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ALTERATION OF ESCHERICHIA COLI RNase D BY INFECTION WITH BACTERIOPHAGE T4*

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SUMMARY: Infection of a variety of E. coli strains with bacteriophage T4 leads to about a 25,000 dalton increase in the apparent molecular weight of RNase D based on gel filtration on Ultrogel AcA44. No alteration occurs when infection is carried out in the presence of chloramphenicol. The change in RNase D is substantially completed by 7.5 min of infection. Chromatography of the altered RNase D on the adsorbant, Affi-gel Blue, restores the enzyme to its original molecular weight of 40,000, indicating that the modification is reversible. Mixing an extract from infected cells with one from uninfected cells converts a portion of the uninfected cell enzyme to the higher molecular weight form. No conversion takes place if the infected cell extract is first treated with phenol to inactivate proteins. Preliminary analysis indicates that the factor in infected cell extracts responsible for the conversion is a heat-labile, relatively low-molecular weight protein, and that RNase D is modified by association with this phage-specific component. The potential role of RNase D in the 3' processing of bacteriophage T4 tRNA precursors, and the involvement of a phage gene product in this process, are discussed.

Bacteriophage T4-infected E. coli synthesize two types of phage-specific tRNA precursors which differ with regard to their 3' terminal structure (2). In what we have termed type I precursors, the -C-C-A sequence is present, and is followed by extra residues which must be removed during processing to generate the mature tRNA species. In the second type, type II precursors, the -C-C-A residues are absent, and are replaced by other nucleotides which must be removed to allow tRNA nucleotidyltransferase to add the -C-C-A sequence of the mature tRNA. Although it is clear that a nucleolytic activity must be involved in these 3' processing events, the nuclease(s) responsible for final

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trimming of the extra residues at the 3' end of each type of precursor has not been conclusively identified.

We previously suggested (3) that the exonuclease, RNase D (4), is the enzyme involved in 3' processing of type I precursors, and have purified this protein to homogeneity using synthetic tRNA precursors as substrates (3,5,6). We also showed that another homogeneous exonuclease, RNase II (7), does not have the properties required for processing type I precursors (3). Using similar procedures we have attempted to identify and purify the enzyme responsible for the 3' processing of type II precursors. For these studies we made use of an E. coli mutant, termed BN (8), which fails to process the type II precursors specified by bacteriophage T4 (9). The defect in strain BN is associated with a decrease in total nucleolytic activity in uninfected cell extracts (ref. 10 and unpublished results), but we have been unable to correlate this decreased activity with the absence of a specific enzyme in the mutant strain compared to the wild type parent, E. coli B (unpublished observation).

For this reason we considered the possibility that strain BN could contain a mutated form of either RNase D or RNase II that has lost its ability to act on type II precursors, but which has otherwise remained functional. Since the "BN phenotype" only becomes apparent during bacteriophage infection, we have been examining the properties of RNase D and RNase II in extracts of bacteriophage T4-infected cells. During the course of these studies we observed that RNase D from infected cell extracts is altered compared to the uninfected cell enzyme. In this paper we present the evidence describing the alteration of RNase D.

EXPERIMENTAL PROCEDURES: E. coli strains B and BN (8) were obtained from Dr. William McClain, and the RNase II-deficient strain, S296 (11), from Dr. David Schlessinger. Cells were grown at 37°C in YT medium (8 g tryptone, 5 g yeast extract, 5 g NaCl per liter) to about 2 x 10^8 cells per ml prior to harvesting or bacteriophage T4 infection. Wild type bacteriophage T4 was purified from lysates by concentration with polyethylene glycol (12) prior to use.

Infection of E. coli with bacteriophage T4 was carried out at a multipli-

city (m.o.i.) of about 5 at 37°C. In all experiments greater than 90% of the cells were killed. Infected cells were generally harvested after 15 min of infection by rapid cooling with ice and centrifugation for 20 min at 6,000 x g. Cells were used immediately or stored frozen. Extracts from infected or uninfected cells were prepared by alumina grinding as described (10).

RNase D and RNase II were separated by gel filtration chromatography on an Ultrogel AcA44 column (3 x 100 cm) equilibrated with 0.01 M Tris-Cl, pH 7.5; 5 mM $MgCl_2$; 0.2 or 0.5 M KCl; 0.1 mM EDTA; 0.1 mM dithiothreitol; 10% glycerol. Fractions of 4.5-5.0 ml were collected at a flow rate of 10-15 ml per hour.

