A NOVEL NUCLEOLYTIC ACTIVITY ASSOCIATED WITH RABBIT LIVER tRNA NUCLEOTIDYLTRANSFERASE[1]

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SUMMARY: Purified preparations of rabbit liver tRNA nucleotidyltransferase contain a nucleolytic activity which removes terminal CMP residues from tRNA-C-C and tRNA-C-C-C. Other tRNA molecules, such as tRNA-C-C-A, tRNA-C-A, tRNA-C-U and tRNA-C are not substrates for this reaction. The activity exhibits a sharp optimum at about pH 10 and a divalent cation (Mg^{++} or Mn^{++}) is required. The reaction is inhibited by ATP, CTP, pyrophosphate and potassium chloride. The relation of this activity to other reactions catalyzed by tRNA nucleotidyltransferase is discussed.

INTRODUCTION: Rabbit liver tRNA nucleotidyltransferase (1) catalyzes the synthesis of tRNA-C-C-A from molecules lacking all, or part, of the specific nucleotide sequence at the 3' terminus (2). In addition, the purified enzyme contains a poly (C) polymerase activity (2,3), and it also can catalyze the addition of an AMP residue to the 3' terminus of a variety of RNA molecules (4). In the presence of pyrophosphate this class of enzymes also removes residues from the 3' terminus of tRNA in a reversal of the synthetic reaction (5,6).

During the course of study of the pyrophosphorolytic reaction, it was found that nucleotides could be released from certain tRNA molecules even in the absence of pyrophosphate. The properties and specificity of this reaction are the subject of this paper.

MATERIALS AND METHODS: Homogeneous rabbit liver tRNA nucleotidyltransferase peak II, used in all the studies presented here, was purified as described

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[1] This paper is number VIII in the series: "Reactions at the 3' Terminus of Transfer Ribonucleic Acid". The previous paper is reference 4.

previously (1). tRNA-C-C-A* (asterisk indicates the labeled residue) was synthesized from tRNA-C-C with ATP and the nucleotidyltransferase; tRNA-C-C*, tRNA-C-A*, tRNA-C-U* and tRNA-C-C*-C* were made from tRNA-C and the appropriate triphosphate; tRNA-C* was synthesized by periodate oxidation of tRNA-C*-C*. Details of the preparation and characterization of specifically-labeled tRNA substrates were reported earlier (4).

Hydrolysis of specifically-labeled tRNAs was determined by the release of acid-soluble radioactivity. Reaction mixtures contained in 0.2 ml: 50 mM glycine-NaOH, pH 9.4; 5 mM $MgCl_2$; 0.1 mg labeled tRNA and enzyme. Details are in the legends. Samples were incubated at 37° for the times indicated and the reaction stopped by the addition of 0.2 ml of carrier RNA (5 mg per ml) and 0.4 ml of 20% trichloroacetic acid. After remaining in ice for 10 min the samples were centrifuged and 0.4 ml of the supernatant fluid was counted in dioxane-based scintillation fluid.

One unit of tRNA nucleotidyltransferase is the amount of enzyme which will incorporate 1 μmole of AMP into tRNA in 1 hour at 37°.

RESULTS: The properties of the nucleolytic reaction with tRNA-C-C* as substrate are presented in Table I. At pH 9.4 there was considerable release of acid-soluble material which was inhibited about 50% by pyrophosphate. In contrast, at pH 7.0, which is the optimum for the pyrophosphorolytic reaction (unpublished results), there was essentially no reaction in the absence of pyrophosphate. Both activities required the presence of a divalent cation, and the reaction at pH 9.4 was more rapid with Mn^{++} compared to Mg^{++}. Intact tRNA-C-C-A, which is a substrate for the pyrophosphorolytic reaction at pH 7.0, was also an inhibitor of that reaction, whereas it had little effect on the reaction at pH 9.4. As shown below, tRNA-C-C-A is not a substrate for the latter reaction.

