*ENZYMATIC HYDROLYSIS OF N-SUBSTITUTED AMINOACYL-TRNA**

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The mechanism of the release of polypeptide chains from the ribosome-messenger RNA complex is not fully understood. It has been reported that free polypeptide chains are formed in cell-free protein-synthesizing systems, directed by polyribonucleotides, only if these polynucleotides contain statistically high frequencies of UAA codons.[1-5] However, we do not know how the chain is released from the tRNA ribosome-mRNA complex after interruption of the translation by the UAA triplet. This release implies a hydrolysis of the ester bond between polypeptide and tRNA which could be catalyzed by a specific enzyme.

The search for such an enzymatic activity necessitates the use of the relatively unstable polypeptidyl-tRNA's. It is difficult and laborious to prepare them in measurable quantities. In contrast, the chemically N-substituted aminoacyl-tRNA's, although having similar characteristics in other respects, are stable and readily synthesized.[6, 7] An enzyme capable of hydrolyzing this ester linkage between *N*-acetylamino-acids and tRNA's has now been found in extracts of *Escherichia coli*. This enzyme was partially purified and several of its characteristics were studied. The enzyme also catalyzes the hydrolysis of di-phenylalanyl-tRNA and N-substituted oligopeptidyl-tRNA's.

Material.—C^{14}-amino acids were obtained from the Commissariat à l'Energie Atomique (France); *E. coli* B tRNA, from General Biochemicals; crystalline pancreatic DNase and RNase, from Mann Research Laboratories; snake venom phosphodiesterase, from British Drug Houses Ltd.; T_1 RNase, from Sigma Corp.

E. coli leucine-specific tRNA of about 50% purity was a gift from Dr. M. Yaniv; and a sample of H^3-diphenylalanyl tRNA, from Dr. C. Ganoza.

The tRNA was charged with different C^{14}-amino acids in the presence of an *E. coli* 105,000 × *g* supernatant. The C^{14}-aminoacyl-tRNA was acetylated with acetic anhydride, as described by Haenni and Chapeville.[7] In all cases it was shown that after acetylation all amino groups of the tRNA-bound amino acids were substituted. When serine and threonine are used it is possible that the OH groups also react with acetic anhydride, forming the corresponding esters.

C^{14}-diphenylalanyl-tRNA was prepared according to Nakamoto and Kolakofsky[8] by incubating C^{14}-phenylalanyl-tRNA in the presence of ribosomes and 105,000 × *g* supernatant without addition of GTP. C^{14}-polylysyl-tRNA was prepared from an incubation mixture of *E. coli* ribosomes with C^{14}-lysyl-tRNA, poly A, GTP, and *E. coli* supernatant.[3]

Methods.—Analysis of the degradation products of N-acetylaminoacyl-tRNA: For most of the *N*-acetylaminoacyl-tRNA's, the method described below for *N*-acetylleucyl-tRNA was used.

N-acetylleucine, leucine, *N*-acetylleucyladenosine (obtained after digestion of *N*-acetylleucyl-tRNA with pancreatic ribonuclease), and *N*-acetylleucyl-tRNA were separated by paper electrophoresis (Fig. 1). Under the same conditions, after treatment with RNase T_1, two *N*-acetylleucyloligonucleotides were separated, one of which migrates with *N*-acetylleucine (Fig. 6). If a similar mixture had to be analyzed, both *N*-acetylleucyloligonucleotides would be converted to *N*-acetylleucyladenosine by treatment with pancreatic RNase before electrophoresis.

N-acetylleucyl-tRNA, *N*-acetylleucyladenylate (*N*-acetylleucyl AMP, obtained after digestion with purified venom phosphodiesterase of *N*-acetylleucyl-tRNA), *N*-acetylleucyladenosine, and

N-acetylleucine were also separated by paper chromatography (*n*-butanol, acetic acid, water; 78:5:17). The R_f's were, respectively, 0, 0.17, 0.70, and 0.92.

