU-3′; [G]5-UdU, 5′-GCUAUGUCUGCUAACUUGUUUUUdU-3′. The following synthetic RNA was annealed to [G]12-U for the experiment in Fig. 8: 5′-AAAAAACAAGUUAGCAGACA-3′.

The synthetic RNAs were gel-purified and phosphorylated at the

* This research was supported in part by Grant AI09102 from the National Institutes of Allergies and Infectious Diseases. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Howard Hughes Medical Inst., University of California, 6780 MacDonald Research Laboratories, 675 Charles Young Dr. S., Los Angeles, CA 90095-1662. Tel.: 310-825-4215; Fax: 310-206-8967; E-mail: simpson@hhmi.ucla.edu.

[1] The abbreviations used are: TUTase, terminal uridylyltransferase; nt, nucleotide; DTT, dithiothreitol; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid.

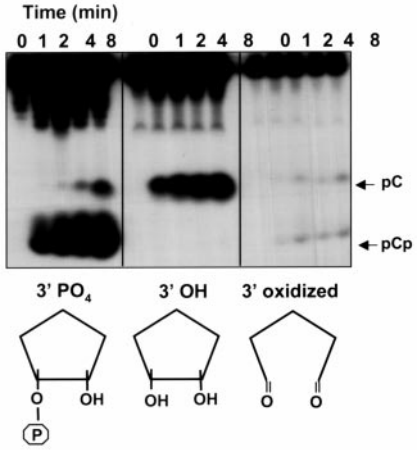

FIG. 1. **Presence of 3′-5′-exoribonuclease activities in mitochondrial extract.** The 3′-end-labeled [G]12-U synthetic RNA was incubated with clarified *L. tarentolae* mitochondrial extract, and the reactions were stopped with an equal volume of formamide with 10 mM EDTA and analyzed on a 20% acrylamide-8 M urea gel. The gel was directly exposed to a phosphorimager plate. The substrate RNA was either 3′-end-labeled with [α-^{32}P]pCp (*left panel*), [α-^{32}P]CTP unmodified (*center panel*), or subjected to periodation after [α-^{32}P]CTP labeling (*right panel*).

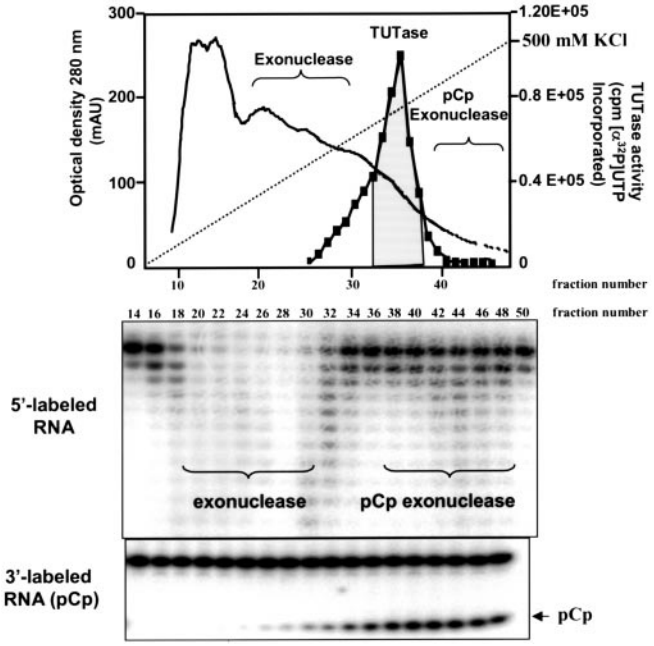

FIG. 2. **Separation of a 3′-phosphate-stimulated exonuclease activity from a nonspecific exonuclease activity by Poros HS20 chromatography.** One hundred g of Renografin-purified mitochondria from *L. tarentolae* were lysed with 1 mM CHAPS and sonication, clarified at 100,000 × *g*, applied to Sepharose Q to remove nucleic acids, and then chromatographed on Poros HS20. The location of the 3′-TUTase peak of activity is indicated, as well as two regions that showed exonucleolytic degradation of a 5′-end-labeled synthetic 30-nt RNA with 12 Us at the 3′-end ([G]12-U). A separate assay of each fraction with [α-^{32}P]pCp 3′-end-labeled RNA is shown in the *bottom panel*.

