Competing Addition and Hydrolysis of the Cytidylylcytidyl-adenosine Terminal Residues of Transfer Ribonucleic Acid Isolated from the Non-lactating Bovine Mammary Gland

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1. The enzyme fraction obtained from the pH 5 enzyme of non-lactating bovine mammary gland between 40 and 100% ammonium sulphate saturation markedly inhibited the AMP-incorporating activity of rat liver nucleotide-incorporating enzyme. This inhibitory effect has been attributed to high nuclease activity which can be partially removed by adsorption of the enzyme fraction on to calcium phosphate gel. 2. The degradation action of the calcium phosphate-purified enzyme is confined mainly to the terminal trinucleotide sequence -pCpCpA of tRNA, its effect being analogous to that of venom phosphodiesterase. This enzyme is heat labile and very readily loses its degradative activity. 3. Treatment of the enzyme fraction with Macaloid results in complete removal of the phosphodiesterase, leaving an enzyme capable of incorporating AMP into tRNA. 4. Transfer RNA extracted from non-lactating bovine mammary gland in the presence of polyvinyl sulphate and Macaloid is able to accept amino acids with an efficiency 30% of that shown by lactating bovine mammary-gland tRNA isolated under identical conditions.

Reports from numerous laboratories have demonstrated the enzymic synthesis of aminoacyl-tRNA derivatives (Hoagland, Stephenson, Scott, Hecht & Zamecnik, 1958; Schweet, Bovard, Allen & Glassman, 1958; Weiss, Acs & Lipmann, 1959) the amino acid of which is linked to either the 2'- or the 3'-hydroxyl group of the terminal nucleotide residue in tRNA. The terminal nucleotide has been conclusively identified as adenylic acid (Hecht, Stephenson & Zamecnik, 1958; Zachau, Acs & Lipmann, 1958), which in turn is adjacent to a dinucleotide sequence of two cytidylic acid residues (Hecht, Stephenson & Zamecnik, 1959). If one or more of the terminal nucleotides is removed, the tRNA loses its ability to accept amino acids (Hecht *et al.* 1958) or to bind to ribosomes (Cannon, Krug & Gilbert, 1963), two processes that are essential for protein synthesis.

The enzyme responsible for the incorporation of the terminal adenylylate and cytidylate residues into tRNA isolated from a 'preincubated pH 5 fraction' of rat liver was partially purified by Herbert (1959) and Canellakis & Herbert (1960). Preiss, Dieckmann & Berg (1961) and Furth, Hurwitz, Krug & Alexander (1961) have also isolated a similar enzyme from *Escherichia coli*. The enzyme was further purified by Daniel & Littauer (1963) who

reported that it was able to incorporate not only adenine and cytosine nucleotides, but also uridine into the terminal positions of tRNA. This enzyme appears to catalyse the reaction with any tRNA regardless of its amino acid specificity or of its source.

Studies on non-lactating bovine mammary-gland tRNA have indicated that the molecule when isolated in the absence of ribonuclease inhibitors is unable to accept amino acids due to the lack of the terminal adenine nucleotide and possibly the terminal -pCpCpA nucleotide sequence (Herrington & Hawtrey, 1970). Once these nucleotides have been replaced by utilizing the rat liver nucleotide-incorporating enzyme in the presence of ATP and CTP, the molecule is able to both accept amino acids and transfer these amino acids to the growing polypeptide chain present on the ribosomes. It is the purpose of this paper to present evidence that the major portion of bovine tRNA, in fact, lacks the terminal trinucleotide sequence -pCpCpA, possibly owing to the action of a nuclease enzyme that is present in the cytoplasm of the mammary-gland cells. A method is also given for the purification of the nucleotide-incorporating enzyme from the pH 5 fraction of non-lactating bovine mammary gland.