Preparation of E. coli extracts: The RNase I-deficient mutant MRE 600 of *E. coli*,[9] obtained through the kindness of Dr. R. Traut, was grown in complete medium and harvested during the logarithmic phase. Cells were suspended in equal volume of Tris-HCl 0.01 *M* buffer, pH 7.4, and disrupted using a French press. Cell debris was removed by centrifugation at 30,000 × *g* for 30 min and the DNA hydrolyzed by the addition of 2 μg/ml of DNase.

Purification of the hydrolase from E. coli: To 77 ml of 30,000 × *g* supernatant prepared from 60 gm of cells, were added: 25 ml of 30% solution of polyethyleneglycol (PEG 6000, purchased from Union Carbide Inc.), 8.5 ml of a 20% solution of Dextrane 500 (Pharmacia), and 0.14 ml of 4 *M* NaCl. The mixture was shaken for 1 hr and centrifuged at 15,000 × *g*, for 10 min. The upper phase was discarded, and 40 ml of Tris-HCl, 0.01 *M*, pH 7.4, were added to the lower phase. After mixing, 16 ml of the PEG 6000 solution and 23.5 gm of NaCl were added. After 1 hr of shaking, the mixture was centrifuged at 15,000 × *g*, for 10 min. The upper phase, containing the enzymatic activity, was dialyzed against three changes of 1 liter of 0.01 *M* Tris-HCl, pH 7.4.

The total volume (105 ml) of the dialyzed solution (fraction I) was passed through a DEAE-cellulose column (8 × 3 cm) previously equilibrated with a solution of Tris-HCl, 0.01 *M*, pH 7.4. The filtrate and 50 ml of washing Tris-HCl buffer were combined and concentrated to 23 ml (fraction II); then 4.1 gm of ammonium sulfate were added. After centrifugation, the upper phase containing most of the PEG was discarded. The lower phase, containing the enzyme activity was dialyzed, and could be kept at −20°C for long periods, or at 0°C for several days.

All operations were accomplished at 0–4°C. Table 1 shows the values for protein content and enzyme activity of the different fractions.

TABLE 1

ENZYMATIC ACTIVITY DURING PURIFICATION

	Total activity (U)	Total protein (mg)	Sp. act. (U/mg protein)
30,000 × *g* supernatant	1.7×10^5	4420	38.6
Fraction I	2.3×10^4	350	65.5
Fraction II	8.5×10^3	2.4	3540

The concentration of protein was determined with the method of Lowry *et al.*,[10] with bovine serum albumin as a standard. Enzyme activity was determined using paper electrophoresis as described under *Methods*. A unit (U) is defined as 1 μμM of *N*-acetylleucine from *N*-acetylleucyl-tRNA in 1 min at 37°C.

Assay for enzymatic activity: The volume of the assay mixture was 0.05 ml and contained: enzyme; 1.7 μmoles barbital-acetate buffer at pH 8.0; *E. coli* tRNA containing 75 μμmoles of *N*-acetylleucyl-tRNA. After 5 min of incubation at 37°C, the results of the reaction were determined by paper electrophoresis. The radioactive spots were located either by autoradiography or by strip-counting, and then were counted in a Tri-Carb liquid scintillation spectrometer.

When the nuclease activity of the enzyme preparation was negligible, the following method was used: after incubation of the reaction mixture, 5 μl of a 20% solution of potassium acetate at pH 5 and 0.5 ml of cold ethanol were added. After centrifugation, the radioactivity of an aliquot of the supernatant (containing the free *N*-acetylleucine) was determined at infinite thinness.

One unit of activity is defined as the amount of enzyme necessary for the formation of 1 μμmole of *N*-acetylleucine from *N*-acetylleucyl-tRNA in 1 min at 37°C.