5′-end with T4 polynucleotide kinase (Life Technologies, Inc.) and [γ-^{32}P]ATP. The 3′-end labeling was carried out with T4 RNA ligase (Life Technologies, Inc.) and [α-^{32}P]pCp or with [α-^{32}P]CTP and yeast poly(A) polymerase (Life Technologies, Inc.) as recommended by the manufacturer. The exonuclease reaction contained 50 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, and 0.1 μM RNA primer. Double-stranded substrates were annealed before adding to the reaction by heating to 90 °C for 1 min followed by a 10-min incubation at room temperature. The reaction was initiated by adding 1 μl of the enzyme fraction to 9 μl of the reaction mixture and was stopped with an equal volume of formamide with 10 mM EDTA at various time intervals. Products were analyzed by 15% acrylamide/urea sequencing gel. The 69-nt ND7X transcript used as a non-complementary RNA for the experiment in Fig. 7 was obtained by T7 transcription (15) and gel-purified.

Enzyme Purification—All chromatography steps were performed at 4 °C. Mitochondria (6 g of total protein, 100 g wet weight) were extracted in 400 ml of buffer QA (50 mM Tris-HCl, pH 8.2, 5 mM $MgCl_2$, 40 mM KCl, 1 mM DTT, and 1 mM CHAPS) with sonication in 40-ml aliquots, three bursts for 30 s. One tablet of EDTA-free protease inhibitors (Roche Molecular Biochemicals) was added per 40 ml of extract. The membrane fraction was precipitated by centrifugation for 1 h at 100,000 × *g*. The supernatant was diluted with an equal volume of 50 mM Tris-HCl, pH 8.2, 10% glycerol, and 1 mM DTT and incubated with 120 ml of Sepharose Q Fast Flow (Amersham Pharmacia Biotech) for 30 min (800 ml). The suspension was transferred to an XK50/20 column (Amersham Pharmacia Biotech), washed with buffer QA containing 70 mM KCl, and eluted with QA containing 400 mM KCl (80 ml). Saturated ammonium sulfate was added to 35%, and the precipitate was collected and dissolved in 50 ml of buffer SA (50 mM HEPES, pH 7.5, 10% glycerol, 1 mM DTT, and 0.1 mM EDTA) and loaded on a 20-ml column packed with Poros HS20 (PerSeptive Biosystems). The column was developed with a 100–500 mM gradient of KCl in 400 ml. Fractions containing activity were desalted against a buffer with 20 mM Tris-HCl, pH 8.0, 50 mM KCl, 10% glycerol, and 1 mM DTT (20 ml) and loaded on an 8-ml Resource Q (Amersham Pharmacia Biotech) column, which was developed with a 50–500 mM gradient of KCl in 150 ml. After the addition of $MgCl_2$ to 10 mM, the active fractions were loaded directly on a 1-ml blue Sepharose column (Amersham Pharmacia Biotech) that was pre-equilibrated with SA buffer supplemented with 100 mM KCl and 10 mM $MgCl_2$. The column was washed with 500 mM KCl, and the activity was eluted with a 1 M KCl step. Saturated ammonium sulfate (4.05 M) was added to the active fractions to a final concentration of 0.5 M (1.5 ml) and loaded on a 5/5 phenyl-Superose column (Amersham Pharmacia Biotech), which was pre-equilibrated with 50 mM HEPES, pH 7.5,1.2 M ammonium sulfate, and 0.1 mM EDTA. The column was developed with 15 ml of a reverse gradient from 1.2 to 0 M ammonium sulfate. Active fractions (2.5 ml) were desalted against SA buffer supplemented with 50 mM KCl and 10 mM $MgCl_2$ and loaded on a 1-ml column of poly(U)-Sepharose (Amersham Pharmacia Biotech). The column was washed with 100 mM KCl and developed with 10 ml of a 100–300 mM salt gradient in the same buffer. Active fractions were dialyzed against SA buffer with 50% glycerol and stored at −20 °C. We found this enzyme to be generally unstable over lengthy isolations or storage and to be easily inactivated by multiple freezing-thawing.