MATERIALS AND METHODS

Chemicals. CTP (disodium salt) and pyruvate kinase were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A., and ATP (disodium salt), crystalline GSH and phosphoenolpyruvate (tricyclohexylammonium salt) from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Streptomycin sulphate was purchased from Calbiochem, Los Angeles, Calif., U.S.A. Macaloid was a gift from Baroid Division, National Lead Co., Houston, Tex., U.S.A. Yeast ^{14}C -labelled protein hydrolysate (1500 $\mu\text{Ci}/\text{mg}$), ^{14}C -labelled CTP (tetralithium salt; 161 mCi/mmol) and ^3H -labelled ATP (tetralithium salt; 4.1 Ci/mmol and 24.5 Ci/mmol) were obtained from Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A. All solutions were prepared before use and adjusted to pH 7.6 with 0.3M-KOH.

Scintillation chemicals. The scintillation fluid contained 0.5% 2,5-diphenyloxazole (PPO) and 0.03% 1,4-bis-(5-phenyloxazol-2-yl)benzene (POPOP) (both purchased from Packard Instrument Co. Inc., La Grange, Ill., U.S.A.) in chromatography-grade toluene.

Preparation of subcellular fractions. Homogenates of rat liver and non-lactating bovine mammary gland were prepared in medium A, containing (final concentrations): 0.25M-sucrose, 5mM-MgCl₂, 25mM-KCl and 50mM-tris-HCl buffer, pH 7.6, as described by Hawtrey, Schirren & Ijckstra (1963) and Herrington & Hawtrey (1969a) respectively.

(1) pH 5 enzyme fractions. These were prepared from the cell sap of rat liver and non-lactating bovine mammary gland as described by Hawtrey *et al.* (1963). The fractions were suspended in medium A, assayed for protein concentration and stored at -15°C until required.

(2) Deacylated tRNA. tRNA was isolated from the pH 5 enzyme fractions and then deacylated as described by Herrington & Hawtrey (1969b).

(3) Twice-pyrophosphorylated rat liver tRNA. This was prepared from the pH 5 enzyme fraction of rat liver according to the procedure of Daniel & Littauer (1963). The sample of RNA was stored at -15°C and thawed just before use.

(4) Aminoacyl-tRNA synthetases. These were prepared from the rat liver pH 5 enzyme according to the method of Hele (1961) as modified by Herrington & Hawtrey (1970).

(5) Nucleotide-incorporating enzyme. Three stages were used for the preparation of rat liver and bovine nucleotide-incorporating enzymes [RNA adenylate (cytidylate) pyrophosphorylase]. The stage of purification for each particular enzyme fraction used in experiments is given in the legends to the Tables and Figures. (a) Stage 1 purification. The first stage of the purification was based upon the method of Daniel & Littauer (1963) in which the protein fraction obtained at 100% $(\text{NH}_4)_2\text{SO}_4$ saturation was dissolved in a minimum volume of medium A and dialysed extensively against medium A for approx. 20h until free of sulphate. (b) Stage 2 purification. In the second procedure the enzyme fractions were further purified by adsorption on to calcium phosphate gel (Keilin & Hartree, 1938). In this method the fraction obtained at 100% $(\text{NH}_4)_2\text{SO}_4$ saturation was dissolved in 10mM-tris-HCl buffer, pH 7.6 and dialysed against the same buffer until free of sulphate. A suspension of

$\text{Ca}_3(\text{PO}_4)_2$ gel was added to the enzyme solution in the proportion of 3.4mg dry wt. of gel/15mg of enzyme protein. After standing for 15min at 4°C , the gel was removed by centrifugation at 2000g for 10min and to the supernatant was added additional gel (10.2mg of gel/15mg of enzyme protein) sufficient to adsorb 80–100% of the enzyme. The suspension was thoroughly stirred, allowed to stand for 15min at 4°C and then centrifuged. The supernatant was discarded and the gel washed twice with 3ml of 10mM-potassium phosphate buffer, pH 7.6. The enzyme was finally eluted with 3ml of 50mM-potassium phosphate buffer, pH 7.6. The process was repeated to ensure complete removal of the enzyme from the gel. (c) Stage 3 purification. In an attempt to remove contaminating nucleases the calcium phosphate gel-purified enzyme was treated with Macaloid in the proportion of 30mg of Macaloid/ml of enzyme solution. After stirring gently for 15min at 4°C , the Macaloid was removed by low-speed centrifugation.