Results.—Enzymatic hydrolysis of the N-acetylleucyl-tRNA: After incubation of *N*-acetylleucyl-tRNA, either with the 105,000 × *g* supernatant of *E. coli* MRE 600 or with the purified fractions, the analysis of the mixture showed free *N*-acetylleucine (Fig. 1). In the experiment depicted in Figure 2, about 70 out of 90 μμmoles of *N*-acetylleucyl-tRNA were hydrolyzed in 30 minutes. When the enzyme preparations were heated to 95°C for ten minutes, prior to the incubation, all activity was destroyed.

The activity of the enzyme was not changed by addition of Mg^{++} ions to a con-

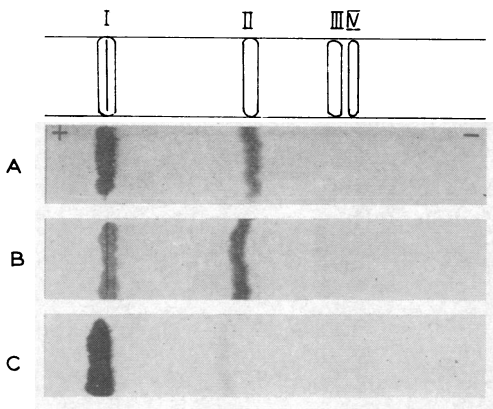

FIG. 1.—Hydrolysis of N-acetyl-C^{14}-leucyl-tRNA in the presence of fraction II. Incubation mixture (total vol 60 μl) contained in (*A*) and (*B*): barbital-acetic acid, pH 8, 2 μmoles; *N*-acetyl-C^{14}-leucyl-tRNA 100 $\mu\mu$moles; fraction II 50 μg proteins; (*C*), as (*A*) and (*B*) except that fraction II was omitted. The mixtures were incubated at 37°C, (*A*) 10 min, (*B*) and (*C*) 30 min. They were electrophorized on Arches 302 paper for 90 min at 20 v/cm in 0.5 *M* formic acid and autoradiographed on Kodirex film for 10 days. Radioactive spots were counted in liquid scintillation counter. *I*, *N*-acetylleucyl-tRNA; *II*, *N*-acetylleucine; *III*, *N*-acetylleucyl-adenosine; *IV*, leucine. The following amounts in $\mu\mu$moles of products were found: in (*A*) I, 66; II, 33; III, 1; IV, 0; in (*B*) I, 30; II, 73; III, 2; IV, 0; in (*C*) I, 93; II, 6; III and IV, 0.

centration of 10^{-2} *M*. High concentrations of certain electrolytes are inhibitory; for example, the enzyme activity was inhibited 60 per cent in 0.37 *M* NaCl. Addition of nucleic acids (uncharged tRNA, ribosomal RNA, poly U, poly A) at concentrations between 1 and 5 mg/ml resulted in a decrease of 20–90 per cent of measured enzymatic activity.

Effect of enzyme concentration: Figure 3 shows that hydrolysis is proportional to the concentration of the purified fraction II, at an enzyme protein concentration of less than 8 μg per 60 $\mu\mu$moles of substrate.

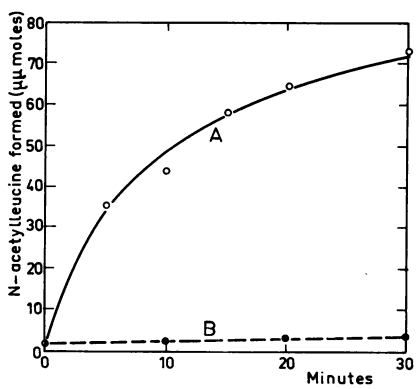

FIG. 2.—Hydrolysis of N-acetyl-C^{14}-leucyl-tRNA as a function of time (*A*) incubation mixture (total vol 250 μl) contained: barbital-acetic acid buffer pH 8, 8.5 μmoles; *N*-acetyl-C^{14}-leucyl-tRNA 90 $\mu\mu$moles; 105,000 × *g* dialyzed *E. coli* extract, 1.25 mg proteins. Incubation at 37°C. At 10-min intervals 25 μl of the mixture were analyzed as shown in Fig. 1. (*B*) Same experiment without *E. coli* extracts.

