Phytochemistry, 1974, Vol. 13, pp. 2065 to 2069. Pergamon Press. Printed in England.

AMINO ACID INCORPORATION ON 80S PLANT RIBOSOMES: THE COMPLETE SYSTEM

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(*Received* 23 *January* 1974)

Key Word Index—*Phaseolus aureus*; *Vicia faba*; Leguminosae; mung bean; broad bean; *Turnip yellow mosaic virus* amino acid incorporation; cell-free systems; aminoacyl *t*RNA; TYMV.

Abstract—A cell-free system directed by poly U or turnip yellow mosaic virus (TYMV)-RNA was obtained from imbibed seeds of *Phaseolus aureus*; this *in vitro* system was dependent upon exogenous *t*RNA. The poly U-directed system functioned in the presence of *t*RNAs from *P. aureus*, *Vicia faba* and yeast, whereas TYMV-RNA was translated only in the presence of *t*RNAs from *P. aureus* or *V. faba*. The pH and Mg^{2+} optima for aminoacylation of *t*RNAs of *P. aureus*, *V. faba* and yeast by leucine and phenylalanine were related to the overall pH and ionic concentration optima for the complete system.

INTRODUCTION

UNLIKE the situation in animals, natural messenger RNAa have not been isolated from plants. An alternative approach to the problem of establishing *in vitro* systems, therefore, is the use of plant viral RNAs as messenger templates.[1] In this report a comparison was made between synthetic and natural messengers using *in vitro* amino acid-incorporating systems from *Phaseolus aureus*.

RESULTS

Acylation of tRNA

Figures 1 and 2 give the pH optima for Leu and Phe respectively, which show *V. faba* *t*RNA to be more active than the other two sources. For Leu acylation by *V. faba*, there was no clear Mg^{2+} optimum between 6 and 20 mM Mg^{2+}; for *P. aureus* and yeast the optima were *ca* 5 mM and 12 mM Mg^{2+} respectively. For Phe acylation by *V. faba*, there was no clear optimum between 2 and 16 mM Mg^{2+}; for *P. aureus* there was no clear optimum between 2 and 10 Mg^{2+}; and for yeast the optimum was *ca* 15 mM Mg^{2+}.

Poly U-directed phenylalanine incorporation. Poly U-directed polyphenylalanine synthesis in the complete system, in the presence of *t*RNA preparations from yeast, *P. aureus* or *V. faba* (Fig. 3). In each case a requirement for the energy system, ATP, phosphocreatine kinase and creatine phosphate, was demonstrated. The Mg^{2+} concentration greatly influenced the amount of poly-Phe synthesized; optima for systems containing *V. faba*-*t*RNA, *P. aureus*-*t*RNA and yeast-*t*RNA, were 10, 12 and 12 mM respectively. There was a clear pH optimum at pH 7·8 with *V. faba*-*t*RNA and less sharp optima at pH 7·8 and pH 8·1 with *P. aureus* and yeast-*t*RNAs, respectively (Fig. 4).

[1] BOULTER, D. (1970) *Ann. Rev. Plant Physiol.* **21**, 91.

TYMV–RNA-directed protein synthesis

TYMV–RNA promoted the incorporation of [^{14}C]leucine with *V. faba* or *P. aureus*-*t*RNAs, but not with yeast-*t*RNA. The time courses for *P. aureus* and *V. faba*-*t*RNAs is given in Fig. 5; no apparent lag phase occurred, typical of a situation with a natural messenger.[2] [^{14}C]Leucine was used in the experiment since it is a major amino acid of the protein shell of the virus.[3] The Mg^{2+} optima for viral-RNA direction with *P. aureus* and *V. faba*-*t*RNAs, were 4–5 mM and <2 mM respectively. Maximum activity of the systems was found over a range of pH values from 7·5 to 8·3 with no optimum value.