At all stages of purification, experiments were conducted on the enzyme fractions by testing for nucleotide-incorporating activity and nuclease activity.

Preparation of radioactively labelled bovine tRNA. ^{14}C -labelled cytosine and ^3H -labelled adenine nucleotides were added to the terminal end of normal deacylated bovine mammary-gland tRNA as follows. The incubation medium contained rat liver nucleotide-incorporating enzyme (1mg of protein), 5mM-phosphoenolpyruvate, 100 μg of pyruvate kinase, 0.15M-sucrose, 31mM-tris-HCl buffer, pH 7.6, 16mM-KCl, 6mM-MgCl₂, 5mg of deacylated bovine tRNA, ^3H -labelled ATP (60 μCi) and ^{14}C -labelled CTP (10 μCi) in a total volume of 22ml. Incubation was at 37°C for 30min. At the end of the incubation the sample was mixed with an equal volume of 90% (w/v) phenol and shaken at room temperature for 90min. The emulsion was broken by centrifugation at 1400g for 30min and the RNA was precipitated from the aqueous phase at -15°C by the addition of 0.1 volume of 20% (w/v) potassium acetate and 2.5 volumes of 96% (v/v) ethanol. The RNA was reprecipitated twice, dissolved in water and dialysed against water for 6–8h.

Measurement of enzyme activity for the incorporation of AMP into tRNA. The reaction medium used for the incorporation of radioactively labelled AMP (and CMP) on to the 3'-terminus end of tRNA is given in the legends to the Tables and Figures. In all cases the reaction was terminated by the addition of an equal volume of cold 10% (w/v) trichloroacetic acid followed by immersion of the tubes in an ice bath. Carrier yeast RNA (200 μg) was then added. After being left for 20min at 4°C the precipitate was washed successively with cold 5% (w/v) trichloroacetic acid, ethanol-ether (1:1, v/v) and ether before being filtered on Millipore HA filters (0.45 μm pore size) (Millipore Filter Corp., Bedford, Mass., U.S.A.). After thorough washing with cold 5% (w/v) trichloroacetic acid, the filters were dried in air for 1h, placed in counting vials with 10ml of scintillation fluid and counted in a liquid-scintillation spectrometer (Packard Tri-Carb model 2002).

Assay of nuclease activity in nucleotide-incorporating enzyme fractions. The nuclease activity present in the nucleotide-incorporating enzyme fractions obtained at different stages of purification was determined by aerobic incubation at 37°C for 20min. Each tube contained the

Table 1. *AMP incorporation into tRNA and nuclease activities exhibited by nucleotide-incorporating enzyme fractions at different stages of purification*

Incorporation of the labelled adenine nucleotide was determined during incubation at 37°C of the indicated nucleotide-incorporating enzyme fraction (approx. 24 µg of protein), 150 µg of twice-pyrophosphorylated rat liver tRNA, 5 µmol of phosphoenolpyruvate, 50 µg of pyruvate kinase, 0.2 µmol of CTP, 0.13 M-sucrose, 26 mM-tris-HCl buffer, pH 7.6, 13 mM-KCl, 6 mM-MgCl₂, 21 mM-potassium phosphate buffer, pH 7.6, and ³H-labelled ATP (2 µCi, 24.5 Ci/mmol) in a total volume 1.4 ml. All incubations were carried out in duplicate at 37°C for 20 min. Experimental details of ribonuclease assays are given in the Materials and Methods section.