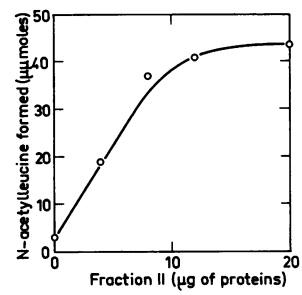

FIG. 3.—Effect of enzyme concentration. Several tubes, each containing in a total volume of 25 μl: barbital-acetic acid buffer, pH 8, 0.5 μmole; *N*-acetyl-C^{14}-leucyl-tRNA about 50% pure, 60 $\mu\mu$M; different quantities of enzymatic fraction II. After incubation at 37°C for 10 min 125 μg of carrier RNA were added and the radioactivity of *N*-acetylleucine was measured following alcohol precipitation as described in *Methods*.

Influence of pH: The rate of hydrolysis was measured at different pH values. The measurement of enzymatic activity is possible up to pH 8.5 but at higher pH's, nonenzymatic hydrolysis becomes important, making the determination of activity difficult. It was observed that, with several *N*-acetylaminoacyl-tRNA's,

the rates of enzymatic hydrolysis increased with pH, the greatest rates being observed at pH 8.5.

Approximation of K_m value: A Michaelis constant was determined, using about 50 per cent pure *N*-acetylleucyl-tRNA (Fig. 4) and the stated conditions of assay. The K_m value is approximately 5×10^{-10} *M*.

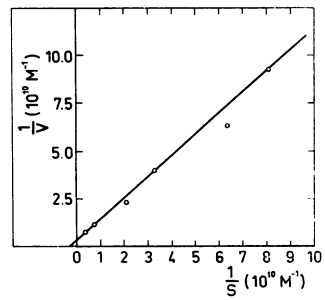

FIG. 4.—Lineweaver-Burk plot for the hydrolase. Incubation at 37°C for 5 min. The mixture contained in total volume of 55 μl: buffer pH 8, enzymatic fraction II, 4 μg of proteins and various amounts (from 10 to 520 μμmoles) of *N*-acetyl-C¹⁴-leucyl-tRNA (50% pure); the formation of *N*-acetyl-C¹⁴-leucine was measured after ethanol precipitation.

Recharge of tRNA after enzymatic deacylation: If the enzymatic deacylation of the tRNA results solely from the hydrolytic cleavage of the ester bond between *N*-acetylamino-acid and the 3′-hydroxyl group of the terminal nucleotide in the tRNA molecule, then the products of the reaction would only be *N*-acetylleucine and an intact tRNA molecule. We have already shown that *N*-acetyl-aminoacid is produced. Following the enzymatic reaction, production of a tRNA molecule (with an intact . . . pCpCpA terminal sequence) could be demonstrated by the ability to recharge the enzymatically stripped tRNA in the presence of amino acid, ATP, and aminoacyl-tRNA-synthetase.

Two identical preparations of *N*-acetylleucyl-tRNA were deacylated, respectively, by alkaline hydrolysis at pH 10 and enzymatically in the manner described previously. From these two preparations, tRNA was recovered by phenol treatment and ethanol precipitation. In each case, the leucine acceptor activity of 70 μg of tRNA was determined in the presence of purified leucyl-tRNA-synthetase.[11] Eighty μμmoles of leucine were esterified with tRNA stripped at pH 10, and 43 μμmoles, with tRNA prepared by enzymatic hydrolysis. This shows that about one half of the accepting capacity of the enzyme-treated tRNA has been lost, as compared with the nonenzymatically stripped tRNA. The contamination of the hydrolase preparation with a nuclease could explain such a difference. Nevertheless it may be concluded that the reaction catalyzed by the enzyme produces a