3′-TUTase Assay—Enzymatic activity was measured as incorporation of [α-^{32}P]UTP (PerkinElmer Life Sciences) into RNA. The 20-μl reaction contained 50 mM Tris-HCl, pH 8.0, 10 mM magnesium acetate, 1 mM DTT, 0.1 mM [α-^{32}P]UTP (1000–2000 cpm/pmol), and 1 μM synthetic 30-mer RNA primer and was incubated at 27 °C for 10 min. The reaction was stopped with 15 μl of 0.5 M NaH_2PO_4 and 0.5% SDS, spotted on DE81 nitrocellulose filters, washed three times with 0.5 M sodium phosphate, and counted in a Beckman scintillation counter.

RESULTS

Purification of a Distributive 3′-5′-Exonuclease—The presence of 3′-5′-exonuclease activity in the crude mitochondrial extract was detected by the trimming of 5′-end-labeled RNA substrates. This assay discriminates 3′-5′-exonuclease from 5′-3′-exonuclease and endonuclease activities, but in crude extracts only nonspecific 3′-5′-RNA degradation could be detected. Substrates with both unmodified or phosphorylated 3′-ends were degraded by incubation with mitochondrial extract; oxidation of the 3′-end of the substrate RNA with sodium periodate (21) blocked this degradation (Fig. 1).

The activity, which removes a 3′-phosphorylated nucleotide (pCp exonuclease), could be separated from the 3′-OH exonuclease activity by cation-exchange chromatography (Fig. 2).

This fractionation also separated both exonuclease activities from a 3′-TUTase activity. The pCp exonuclease was further purified through a heparin column. After this column, the enzyme digested 3′-terminal Us and As equally well, 3′-terminal Cs somewhat less efficiently, and did not digest 3′-terminal Gs (Fig. 3*A*). At the same enzyme and substrate concentrations, the presence of a 3′-phosphate increased the initial velocity of the reaction by more than 10-fold (Fig. 3*B*). This activity was

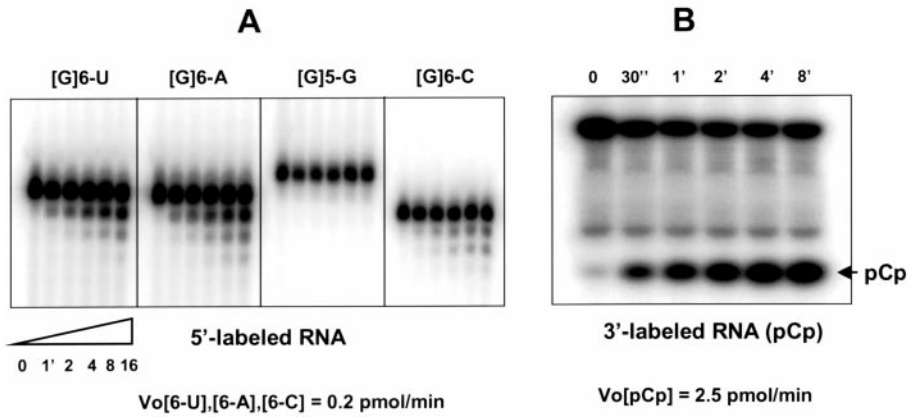

FIG. 3. **Specificity of the pCp-exonuclease activity.** *A,* 5′-end-labeled synthetic 24-nt RNA substrates (*[G]6-U, [G]6-A, [G]5-G,* and *[G]6-C*) were incubated with the partially purified pCp-exonuclease for increasing periods of time, and the degradation products were analyzed on a 20% acrylamide-8 M urea gel. *B,* [α-^{32}P]pCp 3′-end-labeled [G]6-U RNA was used as a substrate with the same enzyme preparation. The location of the released pCp nucleotide is indicated. The initial velocity for 3′-labeled RNA was measured as the rate of pCp release and for 5′-labeled RNAs as the rate of accumulation of the trimmed products.