RNase D and RNase II activities were determined as described previously (6), using [^{32}P] phosphodiesterase-treated tRNA and [^{3}H] poly(A), except that KCl was omitted from the RNase D assays. Details of individual experiments are presented in the Figure legends.

RESULTS: Since extracts of E. coli B contain both RNase II and RNase D, we generally have first separated the two activities by gel filtration on Ultrogel AcA44 so that each enzyme could be examined individually (Fig. 1A). Surprisingly, when this procedure was applied to extracts of bacteriophage T4-infected E. coli B, the peak of activity corresponding to RNase D was absent; however, the peak corresponding to RNase II (based on activity with poly(A) as substrate) was essentially unchanged (Fig. 1B). Quantitation of activity in the RNase II peak using phosphodiesterase-treated tRNA, a substrate for both RNase D and RNase II, revealed more activity than expected based on the amount of RNase II present in the poly(A) assay. This observation suggested that the chromatographic position of RNase D may have changed so that it could no longer be resolved from RNase II.

Further chromatography of the RNase II peak on the adsorbent, Affi-Gel Blue, indicated that this explanation was correct since on this column RNase D could be separated from RNase II (data not shown). In addition, re-chromatography on Ultrogel AcA44 of the separated RNase D gave an apparent molecular weight of 40,000 (data not shown), the same as that for the enzyme from uninfected cells (3). These results indicated that the apparent molecular weight of RNase D is altered after bacteriophage T4 infection, and that the alteration is reversible. Identical results were obtained upon infection of E. coli strain BN.

In order to investigate the alteration of RNase D more easily, and to

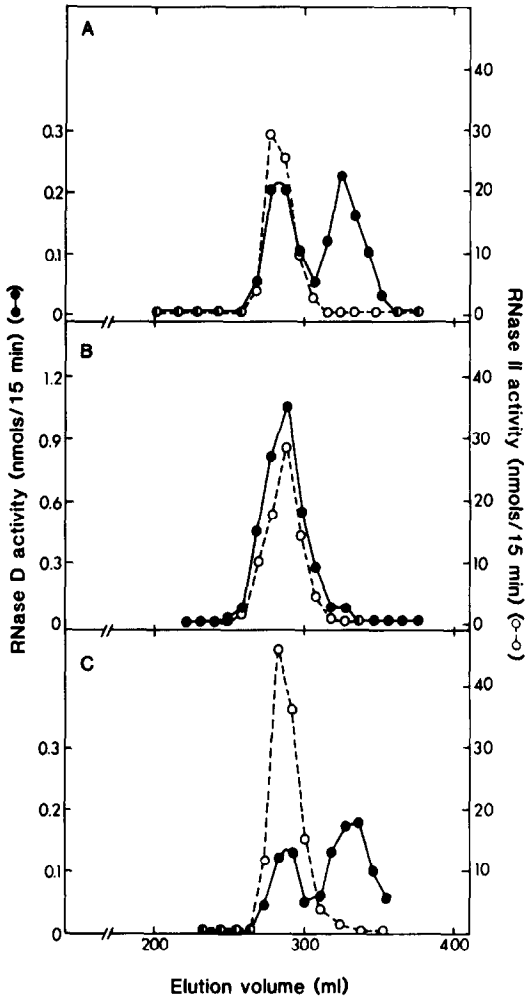

Fig. 1. Chromatography of uninfected and infected E. coli extracts on Ultrogel AcA44. Chromatography was carried out as described in "Experimental Procedures". Aliquots of 10-20 µl were assayed for RNase D and RNase II, but all assays have been normalized to 10 µl of fraction assayed for 15 min at 37°C. A. Uninfected E. coli B; B. E. coli B infected with bacteriophage T4 at a m.o.i. of 5 for 15 min; C. E. coli B infected with bacteriophage T4 at a m.o.i. of 6 for 15 min after a 20 min incubation with 100 µg per ml of chloramphenicol. The first peak containing activity on both poly(A) and phosphodiesterase-treated tRNA is RNase II, the second peak is RNase D. Note the different scale for RNase D activity in panel B.