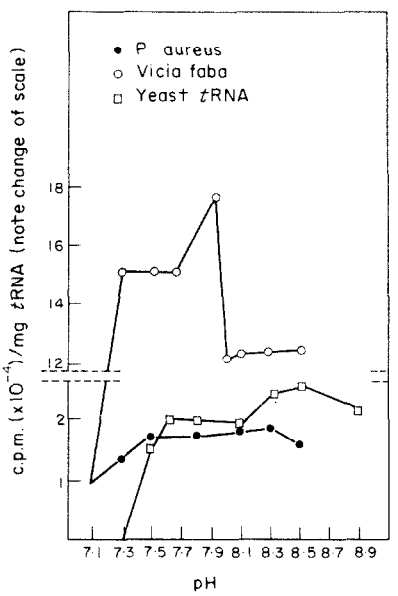

FIG. 1. EFFECT OF pH ON *t*RNA ACYLATION BY LEU. 0·25 ml Incubations contained: 25 μmol Tris–HCl at varying pH at 30°, 0·5 μmol ATP, 5 μmol GSH, 0·05 ml high-speed supernatant, 0·25 mg *t*RNA, 5 nmol [^{14}C]Leu with 0·3 μmol $MgCl_2$ for yeast *t*RNA; 0·15 μmol *V. faba* and *P. aureus* *t*RNAs. Incubations were at 30° and 0·05 ml samples assayed at 0, 15, 30, 45 min. Radioactivity was determined using a Beckman scintillation counter (see Experimental). Confidence limits ±3%. 15 min assays are reported.

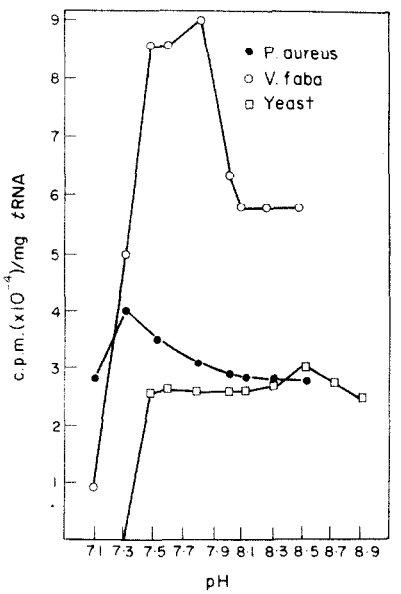

FIG. 2. EFFECT OF pH ON *t*RNA ACYLATION BY PHE. As Fig. 1 except 5 nmol [^{14}C]Phe in place of [^{14}C]Leu, with 4 μmol $MgCl_2$ for yeast and *V. faba* *t*RNAs and 2·5 μmol $MgCl_2$ for *P. aureus* *t*RNA.

DISCUSSION

In this investigation, microbial effects in the *in vitro* system were monitored in every set of incubations by a process of essential component omission and the construction of a kinetic curve of incorporation obtained by sampling the incubation at various times. Assumptions necessitated by one-time assays are, therefore, not applicable and it is concluded from the results that the incorporation experiments described do not result from microbial activity.

[2] SPIRIN, A. S. and GAVRILOVA, L. P. (1969) *The Ribosome*, Springer, New York.
[3] MATTHEWS, R. E. F. and RALPH, R. K. (1966) *Adv. Virus Res.* **12**, 273.

A lag phase was a characteristic feature of the kinetics of poly U direction of polyphenylalanine synthesis. The duration of the lag phase depended upon the source of *t*RNA, incubation temperature and presence of a suitable concentration of components essential to the system. Nevertheless, even in optimal conditions, a lag phase was still discernible. Similar lag phases have been shown in the poly U systems by Nakamoto *et al.*,[4] Allende *et al.*,[5] Nishizuka and Lipmann,[6] and Sander and Matthaei.[7] When considering the poly U-directed complete system, the only *t*RNA species that has to be considered is phenylalanyl-*t*RNA. The overall pH and ionic concentration optima for the complete system will be a compromise between the individual optima for these parameters for each of the separate stages in polyphenylalanine synthesis. If optima governing different reactions are similar, maximum incorporation will result. If they do not, then incorporation is dependent upon a compromise and incorporation will be below the arbitrary maximum. The sharper the parameter optima are, the greater this degree of compromise.