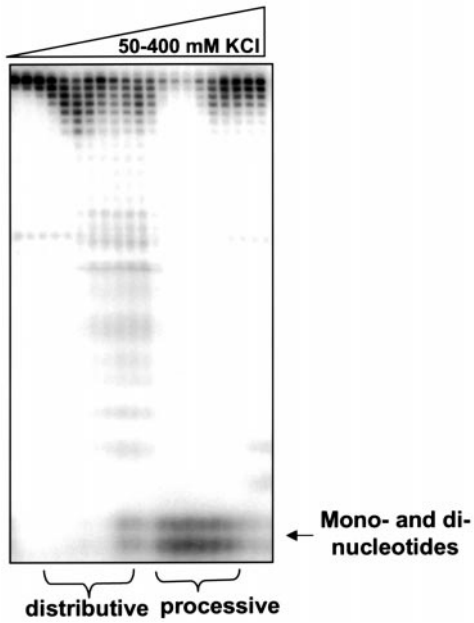

FIG. 4. **Separation of distributive and processive exonuclease activities by anion-exchange chromatography on Resource Q.** The fractions from the column in Fig. 2 eluting between 150 and 250 mM KCl were rechromatographed on an 8-ml Resource Q column (Amersham Pharmacia Biotech). Fractions were eluted with a KCl gradient and assayed for exonuclease activity using 5′-end-labeled [G]6-U RNA as substrate. The two peaks of exonuclease activity are indicated as distributive and processive, based on the oligonucleotide ladder produced by the former and the release of mono- and dinucleotides by the latter.

not purified further because the lack of specificity made it an unlikely candidate for the editing exonuclease.

The exonuclease fractions eluting at 150–250 mM KCl from the Poros S chromatography in Fig. 2 were then pooled and subjected to anion-exchange chromatography on a Resource Q column (Fig. 4). Two exonuclease activities were separated by this procedure, one of which eluted at a lower KCl concentration and produced a ladder of oligonucleotides from the 5′-end-labeled [G]6-U RNA, and the other of which eluted at a higher KCl concentration and degraded the 5′-end-labeled substrate RNA completely to mono- and dinucleotides. The RNA degradation by the processive exonuclease was nonspecific regardless of the RNA substrate used (not shown).

In the subsequent purification steps shown in Table I, the distributive exonuclease activity was enriched 4473-fold with a yield of 5% of the starting material. The final preparation consisted of ~5–7 silver-stained protein bands on an SDS gel (data not shown); a specific band representing the exonuclease has not yet been definitely identified, but gel filtration and glycerol gradient sedimentation experiments indicated a molecular size of 60–80 kDa (data not shown). The enzyme preparation is free of 3′-TUTase (see below), endoribonuclease, RNA ligase, 5′-3′-exonuclease, or poly(A) polymerase activities (data not shown).

The Exonuclease Is U-specific—The purified enzyme was assayed using 5′-end-labeled [G]6-U, [G]6-A, [G]6-C, or [G]5-G synthetic RNAs. As shown in Fig. 5*A*, only the [G]6-U RNA was effectively degraded during the 16-min assay period. A slight nibbling of the terminal nucleotide was observed with the [G]6-A and [G]6-C substrates, and no degradation of the [G]5-G substrate was detected. In a limit digestion, all six 3′-terminal Us were removed from the [G]6-U substrate RNA, leaving a 3′-terminal G residue (Fig. 5*B*).

The 3′-5′-Exonuclease and the 3′-Terminal Uridylyltransferase Are Different Proteins—As shown above in Fig. 2, the anion exchange chromatography step effectively separated the exonuclease activity from the TUTase activity. This is confirmed by the experiment in Fig. 5*C* (*left panel*), in which the addition of 10 μM UTP to the purified exonuclease had no effect on the degradation of the 6-U substrate RNA. Addition of a purified TUTase preparation[2] and UTP, on the other hand, produced 3′-extensions of the [G]6-U substrate RNA of up to several hundred Us (Fig. 5*C*, *right panel*).