TABLE 2

INCUBATION OF FRACTION II WITH *N*-ACETYLLEUCYLADENOSINE

	N-acetyl-C¹⁴-leucyl-tRNA	*N*-acetyl-C¹⁴-leucine (cpm)	*N*-acetyl-C¹⁴-leucyladenosine
Incubation of *N*-acetyl-C¹⁴-leucyl-tRNA in the presence of			
RNase alone 40 min	0	740	4260
RNase alone 10 min then fraction II 30 min	0	715	4285
Buffer 40 min	4585	415	0
Fraction II alone 30 min	870	3650	480

20 μμmoles (about 10,000 cpm) of *N*-acetyl-C¹⁴-leucyl-tRNA were incubated at pH 8 for 10 min in the presence of pancreatic RNase (20 μg/ml). The incubation mixture was then divided into two parts, one of which was supplemented with enzymatic fraction II (50 μg of protein), and both were maintained at 37°C for an additional 30 min. As a control, 10 μμmoles of *N*-acetyl-C¹⁴-leucyl-tRNA were incubated in buffer for 40 min and for another control, 10 μμmoles *N*-acetyl-C¹⁴-leucyl-tRNA were incubated with enzymatic fraction II alone for 30 min. The products of the reactions were, in each case, separated by paper electrophoresis and counted. All values were normalized to 5000 total cpm per incubation mixture.

The slight difference observed in the amounts of *N*-acetylleucine formed in the presence or absence of RNase is significant. It is likely to be due to a contamination of RNase by polypeptidyl-tRNA-hydrolase.

tRNA molecule that is intact at least in those of its structural elements required for recognition by aminoacyl-tRNA-synthetase.

Substrate specificity: (I) *Polynucleotide part:* The activity of the purified fraction II has been tested using different substrates obtained from *N*-acetylleucyl-tRNA by different enzymatic degradations of the tRNA moiety. These substrates are: (1) *N*-acetylleucyl-adenosine, produced by treatment of *N*-acetylleucyl-tRNA with pancreatic RNase;[12] (2) *N*-acetylleucyl-adenosine-5′-P (*N*-acetylleucyl-AMP) produced by venom phosphodiesterase;[12] (3) *N*-acetylleucyloligonucleotides: as previously shown by Berg, Lagerkvist, and Dieckmann with leucyl-tRNA,[13] enzymatic cleavage of *N*-acetylleucyl-tRNA by T_1 RNase gave two *N*-acetylleucyloligonucleotides; the sequences of these two products, which correspond to two different $tRNA_{leu}$'s, are, respectively, CpApCpCpA- and UpApCpCpA-*N*-acetylleucine. These can be separated by paper electrophoresis under the conditions described in Figure 6.

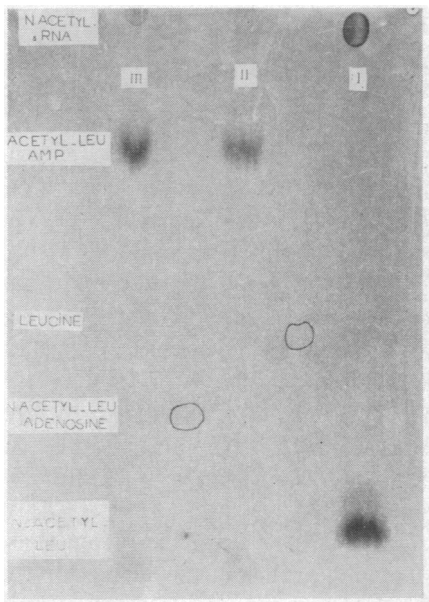

FIG. 5.—Incubation of fraction II with N-acetylleucyl-AMP. The incubation mixture contained, in 80 μl: venom phosphodiesterase, 15 μg; $MgCl_2$, 0.4 μmole; barbital-acetic acid pH 8, 4 μmoles; *N*-acetyl-C^{14}-leucyl-tRNA, 100 μμmoles. After 10 min incubation at 37°C, it was divided into two parts: to one of them 40 μg of proteins of fraction II were added and both were incubated for additional 20 min. The mixtures were then analyzed by paper chromatography as described in *Methods*. The figure shows the corresponding autoradiogram. (*I*) As a control, incubation in the presence of fraction II alone for 30 min; (*II*) phosphodiesterase alone; (*III*) phosphodiesterase and fraction II.