eliminate any involvement of RNase II in this phenomenon, we made use of the RNase II mutant strain, S296 (11). As can be seen in Fig. 2A, no activity on phosphodiesterase-treated tRNA is detectable at the position of RNase II. Activity against poly(A) also is decreased more than 99% (not shown). Infection of S296 with bacteriophage T4 also led to an increase in the apparent

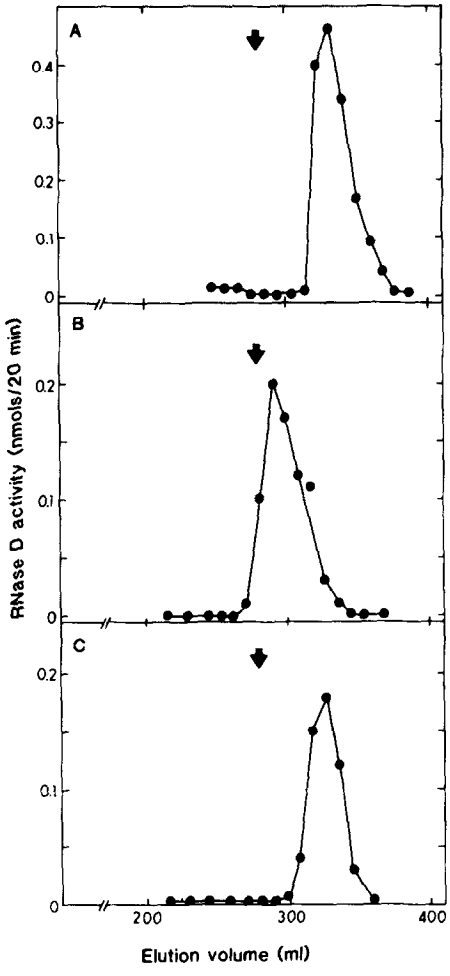

Fig. 2. Chromatography of uninfected and infected E. coli S296 extracts of Ultrogel AcA44. Chromatography and assays were carried out as in Fig. 1 except that 15 µl of each fraction was assayed for 20 min. A. Uninfected E. coli S296; B. E. coli S296 infected with bacteriophage T4 at a m.o.i. of 6 for 15 min; C. E. coli S296 infected with bacteriophage T4 at a m.o.i. of 6 for 15 min after a 20 min incubation with 100 µg per ml of chloramphenicol. The arrow indicates the position of RNase II (molecular weight = 80,000) on this column.

molecular weight of RNase D (Fig. 2B), corresponding to a change in molecular weight from 40,000 to about 65,000 daltons. Thus these results indicate that the alteration of RNase D can also occur in a K12 strain of E. coli, and that it does not involve interaction with a functional RNase II.

Alteration of RNase D does not occur in either E. coli B or S296 when infection is carried out in the presence of chloramphenicol (Figs. 1C and 2C),

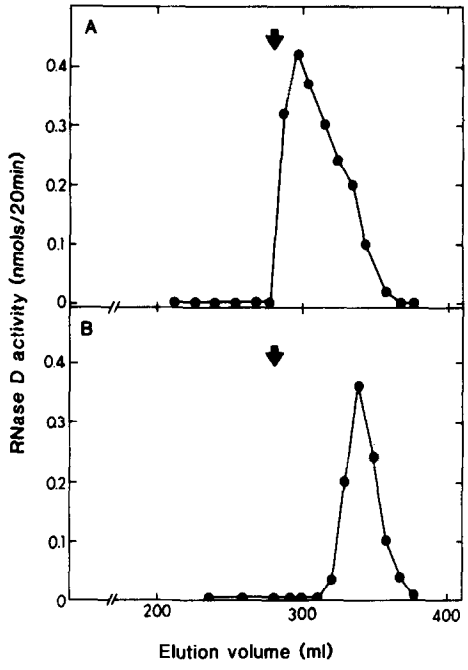

Fig. 3. Chromatography of a mixture of extracts from infected and uninfected cells. A. S30 fractions (750 µl) from infected and uninfected cells were mixed and incubated for 30 min at 0°C prior to chromatography on Ultrogel AcA44. Seventy-five percent of the RNase D activity came from the uninfected cell extract and 25% from the infected cell extract. B. Uninfected cell S30 fraction was incubated for 30 min at 0°C with an equal volume of infected cell S30 fraction that had been extracted 3 times with phenol. Residual phenol was removed by 3 extractions of the aqueous phase with ether. Chromatography and assays were carried out as in Fig. 2. The arrow indicates the position of RNase II on this column.

implicating a phage T4 gene product in the phenomenon. Examination of the time course of RNase D alteration indicated that over 70% of the enzyme is converted to the high molecular weight form by 7.5 min after infection, and that the conversion is essentially complete by 15 min. These results strongly suggest that an early gene product of phage T4 is required for the alteration of RNase D.