Nucleotide-incorporating enzyme	Purification stage	[³ H]AMP incorporated (c.p.m./mg of tRNA)	Nuclease activity (% degradation of tRNA)
Bovine mammary gland	Stage 1	0	71.6
Bovine mammary gland	Stage 2	100	11.8
Bovine mammary gland	Stage 3	5270	0.6
Rat liver	Stage 2	53640	0.8
Rat liver + bovine mammary gland	Stage 2	1340	13.2
Rat liver + bovine mammary gland*	Stage 2	31680	1.7

* The bovine mammary-gland enzyme was heated at 105°C for 10 min before incubation.

indicated enzyme (24–30 µg of protein), 500 µg of deacylated rat liver tRNA (8.52 E_{260} units), 0.16 M-sucrose, 33 mM-tris-HCl buffer, pH 7.6, 16 mM-KCl, 8 mM-MgCl₂ and 14 mM-potassium phosphate buffer, pH 7.6, in a total volume of 1.1 ml. The incubation was stopped by the addition of 2 ml of 1 M-HClO₄. After 20 min at 4°C the protein and unhydrolysed RNA were precipitated by centrifugation at 1400g for 10 min and the supernatant was assayed for acid-soluble nucleotides by reading the extinction at 260 nm. The percentage degradation of RNA was calculated with no corrections being made for hypochromicity.

Determination of RNA and protein. Protein was determined by the biuret method of Gornall, Bardawill & David (1949) with bovine serum albumin as standard. The concentration of RNA was measured spectrophotometrically at 260 nm ($E_{1\%}^{1\text{cm}}$ 170) (Hawtrey, Nourse & King, 1966).

RESULTS

Characterization of bovine nucleotide-incorporating enzyme fractions at different stages of purification. The nucleotide-incorporating enzyme was prepared from non-lactating bovine mammary gland in three different stages, each stage increasing in the degree of purification. Each fraction was then tested for its ability to incorporate AMP terminally into either twice-pyrophosphorylated rat liver RNA or normal deacylated bovine tRNA and also its tendency to degrade tRNA.

(1) Stage 1 purification. The results presented in Table 1 show that the enzyme fraction prepared from non-lactating bovine mammary gland up to stage 1 purification is totally incapable of incorporating AMP into the 3'-terminus position of twice-pyrophosphorylated rat liver tRNA. This inability is presumably due to the high nuclease

activity of the fraction which results in approx. 71.6% degradation of the tRNA molecule.

(2) Stage 2 purification. In an attempt to determine whether non-lactating bovine mammary gland contains an enzyme capable of incorporating AMP and CMP into tRNA, further fractionation was adopted in the hope of removing contaminating nucleases. Adsorption of the bovine mammary-gland enzyme fraction on to calcium phosphate gel and elution with 50 mM-potassium phosphate buffer, pH 7.6, resulted in a marked decrease in nuclease activity from approx. 70% degradation of deacylated rat liver tRNA to between 10 and 15%. Nevertheless, the results presented in Table 1 show that this enzyme fraction is still incapable of incorporating AMP on to tRNA. In addition, the decrease in nuclease activity is insufficient to prevent the inhibition exerted by the bovine mammary-gland enzyme on the ability of the rat liver enzyme to incorporate the nucleotides into tRNA components lacking the terminal -pCpCpA sequence. Treatment of the bovine mammary-gland enzyme fraction at 105°C for 10 min before incubation in the presence of the rat liver enzyme resulted in almost total loss of nuclease activity with a concomitant increase in AMP incorporation.

