PARTICIPATION OF AN RNA FRACTION IN PEPTIDE SYNTHESIS IN THE PRESENCE OF A PURIFIED ENZYME SYSTEM FROM ALCALIGENES FAECALIS

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In our previous papers we have described an enzymic system capable of synthetizing various peptides in the presence of ribonucleoside triphosphates using free C^{14}-labeled amino acids (1). This system, purified from Alcaligenes faecalis involves four different enzymes each specific for one ribonucleoside triphosphate (2). Since these enzymes catalyze amino acid dependent P_i release from nucleotides, we have proposed an over all reaction similar to that described by Snoke and Bloch for glutathione synthesis (3).

$$X\text{-}R\text{-}P\text{-}P\text{-}P + AA \xrightarrow{E} X\text{-}R\text{-}P\text{-}P + \text{peptides} + E + P_i$$

In this paper we present evidence showing that an RNA fraction is required for amino acid dependent release of P_i from ribonucleoside triphosphates and for peptide synthesis. We also report the finding that in the presence of the enzymes and nucleoside triphosphates C^{14}-amino acids become linked covalently to the RNA fraction.

Active polypeptide synthetase preparations used in our earlier experiments contained between 1 to 3 per cent nucleotide material. Such preparations activated P_i release and peptide synthesis in the absence of added RNA. We have now obtained further purified enzyme preparations which contain not more than 0.1 % nucleotides. Using these, a requirement for RNA could be demonstrated. Several RNA preparations were tested. "Ribosomal RNA" (r-RNA) and transfer RNA (s-RNA) were isolated from washed ribosomes and 105.000 x g supernatant respectively, using the phenol method and the method described by Berg (4). In both cases, the RNA preparations were further treated with DNase and deproteinized by the Sevag method (5). Details of these techniques will be described elsewhere. The methods for P_i release from ribonucleoside triphosphates, peptide formation and their analysis are the same as already described (1).

C^{14}-amino-acyl-RNA was separated from free C^{14}-amino acids by precipitation and washing with $HClO_4$ (4) (6).

In Table I are summarized results showing that the amino acid

TABLE I

	μMole P_i released			
	ATP	GTP	aza-GTP	
Complete system	0.40	0.21	0.07	Concentrations of reac-
Omit Mg^{++}	0.01	0.01	0.01	tants in μmoles per ml:
Omit Ala	0.01	0.01	0.01	ATP 2.0 or GTP 2.0 ;Ala
Omit r-RNA	0.10	0.04	0.01	6.0 ;Tris buffer pH 8.0
r-RNA after RNase	0.11	0.04	-	100 ;$MgCl_2$ 5.0 ; r-RNA
s-RNA instead of r-RNA	0.12	0.05	-	30 μg and enzyme 300 μg.
DNA instead of r-RNA	0.12	0.04	-	Incubation 1 hour at 32°

dependent P_i release from nucleoside triphosphates is several times higher in the presence of r-RNA, while DNA or s-RNA seem to have no effect. RNase pretreated r-RNA is inactive. Residual activity observed in the absence of r-RNA is not decreased by treatment of the enzyme preparations with RNase.

The results in Table II further show that the formation of peptides with the purified enzymic system in the presence of ATP or GTP is equally dependent on an r-RNA fraction.

TABLE II

Analysis of C^{14}-alanine peptides formed in the presence of ATP

	μmoles of C^{14}-Ala in peptides	μmoles in di-peptides	μmoles in tetra-peptides	Reactants in μmoles $MgCl_2$ 5;Tris buffer pH 8.0 100 ; ATP or GTP 2.5 ; 1-alanine 6 (1.5 - 2.0 x 105 cpm) ; nucleic acid 30 μg; enzyme 400 μg.Total vol.1 ml. Incub. 2 hrs at 32°.
Complete system	0.94	0.16	0.78	
Omit r-RNA	0.12	0.12	no	
s-RNA instead of r-RNA	0.12	0.12	no	
DNA instead of r-RNA	0.16	0.16	no	

In the presence of GTP the bulk incorporation of C^{14}-alanine occurs in a tripeptide.

These results indicate that an RNA fraction present in the r-RNA preparation is an essential factor for amino acid dependent P_i release from ribonucleoside triphosphates and for peptide synthesis.These ob-

servations also suggest that RNA might serve as intermediate in the formation of peptide linkages. This is confirmed by the observations summarized in Table III which shows that in the presence of enzyme and ribonucleoside triphosphates in the complete system C^{14}-alanine becomes bound with the "r-RNA" fraction. This binding requires conditions similar to those for P_i release and peptide formation by the system. It will be noted that s-RNA appears to be virtually inactive in P_i release and peptide synthesis ; some binding however occurs in its presence. Figure 1 shows the course of C^{14}-alanine incorporation into the r-RNA fraction in the presence of different ribonucleoside triphosphates.The correlation between P_i release from ribonucleoside triphosphates,peptide synthesis in the presence of r-RNA and C^{14}-alanine-RNA complex formation strongly suggest that the transfer of C^{14}-alanine to r-RNA is an essential step in the present system.