Although the mechanism of the phage-induced alteration of RNase D remains to be elucidated, it apparently involves a protein found in extracts of phage-infected cells. Thus, mixing extracts from uninfected and infected cells, and incubating for 30 min in ice, leads to conversion of a substantial portion of the uninfected cell RNase D to the higher molecular weight form (Fig. 3A). In contrast, the same infected cell extract treated with phenol to remove protein

does not cause alteration of RNase D (Fig. 3B). Preliminary studies indicate that the component in infected cell extracts responsible for the conversion of RNase D is heat-labile (65°C for 5 min) and relatively low molecular-weight (10,000-20,000). Studies are in progress to purify this component, and to determine its mechanism of action.

We have not observed any change in the specificity of RNase D with respect to hydrolysis of phosphodiesterase-treated tRNA or the synthetic precursor, tRNA-C-U (5) in extracts of infected cells. However, detailed comparisons of RNase D from uninfected and infected cells must properly await purification of the latter enzyme.

DISCUSSION: The data presented here suggest that the structure of RNase D is altered upon infection of E. coli with bacteriophage T4. Although the basis for this alteration is not yet completely understood, our results suggest that the most likely possibility is that RNase D associates with one or more low-molecular weight, phage-specific proteins. We favor this explanation because upon infection a) the apparent molecular weight of RNase D increases by about 25,000 daltons; b) the molecular weight change is reversible; c) the alteration does not occur if infection is carried out in the presence of chloramphenicol; d) an extract from infected cells can alter RNase D in uninfected cell extracts, and the alteration is abolished by phenol extraction of the infected cell extract; and e) the factor in the infected cell extract responsible for the alteration is a heat-labile, relatively low-molecular weight component. It should be pointed out that our results only show that alteration of RNase D occurs in vitro, and that a physiological role for such an alteration has not yet been demonstrated. However, modification of host enzymes by association with phage-specific, low-molecular weight proteins has previously been observed for RNA polymerase (13) and valyl-tRNA synthetase (14), suggesting that this method of phage-controlled modification of host enzymes may be quite general.

Inasmuch as RNase D has been implicated in 3' processing of type I tRNA precursors (3), we presume it serves a similar function in infected cells. However, this still leaves open the question of which enzyme processes the type II precursors that are synthesized in T4-infected cells. We have examined both uninfected and infected cell extracts, using a variety of chromatographic techniques, and have been unable to detect any activity, other than RNase II and RNase D, which can act on the synthetic type II precursor, tRNA-C-U (unpublished observation). In addition, we have not found any activity in E. coli B which is absent in the type II-processing mutant, strain BN, either in uninfected or infected cells, despite the fact that total activity against tRNA-C-U is decreased in the mutant strain (ref. 10 and unpublished observation). Thus, we have considered the possibility that the mutation in strain BN could be a subtle defect in RNase D (or RNase II) which only affects its ability to act on type II precursors. Experiments carried out in collaboration with Dr. William McClain have shown that purified RNase D can accurately process bacteriophage T4 type II precursors in vitro, and studies in our laboratory have suggested that RNase D activity is affected in strain BN (unpublished observations). However, as noted in Results, the phage-induced alteration of RNase D also occurs in strain BN. Nevertheless, it is worthwhile considering that the alteration in RNase D upon bacteriophage infection serves to increase the enzyme's efficiency on type II precursors, and that the proper functional alteration does not take place in strain BN. We are in the process of purifying RNase D from phage-infected cells to test these possibilities.

Finally, it should be noted that the type II tRNA precursors specified by bacteriophage T4 which require 3' trimming prior to addition of the -C-C-A sequence ($tRNA^{Pro}$, $tRNA^{Ser}$, $tRNA^{Ile}$) (15), are the same tRNAs that are affected by mutations in the T4 gene, mb (also called M1), which has been implicated in tRNA processing or stability (16,17). This raises the interesting possibility that it is this T4 gene that specifies the alteration of RNase D to allow efficient processing of type II precursors.

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