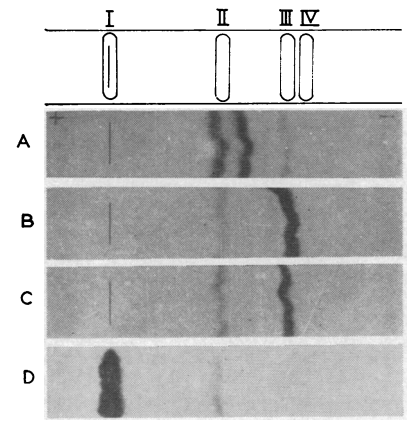

FIG. 6.—Incubation of enzymatic fraction II with T_1 RNase digest of N-acetyl-C^{14}-leucyl-tRNA. Incubation mixture (total vol 100 μl): barbitalacetic acid pH 8, 4 μmoles; *N*-acetyl-C^{14}-leucyl-tRNA, 250 μμmoles; T_1 RNase, 100 units. After 15 min incubation at 37°C, an aliquot was analyzed by paper electrophoresis (*A*); the autoradiogram shows three spots: from left to right, two major spots containing respectively 35 and 63%, and a minor spot containing 3% of the total radioactivity. The first two are *N*-acetyl-C^{14}-leucyloligonucleotides, and the third, *N*-acetyl-C^{14}-leucyladenosine. The remaining mixture was divided into two parts (*B* and *C*), one of which (*C*) was supplemented with enzymatic fraction II (50 μg of proteins). After an additional 30 min incubation, 2 μg of pancreatic RNase were added to both mixtures. They were again incubated for 5 min and subjected to electrophoresis. The autoradiogram shows a minor spot of *N*-acetyl-C^{14}-leucine (*II*), and a major spot of *N*-acetyl-C^{14}-leucyladenosine (*III*). The proportions of *N*-acetylleucine were, in (*B*), 9%, and in (*C*), 24% of the total. (*D*) was the same control as (*C*) in Fig. 1.

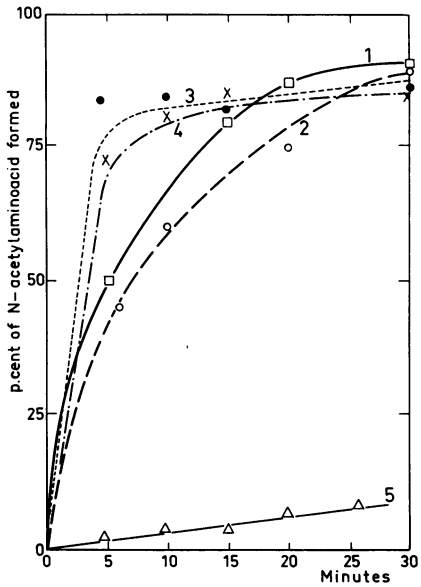

FIG. 7.—Enzymatic hydrolysis of different N-acetyl-C^{14}-aminoacyl-tRNA's. Incubation mixtures (total vol 250 μl) containing barbital-acetic acid pH 8, 10 μmoles, *N*-acetyl-C^{14}-amino acid, 150 $\mu\mu$moles, and fraction II (50 μg of protein), were incubated at 37°C. At different times, aliquots were taken and per cent of ethanol soluble C^{14} material was determined as described in *Methods*. (1) *N*-acetyl-C^{14}-leucyl-tRNA; (2) *N*-acetyl-C^{14}-valyl-tRNA; (3) *N*-acetyl-C^{14}-seryl-tRNA; (4) *N*-acetyl-C^{14}-threonyl-tRNA; (5) was a control with *N*-acetyl-C^{14}-leucyl-tRNA without fraction II. The non-enzymatic hydrolysis for the others was similar or lower than in 5.