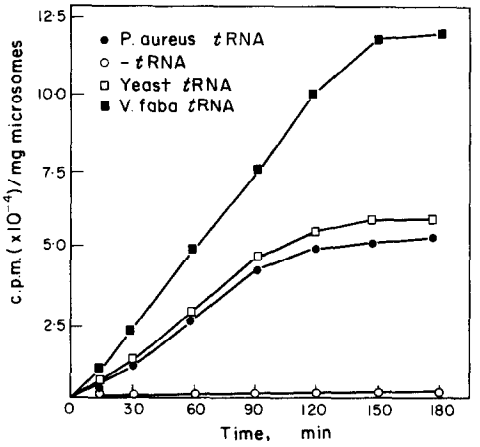

FIG. 3. POLY U-DIRECTED [^{14}C]PHE INCORPORATION WITH *t*RNAs FROM *V. faba*, *P. aureus* AND YEAST.
0·5 ml Incubations contained: 30 μmol Tris–HCl pH 7·8 for bean *t*RNA or 8·1 for yeast *t*RNA at 30°, 35 μmol KCl, 2 μmol ATP, 5 μmol creatine phosphate, 10 μg phosphocreatine kinase, 0·1 μmol GTP, 5 μmol GSH, 10 nmol [^{14}C]Phe, 10 nmol 19 [^{12}C]-amino acid mixture, 0·5 mg microsomes, 0·1 mg poly U, either 0·2 mg yeast *t*RNA with 6 μmol $MgCl_2$ and 0·04 ml high-speed supernatant, or 0·2 mg *V. faba* *t*RNA with 5 μmol $MgCl_2$ and 0·02 ml high-speed supernatant, or 0·2 mg *P. aureus* *t*RNA with 6 μmol $MgCl_2$ and 0·04 ml high-speed supernatant. Incubation was at 30° and 0·05 ml samples assayed at times indicated. Radioactivity/disc determined as in Fig. 1.

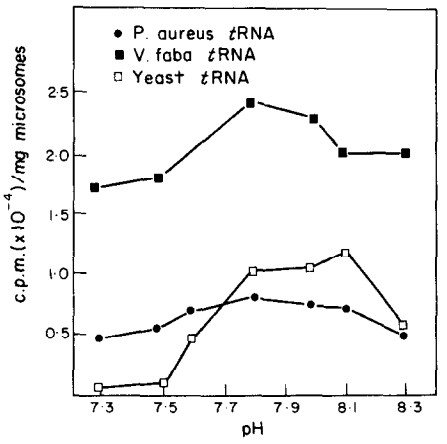

FIG. 4. EFFECT OF pH ON POLY U-DIRECTED [^{14}C]PHE INCORPORATION.
0·25 ml Incubations contained: 15 μmol Tris–HCl at varying pH at 30°, 17·5 μmol KCl, 1 μmol ATP, 2·5 μmol creatine phosphate, 5 μmg phosphocreatine kinase, 0·05 μmol GTP, 5 nmol [^{14}C]Phe, 5 nmol 19 [^{12}C]amino acid mixture, 0·25 mg microsomes, 0·05 mg poly U, 0·03 ml high-speed supernatant, and either 0·1 mg yeast or *P. aureus* *t*RNA with 3 μmol $MgCl_2$ or 0·1 mg *V. faba* *t*RNA with 2·5 μmol $MgCl_2$. Radioactivity/disc determined as in Fig. 1. 30 min assays are reported.