The 3′-5′-Exonuclease Requires a 3′-OH and Terminates Digestion at a G or A Residue—The exonucleolytic degradation of substrate RNAs with six terminal Us preceded by a G or an A preferentially terminated at the first non-U residue (Fig. 6*A*). However, the degradation of a substrate with six terminal Us preceded by a C also showed some removal of the C residue in an extended digestion. The presence of a dU residue at the 3′-end completely inhibited the exonuclease activity (Fig. 6*B*). Oxidation of the 3′-OH of the substrate RNA by periodation also blocked the exonuclease activity (data not shown).

The 3′-5′-Exonuclease Has a Preference for a 3′ Single-stranded Oligo-U Overhang—A duplex RNA with a 3′-oligo(U) overhang was produced by annealing two synthetic complementary RNAs, and this was tested as a substrate for the 3′-5′-exonuclease (Fig. 7). A control digestion with no antisense RNA added showed complete degradation of the 12 terminal Us, as did a control digestion of the substrate annealed with a 10-fold molar excess of a non-complementary RNA. However, use of the substrate RNA annealed with 2-, 5-, and 10-fold molar excess of antisense RNA showed a limitation of exonuclease digestion to the six non-base-paired 3′-terminal Us. The limited extent of nibbling of the adjacent base-paired Us was probably due to breathing of the duplex.

[2] R. Aphasizhev, M. Peris, S. Sbiego, S. Jang, I. Aphasizheva, A. Simpson, and L. Simpson, unpublished results.

TABLE I
Purification of 3′–5′ U-specific exoribonuclease
The purification was monitored by following the degradation of 5′-end-labeled substrate RNA [A]6-U.

Step	Exo U[a]	Total protein	Specific activity	Yield	Purification
	units	*mg*	*units/mg*	*%*	*-fold*
Extract	920	6200	0.15	100	
S100	1010	1200	0.84	109	5.4
Sepharose Q	1120	680	1.65	121	11
Poros 20 HS	735	65	11.3	79	75
Resource Q	450	5.1	88.2	49	588
HP-Sepharose Blue	120	0.8	150	13	1000
Resource Phenyl	65	0.16	406	7	2700
Poly(U)-Sepharose	47	0.07	671	5	4473

[a] Exo U, nanomoles of UMP released per minute.

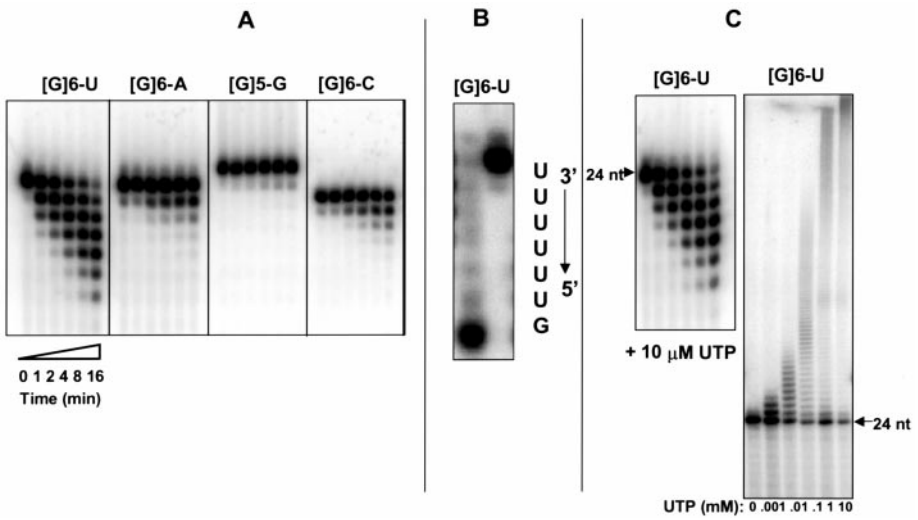

FIG. 5. **U specificity of the partially purified exoribonuclease.** *A,* 5′-end-labeled [G]6-U, [G]6-A, [G]6-C, and [G]5-G synthetic RNAs were incubated with partially purified exonuclease, and the digestion products were analyzed on a 15% acrylamide/urea gel. *B,* a limit digestion of the [G]6-U substrate RNA. *C,* the *left panel* shows the lack of effect of addition of 10 μM unlabeled UTP on the digestion of the 5′-end-labeled [G]6-U substrate RNA. The *right panel* shows the extension products of the same RNA obtained by incubating with purified 3′ *L. tarentolae* TUTase and different concentrations of unlabeled UTP.