The experiments reported in Table 2 and in Figure 5 show that free *N*-acetylleucine was not formed, either from *N*-acetyl-leucyladenosine, or from *N*-acetyl-leucyl-AMP.

In contrast, in the presence of enzymatic fraction II, the *N*-acetylleucylpentanucleotides produced after T_1 RNase treatment were hydrolyzed with the formation of *N*-acetylleucine (Fig. 6). However, the extent of the hydrolytic reaction was less with these as substrates than with undegraded *N*-acetylleucyl-tRNA as substrate; five times more *N*-acetylleucine was produced under the same conditions from *N*-acetyl-leucyl-tRNA.

Thus, the enzyme acts most efficiently on the relatively long polynucleotide chain of tRNA.

(II) *Aminoacyl residue:* All the experiments described above have been performed using either *N*-acetylleucyl-tRNA, or a derivative of this compound as a substrate. Using the same procedure, it has also been possible to show an extensive enzymatic cleavage of different *N*-acetyl-aminoacyl-tRNA's. Figure 7 shows the kinetics of the reaction catalyzed by the purified fraction II for *N*-acetylseryl-, *N*-acetylvalyl-, *N*-acetylthreonyl-, and *N*-acetylleucyl-tRNA.

It is not possible to conclude from these results whether fraction II contains a unique enzyme with low specificity for the aminoacyl residue, or several enzymes, each specific for a given amino acid. However, using the crude extract or fraction II, we observed that the ratios of activities toward *N*-acetylvalyl-, *N*-acetylleucyl-, and *N*-acetylphenylalanyl-tRNA's were identical, suggesting that a single enzyme was acting on these different substrates.

(III) *Unacetylated aminoacyl-tRNA's:* Spontaneous hydrolysis of the ester bond occurs very rapidly at pH 8 for most of the aminoacyl-tRNA's;[6] only isoleucyl-, valyl-, and threonyl-tRNA are stable enough so that a test for enzymatic cleavage at that pH is possible.

As shown in Figure 8, in five minutes, the hydrolysis of *N*-acetylthreonine-tRNA was at least 100 times greater in the presence of the enzyme than in its absence. With threonyl-tRNA the enzymatic hydrolysis was only double and this difference may be due to the action of the nuclease which, we know, is present as a contaminant of fraction II. Similar results were obtained with valyl-tRNA and isoleucyl-tRNA. It appears that the enzyme does not catalyze the hydrolysis of the ester bond in the presence of free $-NH_2$ group of tRNA-esterified amino acid.

(IV) *Polypeptidyl-tRNA's:* *N*-acetyl-C^{14}-diphenylalanyl-tRNA and *N*-acetyl-leucyl-glycyl-C^{14}-phenylalanyl-tRNA, both prepared according to Lapidot *et al.*,[14] and C^{14}-diphenylalanyl-tRNA and C^{14}-polylysyl-tRNA were incubated with fraction II for 30 minutes. After incubation, free di- or polypeptides were separated from tRNA-bound material by paper electrophoresis or by chromatography. In all cases, except for polylysyl-tRNA, the enzyme catalyzed the hydrolysis of polypeptidyl-tRNA's. The details of these experiments will be reported later.[15]