The condition of "matching" is illustrated by the identical pH optima for aminoacylation (Figs. 1 and 2) and incorporation (Fig. 4) using *V. faba*-*t*RNA^{Phe} and a condition of mutual concession is exemplified by the dissimilar pH optima of aminoacylation (Figs. 1

[4] NAKAMOTO, T., CONWAY, T. W., ALLENDE, J. E., SPYRIDES, G. J. and LIPMANN, F. (1963) *Cold Spring Harb. Symp. quant. Biol.* **28**, 227.
[5] ALLENDE, J. E., MONRO, R. and LIPMANN, F. (1964) *Proc. Nat. Acad. Sci. U.S.* **51**, 1211.
[6] NISHIZUKA, Y. and LIPMANN, F. (1961) *Proc. nat. Acad. Sci. U.S.* **55**, 212.
[7] SANDER, G. and MATTHAEI, H. (1969) *FEBS Lett.* **2**, 293.

and 2) and incorporation with *P. aureus* and yeast-*t*RNAs^{Phe} (Fig. 4). However, it should be noted that in the poly U-directed complete system a broad pH range, related specifically to the source of *t*RNA, allows significant polyphenylalanine synthesis (Fig. 4), characteristic of other plant-derived *in vitro* systems.[8,9]

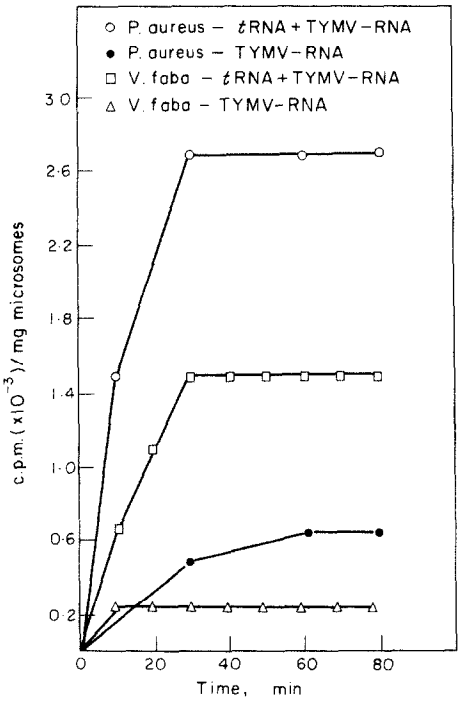

FIG. 5. TIME COURSE OF TYMV–RNA-DIRECTED [^{14}C]LEU INCORPORATION.
0·5 ml Incubations contained: 30 μmol Tris–HCl pH 7·8 at 30°, 35 μmol KCl, 2 μmol ATP, 5 μmol creatine phosphate, 10 μg phosphocreatine kinase, 0·1 μmol GTP, 2·5 μmol $MgCl_2$, 5 μmol GSH, 0·01 μmol [^{14}C]Leu, 10 nmol 19 [^{12}C]amino acid mixture, 0·5 mg microsomes, 0·13 mg TYMV–RNA, 0·2 mg *V. faba* *t*RNA and 0·04 ml high-speed supernatant. Radioactivity/disc determined as in legend of Fig. 1.

Various authors have shown that a relatively high Mg^{2+} concentration is required for activity in the poly U-directed system, since poly U does not have an initiating codon. No critical Mg^{2+} optima were recorded within the range of 2–16 mM for esterification of phenylalanine to *t*RNAs of *V. faba* and *P. aureus*. Therefore, the Mg^{2+} requirement of the latter would not appear to compromise Mg^{2+} requirements of the complete system. The Mg^{2+} optimum for the esterification of phenylalanine to yeast *t*RNA was sharper at 15 mM compared with the Mg^{2+} optimum for the complete system of 12 mM, possibly another example of a condition of concession.

It would appear, therefore, that for maximal synthesis of polyphenylalanine by poly U-direction in the *P. aureus* microsomal system, an heterologous *t*RNA, i.e. that of *V. faba*, is better able to meet the requirements necessary for the synthesis than the homologous *t*RNA. The significance of this finding may simply be that the synthetic messenger imposes on the system a set of non-physiological parameters to which *V. faba*-*t*RNA conforms more easily than do the *t*RNAs from other sources.

[8] PAYNE, F. S. (1970) Ph.D. Thesis, University of Durham.
[9] BEEVERS, L. and POULSON, R. (1972) *Plant Physiol.* **49**, 476.