The nucleolytic reaction at pH 9.4, was strongly inhibited by ATP, CTP and KCl, whereas pyrophosphorolysis was less sensitive to these substances. The potent inhibition by ATP was due to the synthesis of tRNA-C-C*-A which was then no longer a substrate for the reaction. ATP had much less effect on the

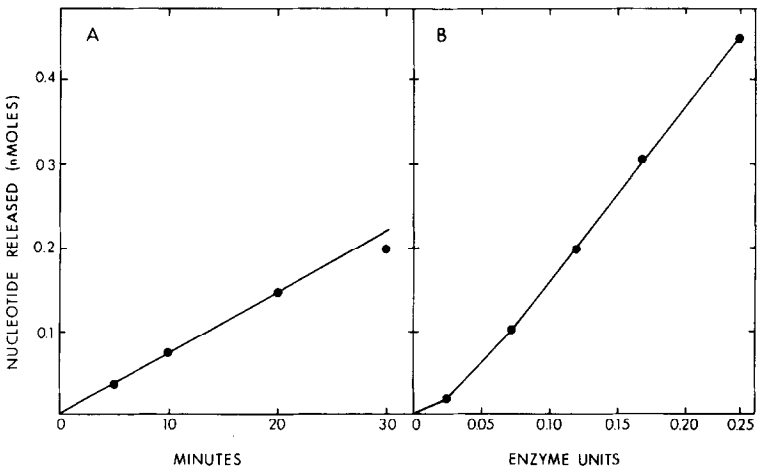

Fig. 1. Effect of time and enzyme concentration on nucleolytic activity. Reaction mixtures were as described in Methods, with 0.12 unit of enzyme for experiment A and 30 minutes of incubation for experiment B.

reaction at pH 7.0 because ATP incorporation at this pH in the presence of pyrophosphate is relatively low (unpublished results). The inhibition by CTP was similar in both reactions and was probably due to the action of poly (C) polymerase which covered up the labeled residue.

As shown in Figure 1, the nucleolytic reaction was linear for at least 30 minutes. In the presence of more enzyme and incubation for longer times, as much as 70% of the terminal residue of the tRNA-C-C* substrate was rendered acid-soluble. The reaction also responded to increasing enzyme concentrations, although it has been observed repeatedly that the reaction is relatively less effective at low enzyme levels. The explanation for this phenomenon is not known.

The nucleolytic reaction had a sharp pH optimum at about pH 10 with essentially no activity below pH 8 (Fig. 2).

The reaction was highly specific for tRNA molecules with certain 3' terminal sequences (Table II). Only tRNA-C-C* and tRNA-C-C-C* of the molecules tested were hydrolyzed. tRNA-C-C-A*, tRNA-C-A*, tRNA-C-U* and tRNA-C* were totally resistant to the nucleolytic activity. However, each of these latter tRNAs was a substrate for pyrophosphorolysis, as shown by the release of acid-

TABLE I

PROPERTIES OF THE REACTION

	Additions	Nucleotide released pmoles/30 min
Expt. 1 - pH 9.4		
complete	-	137
"	+ 2 mM PP_i	72
"	omit Mg^{++}	< 2
"	omit Mg^{++}, + 1 mM Mn^{++}	200
"	+ 0.1 mg tRNA-C-C-A	123
"	+ 1 mM ATP	< 2
"	+ 1 mM CTP	45
"	+ 0.25 M KCl	5
"	omit enzyme	< 2
Expt. 2 - pH 7.0		
complete	-	3
"	+ 2 mM PP_i	497
"	" , omit Mg^{++}	< 2
"	" , + 0.1 mg tRNA-C-C-A	318
"	" , + 1 mM ATP	298
"	" , + 1 mM CTP	258
"	" , + 0.25 M KCl	422
"	" , omit enzyme	< 2

Reaction mixtures were as described in Methods with glycine (pH 9.4) or Tris (pH 7.0) buffer, tRNA-C-C* (10^4 cpm/nmole) and 0.10 unit of tRNA nucleotidyltransferase. PP_i is sodium pyrophosphate.

soluble material in the presence of pyrophosphate. In contrast, the release of radioactive material from tRNA-C-C* and tRNA-C-C*-C* was strongly inhibited by pyrophosphate. These results demonstrate the distinct nature of the nucleolytic reaction with the latter two substrates.