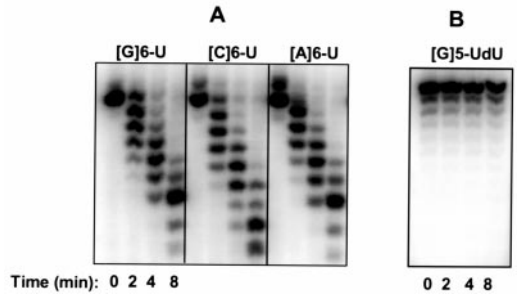

FIG. 6. **Digestion of a 3′-terminal oligo(U) stretch does not efficiently proceed past a G, A, or C residue and is blocked by a 3′-terminal dU residue.** *A,* 5′-end-labeled [G]6-U, [C]6-U, [C]6-U, and [A]6-U 24-nt synthetic RNAs were incubated with the partially purified exonuclease, and the digestion products were analyzed on a 15% acrylamide/urea gel. *B,* the [G]6-U RNA was synthesized with a deoxyuridine at the 3′-end (= *[G]5-UdU*) and used as substrate for the exonuclease reaction.

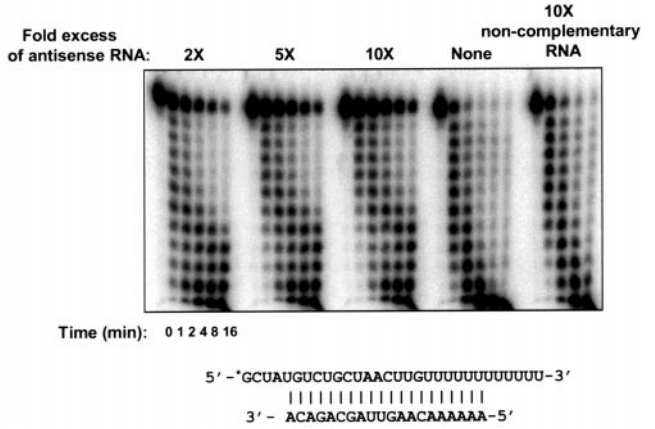

FIG. 7. **The U-specific exoribonuclease prefers a single-stranded 3′-overhang and is blocked by duplex RNA.** 5′-End-labeled (*) [G]12-U synthetic RNA was annealed with 2-, 5-, and 10-fold molar excess of a 20-nt complementary RNA (see "Experimental Procedures") that leaves the 3′-terminal 6-U single-stranded. One reaction used the substrate RNA alone, and one used the substrate RNA annealed with 10-fold excess of a non-complementary RNA (69-nt ND7X T7 transcript). The exonuclease assay was performed for the indicated times, and the products were analyzed on a 15% acrylamide/urea gel.

DISCUSSION

The distributive exonuclease is the first mitochondrial 3′-5′-exoribonuclease purified from a kinetoplastid protozoan. The enzyme has a strong preference for single-stranded oligo(U) 3′-overhangs. The origin of U specificity may reside either in a higher affinity of the exonuclease for stretches of Us or discrimination of a U from a non-U base during the catalytic reaction. The data from Fig. 6*A* indicate that even after a stretch of Us the enzyme stops more efficiently at purine bases. A C residue is discriminated less efficiently than larger purine bases. Competitive inhibition experiments with all four homoribopolynucleotides showed no specific effect on the degradation of the [G]12-U substrate RNA (data not shown). In addition, the chromatographic properties of the enzyme on poly(U) and poly(A) Sepharose-based media were virtually identical (data not shown). Taken together, these results strongly favor a specific recognition of a U at the active center.

Although the enzyme was not yet purified to homogeneity, the fact that the activity migrated as a single, fairly sharp peak during the course of the fractionation and was shown to have an apparent molecular size of 60–80 kDa suggests that the

observed specificity is a property of the enzyme and not due to copurifying auxiliary factors.