When twice-pyrophosphorylated rat liver tRNA was incubated with ³H-labelled ATP and the nucleotide-incorporating enzyme from rat liver in the presence of increasing concentrations of the bovine mammary-gland enzyme fraction (stage 2 purification), the results shown in Fig. 1 were obtained. After 20 min incubation, 70% inhibition of AMP incorporation into tRNA resulted due to the presence of 4 µg of protein of bovine mammary-gland enzyme. This inhibition increased rapidly

Fig. 1. Effect of the nucleotide-incorporating enzyme fraction from non-lactating bovine mammary gland (stage 2 purification) on the addition of the terminal adenine nucleotide to twice-pyrophosphorylated rat liver tRNA. The incubation medium contained $5\mu\text{mol}$ of phosphoenolpyruvate, $50\mu\text{g}$ of pyruvate kinase, $0.2\mu\text{mol}$ of CTP, 0.13M -sucrose, 26mM -tris-HCl buffer, pH 7.6, 13mM -KCl, 6mM -MgCl₂, 21mM -potassium phosphate buffer, pH 7.6, $150\mu\text{g}$ of twice pyrophosphorylated rat liver tRNA, ^3H -labelled ATP ($2\mu\text{Ci}$, $24.5\text{Ci}/\text{mmol}$), rat liver nucleotide-incorporating enzyme ($24\mu\text{g}$ of protein) and the indicated amount of corresponding non-lactating bovine mammary-gland enzyme in a total volume of 1.4ml . Both enzymes were purified by the $\text{Ca}_3(\text{PO}_4)_2$ step. All incubations were carried out in duplicate at 37°C for 20 min.

to a maximum of between 97 and 100% when both enzymes were present in the incubation medium at equal proportions.

(3) Stage 3 purification. Treatment of the calcium phosphate gel-purified enzyme with Macaloid results in adsorption of the unwanted nucleases (Table 1) leaving behind an enzyme that is now capable of incorporating AMP on to tRNA lacking the terminal -pCpCpA sequence. Hence, the nuclease activity of the bovine mammary-gland enzyme fraction has been decreased from 71.6% degradation of tRNA to approx. 0.6% as the stage of purification has increased and this in turn is accompanied by an increase in incorporation of $[^3\text{H}]\text{AMP}$ into tRNA. The results given in Fig. 2 show that the rate of incorporation of $[^3\text{H}]\text{AMP}$ into tRNA is a direct function of the purified enzyme concentration.

Effect of bovine nucleotide-incorporating enzyme (stage 2 purification) on the terminal nucleotide

Fig. 2. Rate of incorporation of $[^3\text{H}]\text{AMP}$ into pyrophosphorylated rat liver tRNA as a function of enzyme concentration. The incubation medium contained $5\mu\text{mol}$ of phosphoenolpyruvate, $50\mu\text{g}$ of pyruvate kinase, $0.2\mu\text{mol}$ of CTP, 0.14M -sucrose, 30mM -tris-HCl buffer, pH 7.6, 14mM -KCl, 7mM -MgCl₂, 12mM -potassium phosphate buffer, pH 7.6, $500\mu\text{g}$ of twice-pyrophosphorylated rat liver tRNA, ^3H -labelled ATP ($2\mu\text{Ci}$, $24.5\text{Ci}/\text{mmol}$) and the indicated amount of non-lactating bovine mammary-gland enzyme (stage 3 purification) in a total volume of 1.28ml . All incubations were carried out in duplicate at 37°C for 20 min.

sequence of modified bovine tRNA. To determine the effect of bovine mammary-gland nucleotide-incorporating enzyme on the terminal sequence of tRNA, deacylated bovine tRNA was labelled in the 3'-terminus position with $[^{14}\text{C}]\text{CMP}$ and $[^3\text{H}]\text{AMP}$. As shown in Table 2, treatment of the radioactively labelled bovine tRNApCpCpA with 10mM -inorganic pyrophosphate and the rat liver nucleotide-incorporating enzyme results in the complete removal of the terminal adenine nucleotide but not the cytosine nucleotides. When the same RNA component, however, is treated with the corresponding bovine mammary-gland enzyme fraction, the terminal AMP is removed as well as most of the CMP (approx. 67%). The presence of the rat liver enzyme has no effect on preventing the terminal degradation by the bovine mammary-gland enzyme.