TABLE II

ACTIVITY WITH DIFFERENT tRNA SUBSTRATES

	tRNA Substrate	Nucleotide released pmoles/30 min	
		$-PP_i$	$+PP_i$
Expt. 1			
	tRNA-C-C-A*	< 2	36
	tRNA-C-C*	256	124
	tRNA-C-A*	< 2	13
	tRNA-C*	< 2	30
Expt. 2			
	tRNA-C-C*	141	73
	tRNA-C-U*	2	34
	tRNA-C-C*-C*	238	132

Reactions were carried out as described in Methods at pH 9.4. Experiment 1 contained 0.19 unit of enzyme, and experiment 2, 0.10 unit.

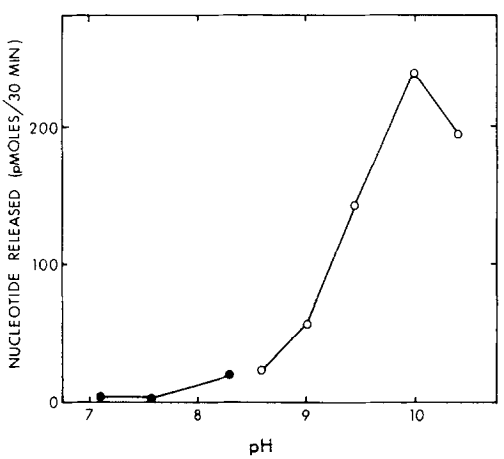

Fig. 2. Effect of pH on enzyme activity. Reaction mixtures contained 50 mM Tris or 50 mM glycine buffer of the indicated pH, 5 mM $MgCl_2$, 0.1 mg tRNA-C-C* and 0.10 unit of enzyme. Incubation was for 30 min at 37°. Tris (●-●), glycine (O-O).

Analysis on DEAE paper of the acid-soluble material released by hydrolysis of tRNA-C-C* indicated that CMP was the only product. This contrasts with the pyrophosphorolytic reaction in which the product was shown to be CTP (5,6).

DISCUSSION: The data presented here indicate that homogeneous preparations of liver tRNA nucleotidyltransferase contain a novel nucleolytic activity specific for tRNA-C-C* and tRNA-C-C*-C*. Other tRNA molecules in which only the 3' terminal residue has been changed are totally inactive. In an earlier paper (7), in order to account for the synthesis of a -C-C-A sequence by a single protein, it was suggested that this enzyme contains three triphosphate binding sites arranged in tandem. tRNA molecules would bind opposite one of the sites for acceptance of the appropriate nucleotide residue. The results in this paper lend support to such a model since the data could be explained if the nucleolytic activity was associated with only one of the three binding sites, the second site for CTP. Presumably, only the tRNA-C-C* and tRNA-C-C*-C* substrates bind to the enzyme in such a manner that their terminal residue is in this site and accessible to the nucleolytic activity. The other tRNA molecules might be expected to bind in the other triphosphate sites; for example, tRNA-C-A* and tRNA-C-C-A* would bind with their terminal AMP residue in the ATP site. In addition, since tRNA-C-U* is not a substrate, it probably binds with its terminal residue in the first, rather than the second, CTP site. Such a mode of binding is consistent with the observation that tRNA-C-U is a poor acceptor for AMP incorporation (4), and that low concentrations of CTP completely abolish UMP incorporation (7).

It is also curious that the properties of the nucleolytic activity are very similar to those of the poly (C) polymerase associated with tRNA nucleotidyltransferase (3). Both reactions are stimulated by Mn^{++}, are inhibited by ATP, and are sensitive to increased ionic strength. In addition, the rates of these two activities are almost identical, being only one percent, or less, of the normal CMP-incorporating activity. This similarity in the properties

of the two activities suggests that the same CTP-binding site may be involved in both reactions.

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