The enzymatic properties of this exonuclease are consistent with a possible role in the U-deletion RNA editing that occurs in these mitochondria, in that this reaction involves the specific removal of unpaired Us at the 3′-end of the 5′-mRNA cleavage fragment prior to religation (10, 12, 13). The removal of adjacent non-U nucleotides would produce a misedited sequence that could not be corrected by editing because U is the only nucleotide that can be added (22). The minor lack of specificity with an unpaired terminal C residue may be overcome *in vivo* because the adjacent nucleotides at the 3′-end of the 5′-cleavage fragment always have complementary nucleotides in the guide RNA and could form a short duplex region that inhibits the exonuclease digestion (23, 24). The sensitivity of the enzyme to the chemical nature of the nucleotide rather than affinity to a stretch of Us is also consistent with the presence of editing sites where deletion of only one U occurs *in vivo*. We propose that the specificity of U-deletion editing is determined by both the specificity of the exonuclease toward the U base and also by the preference for a single-stranded 3′-overhang.

It is of course possible that this enzyme is involved with other aspects of RNA metabolism in the mitochondrion of *Leishmania*, and further work is required to determine the precise role.

Acknowledgment—We acknowledge the helpful advice and assistance of everyone in the Simpson laboratory.

REFERENCES

1. Mitchell, P., Petfalski, E., and Tollervey, D. (1996) *Genes Dev.* **10,** 502–513
2. Papadimitriou, A., and Gross, H. J. (1996) *Eur. J. Biochem.* **242,** 747–759
3. Korner, C. G., and Wahle, E. (1997) *J. Biol. Chem.* **272,** 10448–10456
4. Beelman, C. A., and Parker, R. (1995) *Cell* **81,** 179–183
5. Trippe, R., Sandrock, B., and Benecke, B. J. (1998) *Nucleic Acids Res.* **26,** 3119–3126
6. Booth, B. L., Jr., and Pugh, B. F. (1997) *J. Biol. Chem.* **272,** 984–991
7. Huang, H., Liao, J., and Cohen, S. N. (1998) *Nature* **391,** 99–102
8. Mitchell, P., Petfalski, E., Shevchenko, A., Mann, M., and Tollervey, D. (1997) *Cell* **91,** 457–466
9. Moser, M. J., Holley, W. R., Chatterjee, A., and Mian, I. S. (1997) *Nucleic Acids Res.* **25,** 5110–5118
10. Blum, B., Bakalara, N., and Simpson, L. (1990) *Cell* **60,** 189–198
11. Kable, M. L., Seiwert, S. D., Heidmann, S., and Stuart, K. (1996) *Science* **273,** 1189–1195
12. Seiwert, S. D., and Stuart, K. (1994) *Science* **266,** 114–117
13. Seiwert, S. D., Heidmann, S., and Stuart, K. (1996) *Cell* **84,** 831–841
14. Frech, G. C., and Simpson, L. (1996) *Mol. Cell. Biol.* **16,** 4584–4589
15. Byrne, E. M., Connell, G. J., and Simpson, L. (1996) *EMBO J.* **15,** 6758–6765
16. Cruz-Reyes, J., and Sollner-Webb, B. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 8901–8906
17. Rusché, L. N., Cruz-Reyes, J., Piller, K. J., and Sollner-Webb, B. (1997) *EMBO J.* **16,** 4069–4081
18. Cruz-Reyes, J., Rusché, L. N., and Sollner-Webb, B. (1998) *Nucleic Acids Res.* **26,** 3634–3639
19. Piller, K. J., Rusché, L. N., and Sollner-Webb, B. (1996) *J. Biol. Chem.* **271,** 4613–4619
20. Braly, P., Simpson, L., and Kretzer, F. (1974) *J. Protozool.* **21,** 782–790
21. Kapushoc, S. T., and Simpson, L. (1999) *RNA* **5,** 656–669
22. Sturm, N. R., Maslov, D. A., Blum, B., and Simpson, L. (1992) *Cell* **70,** 469–476
23. Simpson, L., Wang, S. H., Thiemann, O. H., Alfonzo, J. D., Maslov, D. A., and Avila, H. A. (1998) *Nucleic Acids Res.* **26,** 170–176
24. Burgess, M. L., and Stuart, K. (2000) *RNA* **6,** 1492–1497