Bovine tRNA. In a previous paper (Herrington & Hawtre, 1970) it was shown that tRNA isolated from non-lactating bovine mammary gland by phenol-sodium dodecyl sulphate extraction is unable to accept amino acids due to the absence of the terminal trinucleotide sequence. The results presented in Table 3, however, illustrate that tRNA isolated from lactating bovine mammary gland is quite capable of accepting amino acids to a degree more efficient than that of deacylated rat liver

Table 2. *Liberation of adenine and cytosine ribonucleotides into the acid-soluble fraction by treatment of bovine tRNA^pCpCpA with different nucleotide-incorporating enzymes*

The incubation medium contained 200 μ g of radioactively labelled bovine tRNA^pCpCpA (see the Materials and Methods section for preparation), 0.15 M-sucrose, 30 mM-tris-HCl buffer, pH 7.6, 15 mM-KCl, 7.5 mM-MgCl₂, 13 mM-potassium phosphate buffer, pH 7.6, and the indicated nucleotide-incorporating enzyme fraction (stage 2 purification) in a total volume of 1.2 ml. All incubations were carried out in duplicate at 37°C for 30 min.

Incubation system	Radioactive nucleotide present (μ mol/mg of tRNA)	
	[¹⁴ C]CMP	[³ H]ATP
Bovine tRNA ^p CpCpA	7.35	0.900
Bovine tRNA ^p CpCpA+rat liver enzyme	6.65	0.885
Bovine tRNA ^p CpCpA+rat liver enzyme+10 mM-PP _i	8.40	0
Bovine tRNA ^p CpCpA+rat liver enzyme+bovine enzyme	2.70	0
Bovine tRNA ^p CpCpA+bovine enzyme	2.25	0

Table 3. *Ability of tRNA fractions isolated under different conditions to form aminoacyl-tRNA complexes*

The incubation medium contained 3 μ mol of ATP, 1.6 μ mol of GSH, 0.19 M-sucrose, 38 mM-tris-HCl buffer, pH 7.6, 19 mM-KCl, 8 mM-MgCl₂, yeast ¹⁴C-labelled protein hydrolysate (1 μ Ci), rat liver aminoacyl-tRNA synthetases and 200 μ g of the indicated tRNA in a total volume of 0.9 ml. All incubations were carried out in duplicate at 37°C for 20 min.

tRNA component added	Extraction procedure of tRNA	¹⁴ C-labelled amino acids incorporated into tRNA (c.p.m.)
Deacylated rat liver tRNA	Phenol-sodium dodecyl sulphate extraction	17 279
Twice-pyrophosphorylated rat liver tRNA	as described in the Materials and Methods	0
Bovine tRNA (non-lactating)	section	309
Bovine tRNA (non-lactating)	Tissue homogenized in medium A containing	8 236
Bovine tRNA (lactating)	20 μ g of polyvinyl sulphate/ml+1% (w/v) of Macaloid. pH5 enzyme prepared and tRNA extracted by phenol in the presence of 0.5% Macaloid and sodium dodecyl sulphate	27 231

tRNA. It is apparent, therefore, that tRNA isolated under the defined conditions from non-lactating bovine mammary gland, lacks the terminal trinucleotide -pCpCpA sequence, whereas in lactating mammary gland this sequence is intact.

If, however, tRNA is isolated from non-lactating bovine mammary gland in the presence of polyvinylsulphate and Macaloid (both of which inhibit ribonuclease activity), it is capable of accepting some amino acids with approx. 30% efficiency. Nonetheless the major portion of tRNA still appears to lack the -pCpCpA trinucleotide sequence and as such is incapable of accepting amino acid.

DISCUSSION

Attempts to demonstrate the incorporation of amino acids into protein by subcellular fractions derived from non-lactating bovine mammary gland

have met with little success (Herrington & Hawtrey, 1969a). This failure has been attributed to the presence of active nucleases in the cytoplasm and to tRNA, which appears to be unable to accept amino acids due to the absence of the terminal trinucleotide sequence -pCpCpA (Herrington & Hawtrey, 1970).