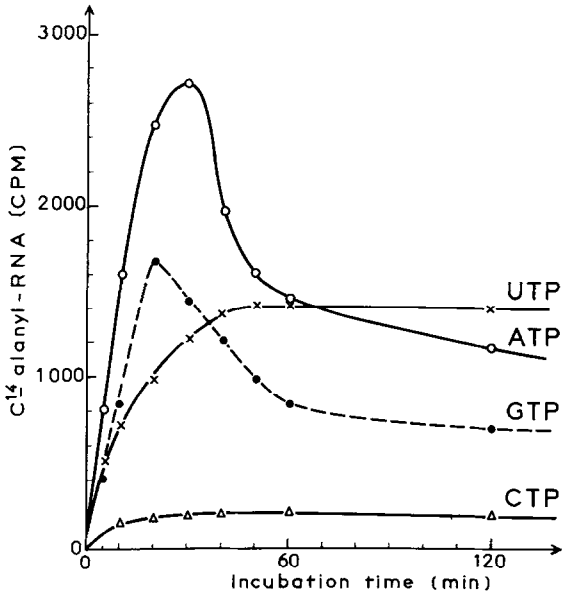

TABLE III

Conditions	ATP	GTP	UTP	CTP
Complete system	0.81	0.45	0.40	0.07
Omit nucleotide	0.02	0.01	0.01	0.01
Omit Mg^{++}	0.01	0.01	0.01	0.01
Omit enzyme	0.01	0.01	0.01	0.01
Omit r-RNA	0.01	0.01	0.01	0.01
r-RNA after RNase	0.02	0.02	0.02	0.01
r-RNA heated at 100°; 15 mn ;pH 7	0.79	0.44	0.39	0.05
s-RNA	0.17	0.17	0.16	0.05
r-RNA + s-RNA (100 µg)	0.82	0.44	0.40	0.06

Complete system contained in µmoles ; $MgCl_2$ 5; Tris buffer pH 8.0 100 ; ribonucleoside triphosphate, 0.5 ; C^{14}-amino acid 0.03 (1 x 10^5 cpm or 1.5 x 10^5 cpm) and ribosomal RNA 200 µg ; enzyme 50 µg ; total vol.1 ml; incubation for 20 mn. at 30° (30 mn. for UTP).

The above results raise the question of the type of binding which occurs between amino acid and RNA in the system. The fact that mild alcali treatment liberates C^{14}-alanine suggests that the amino acid is bound to r-RNA in ester linkage. Pretreatment of the "r-RNA" fraction with excess periodate, reduces its ability to bind the C^{14}-amino acids so far tested : Ala, Leu, His, Phe, Pro (Table IV).

TABLE IV

	mumoles of C^{14}-amino-acyl-RNA formed			
RNA used	ATP(Ala)	UTP(Leu)	CTP(His)	ATP + UTP + CTP (Ala+Leu+His)
r-RNA	0.82	0.83	0.39	3.0
r-RNA pre-treated HIO_4	0.28	0.29	0.18	1.36
r-aza-RNA	0.28	0.38	0.21	1.05
r-5-FU-RNA	0.45	0.22	0.62	1.65

This observation suggests that the major fraction of the amino acid is bound to $2^{\prime}$- or $3^{\prime}$- hydroxyl groups of terminal nucleotides. However, results obtained by electrophoretic separation at pH 3.2 (7) of C^{14}-amino acid "precharged" r-RNA, treated briefly with RNase, do not reveal any appreciable release of nucleoside -bound radioactivity. This may be considered to indicate that while the binding of the amino acid to r-RNA may not be primarily terminal, the presence of intact hydroxyl groups on the terminal nucleotides is important in the reactions.

It is known that 5-FU and other base analogs incorporated in vivo into RNA may inhibit protein biosynthesis or alter the structure of protein in microorganisms (8) (9) (10). It is of particular interest to note that "r-RNA" prepared from organisms grown in the presence of base analogs exhibits a profoundly modified capacity for accepting C^{14}-amino acids (Table IV). For instance "r-RNA " containing 5-FU, accepts 75 per cent less leucine (in the presence of UTP) but over 30 per cent more histidine (in the presence of CTP) than normal "r-RNA". Azaguanine containing "r-RNA" is a much less active acceptor for many amino acids than is normal r-RNA , or 5-FU-RNA .

The "r-RNA" preparations found to be active in the present system presumably contain different fractions of RNA , including in particular "messenger RNA "(11). It will therefore be of particular interest to determine whether one of these fractions is more specifically involved in the reactions described above.

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