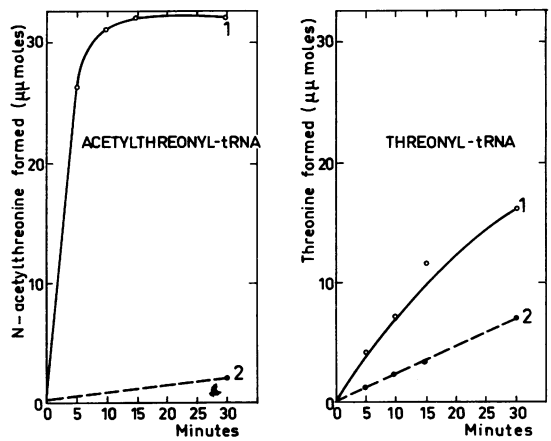

FIG. 8.—Enzymatic hydrolysis of acetylated and unacetylated C^{14}-threonyl-tRNA. Incubation mixtures (total vol 60 μl) containing barbital-acetic acid pH 8, 2 μmoles; C^{14}-threonyl-tRNA or *N*-acetyl-C^{14}-threonyl-tRNA, 45 μμmoles, were incubated at 37°C in the presence or in the absence of fraction II (10 μg of proteins). At different times, the formation of free C^{14}-threonine or *N*-acetyl-C^{14}-threonine was measured after ethanol precipitation of unreacted material.

Discussion.—The enzyme described in this report is a specific hydrolase, which cleaves the ester linkage between tRNA and N-substituted aminoacyl residues (either *N*-acetyl- or polypeptidyl-tRNA). The enzyme requires, in the substrate, the presence of a relatively long polynucleotide chain, providing evidence that it is not a nonspecific hydrolase. It shows a low level of specificity with respect to particular amino acids, except that it does not cleave polylysyl-tRNA (perhaps because of the presence of free ε-amino groups). However, unsubstituted aminoacyl-tRNA's are not acted on to a significant extent.

All these properties are in good agreement with those expected for an enzyme involved in the mechanism of chain termination during protein biosynthesis. Whether enzymatic cleavage of the polypeptidyl-t-RNA occurs on the ribosome or after its release in the soluble fraction has not yet been firmly established. However, Ganosa[3] reported that the release of the polypeptide chain was lost during purification and presented some arguments for cleavage while the polypeptidyl-tRNA is situated on the ribosome. If the hydrolase acts at that level, an efficient control mechanism must operate in order to avoid any interruption of chains during the growth. The UAA triplet would not only stop the translation but in some unknown way would trigger the cleavage by the hydrolase of the ester linkage between the polypeptide and tRNA.

Another possible role for the hydrolase would be to regenerate the tRNA's from unfinished polypeptides which could have "accidentally" escaped from the ribosomal complex.

In the second model, it has to be assumed that the chain associated with the ribosome-messenger complex is protected against enzymatic hydrolysis. One may note that nascent polypeptide chains are protected against proteolytic activity.[16]

The properties of the enzyme described in this paper are very similar to those of the D-tyrosyl-tRNA-deacylase recently described by Calendar and Berg.[17] Since these authors did not check possible hydrolytic activity against *N*-acetyl-L-tyrosyl-tRNA, a direct comparison of the two enzymes cannot be made. It would not be too surprising if both enzymes would prove to be analogous or even identical: the D-tyrosyl-tRNA-deacyclase cleaves the ester bond between D-amino acids (either tyrosine or phenylalanine) and tRNA's. The structure of the D-amino acid, with its NH_2-group in opposite configuration, as compared with the L form, could be operationally equivalent to a substitution of the amino group for the recognition of the substrate by the enzyme molecule.

Capecchi[18] has recently isolated a protein factor, the charge properties of which are opposed to those of the hydrolase described here and which is required for the chain determination triggered by the UAG triplet. It remains to be shown whether there is any relation between these two enzymes in polypeptide chain termination.

Abbreviations used: poly A, polyadenylic acid; poly U, polyuridylic acid; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; AMP, adenosine 5′-phosphate; DEAE-cellulose, *O*-(diethylaminoethyl) cellulose.

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