Considerable work has been carried out on the enzymes that catalyse the reaction for the incorporation of ribonucleotides into the terminal position of tRNA (Preiss *et al.* 1961; August, Ortiz & Hurwitz, 1962; Daniel & Littauer, 1963). Initial attempts to isolate a similar enzyme from non-lactating bovine mammary gland met with little success since the possible presence of such an enzyme would be masked by active nucleases which would consequently remove the terminal -pCpCpA sequence as soon as it had been incorporated. Treatment of the enzyme fraction with calcium

phosphate gel considerably decreased the RNA degradative activity, although a very small percentage remained which would nevertheless still be capable of inhibiting AMP incorporation into tRNA.

It appears that one or more of the nuclease enzymes present in the nucleotide-incorporating enzyme fraction (stage 1 purification) from non-lactating bovine mammary gland is capable of removing nucleotides from the 3'-terminal end of tRNA. Experimental evidence suggests that only the terminal -pCpCpA grouping is removed (Table 2).

Cannon (1966) reported that during exponential growth of *E. coli* the terminal adenosine of tRNA is unstable and exhibits a very slow turnover. This turnover phenomenon has been implicated with the action of an enzyme, ribonuclease II. A possible explanation for the adenosine turnover was originally put forward by Rosset & Monier (1963) who proposed that tRNA molecules, charged with amino acids could be resistant to exonucleolytic attack from the 3'-end, but once the amino acids had been removed they became susceptible. Hence in mammary-gland tissue that is no longer synthesizing milk protein, the RNA will become more susceptible to nuclease attack resulting in the removal of the terminal -pCpCpA sequence. During milk production, however, where there is a rapid exchange of amino acids from tRNA into protein the damage could be repaired quickly by a highly functional nucleotide-incorporating enzyme system, since Bucovaz & Davis (1961) have shown that lactating mammary gland readily forms aminoacyl-tRNA. This is substantiated by the results presented in Table 3 in which tRNA isolated from lactating mammary gland is readily able to accept amino acids to form aminoacyl-tRNA complexes.

The results reported in the present paper suggest that the nuclease activity exhibited by the calcium phosphate gel-purified enzyme (stage 2 purification) is similar to that of phosphodiesterase or ribonuclease II present in *E. coli* since, when reacted with tRNA it completely removes the terminal adenylate residue and almost all of the cytidylate units (see Table 2).

Definite proof, however, is given for the existence of an active and functional nucleotide-incorporating enzyme in non-lactating bovine mammary gland but its effect appears to be completely masked by the presence of phosphodiesterases. However, once the contaminating nucleases have been removed by treatment of the enzyme fraction with Macaloid, the remaining enzymes are able to terminally incorporate AMP into tRNA.

When tRNA is isolated from non-lactating bovine mammary gland in the presence of polyvinyl sulphate and Macaloid, approx. 30% of the total

tRNA is able to accept amino acids. This suggests, contrary to previous assumptions, that at least a proportion of the tRNA molecules contain intact terminal trinucleotide sequences at the 3'-end (Herrington & Hawtre, 1970). tRNA from lactating mammary gland, however, is able to accept amino acids, even more efficiently than deacylated rat liver tRNA.

From the evidence presented it appears that the nucleases in non-lactating bovine mammary gland mask the effect of the nucleotide-incorporating enzymes, thereby playing an important role in the control of synthesis, since the biological activity of tRNA with respect to amino acid acceptance and binding to ribosomes is critically dependent upon the integrity of its terminal nucleotide sequence. One may therefore suggest that the initiation of lactation results in a decrease in nuclease activity followed by an increase in activity of the nucleotide-incorporating enzyme, with the consequent formation of RNA components containing the terminal nucleotide sequence -pCpCpA. These molecules would once again be capable of forming aminoacyl-tRNA complexes, thereby allowing milk protein synthesis to proceed normally.

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