In a complete system directed by TYMV-RNA, consideration has to be given not only to the conditions for initiation of protein synthesis, but also to those for aminoacylation of the twenty "protein" amino acid *t*RNAs. It has been shown that at concentrations greater than 9 mM,[2] association of the ribosome with the template and the *t*RNAs becomes stronger and the ribosome begins to form the initial complex less specifically and independently of the presence or absence of an initiating codon.

The Mg^{2+} optima of about 4–5 and 2 mM required for TYMV–RNA direction reported in this investigation are similar to that of 3·4 mM found by Marcus[10] using a tobacco mosaic viral-RNA wheat embryo ribosomal system, and that of 3 mM by Klein *et al.*[11] in a satellite tobacco necrosis viral-RNA wheat embryo ribosomal system.

The effect of Mg^{2+} concentration on acylation of leucyl-*t*RNA of *V. faba* can be contrasted with that on yeast leucyl-*t*RNA. At 2 mM Mg^{2+} virtually no yeast leucyl-*t*RNA was charged, whereas charging of the *V. faba* leucyl-*t*RNA was little affected from its optimum rate at this Mg^{2+} concentration. With *P. aureus*-*t*RNA there was considerable activity at 2 mM, even though it was somewhat reduced from the optimum. The results given in Figs. 1 and 2 and the effects of Mg^{2+} concentration bring out clearly the very different optimal conditions in the charging of different amino acids to *t*RNA in the same species, and also between species. The Mg^{2+} optimum for amino acid incorporation by 80S ribosomes was a compromise between the individual optima for stages in the synthesis.

EXPERIMENTAL

Biological materials. Imbibed seeds of *P. aureus* were used as a source of microsomes and enzymes, and 6-day-old seedlings of *P. aureus* and *V. faba* cv. Triple White for *t*RNA. Turnip yellow mosaic virus was the Cambridge strain propagated by Dr. R. E. F. Matthews in New Zealand. TYMV was cultivated in *Brassica pekinensis* cv. Wong Bok and the leaves were harvested 4–8 weeks after inoculation.

Chemicals. Adenosine 5′ triphosphate, Na salt; guanosine 5′ triphosphate, Na salt; creatine phosphokinase; phosphocreatine, Na salt; polyuridylic acid, potassium salt; L-amino acids; reduced glutathione; bovine serum albumin; K salt; and trizma base, anal. grade, were obtained from Sigma. Yeast *t*RNA from Boehringer Corporation, London, U.K. [^{14}C]DL-leucine 55 mCi/mmol; [^{14}C]DL-phenylalanine 59 mCi/mmol; [U^{14}C]amino acid mixture (CFB 104), specific activity 52 mCi/m Atom carbon, from The Radiochemical Centre, Amersham, Bucks.

Methods. TYMV was extracted by the method of Dunn and Hitchborn.[12] RNA was extracted from TYMV by the procedures of Haselkorn[13] and Fraenkel-Conrat[14], and shown to be undegraded by examination in the Spinco E analytical ultracentrifuge. *t*RNA was extracted and purified as described in Payne *et al.*[15] Microsomes and supernatant enzyme fractions were prepared from *P. aureus* after the method of Payne *et al.*[15] Preparation of charged transfer RNA was by the method of Ravel *et al.*[16] Cell-free amino acid incorporating systems and measurement of radioactivity were as described in Payne *et al.*[15] Radioactivity/disc was determined by the method of Mans and Novelli.[17,18]

[10] MARCUS, A. (1970) *J. Biol. Chem.* **245,** 955, 962.
[11] KLEIN, W. H., NOLAN, C., LAZAR, J. M. and CLARK, J. M. (1972) *Biochemistry* **11,** 2002.
[12] DUNN, D. B. and HITCHBORN, J. H. (1965) *Virology* **25,** 171.
[13] HASELKORN, R. (1962) *J. Mol. Biol.* **4,** 357.
[14] FRAENKEL-CONRAT, H. (1969) *Chemistry and Biology of Viruses.* Academic Press, London.
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