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AMINO ACID INCORPORATION BY FIG FRUIT RIBOSOMES IN A CELL-FREE SYSTEM*

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Abstract—An amino acid-incorporating system from fig fruit tissue has been prepared and the requirements needed for such an activity were established. The pH 5 enzymes, prepared from the same tissue, successfully supported the incorporation of ^{14}C-L-phenylalanine by ribosomes. Puromycin and RNAse, but not DNAse, inhibited the protein synthesis activity. The addition of poly U increased the incorporation rate. A gradual increase in the rate of ^{14}C-L-phenylalanine incorporation began in the dimer (120 S) fraction and continued in the higher polysomal aggregates.

INTRODUCTION

THE CELL-FREE incorporation of amino acids into plant protein was first reported by Stephenson *et al.*[1] who worked with tobacco leaf extracts. A large number of cell-free amino acid incorporating systems from different plant sources, including those derived from cytoplasm and from cell organelles (nuclei, chloroplasts and mitochondria),[2,3] have since been reported. No reports, however, have been found in the literature describing *in vitro* protein synthesis by a system prepared from fruit tissues. Furthermore, except for a limited number of publications,[4–7] the process of protein synthesis *in vivo* in relation to fruit ontogeny has not been investigated at the molecular level. This is a particular void since protein synthesis, particularly enzymes, has been proposed to regulate the physiological transitions (tissue differentiation, growth, maturation and senescence) characteristic of fruits.[8,9] The successful isolation of ribosomes from fig (*Ficus carica* L., cv. Mission) fruits[10] prompted us to examine the possibility of using this sub-cellular fraction in a system to incorporate amino acids into protein *in vitro*.

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TABLE 1. CHARACTERISTICS OF THE INCORPORATION OF ^{14}C-L-PHENYLALANINE INTO PROTEIN BY THE CELL-FREE SYSTEM FROM THE FIG FRUITS*

Additions or ommissions	Total activity $\mu\mu$moles phenylalanine/ mg RNA	Activity (%)
Complete system	31·8	100·0
$-Mg^{2+}$	1·3	4·0
+DNAse (10 μg)	29·8	93·7
+RNAse (10 μg)	1·4	4·4
+Puromycin (0·5 μmole)	0·8	2·5
−Fraction pH 5	5·6	17·6
−Ribosomes	0·0	0·0
−GTP	4·0	12·5
−ATP	3·4	8·1
Complete system + 50 μg Poly U	42·8	100·0
$-Mg^{2+}$	1·5	3·5
+DNAse (10 μg)	39·1	91·3
+RNAse (10 μg)	1·1	2·5
+Puromycin (0·5 μmole)	1·4	3·3
−Fraction pH 5	7·4	18·0
−Ribosomes	0·0	0·0
−GTP	3·1	7·1
−ATP	3·0	7·0

* The incubation conditions are outlined in the Experimental. The amounts of the following components in the incubation medium were changed to their optimum conditions established during the course of the study: Mg^{2+}, 12 mM; pH 5 enzyme fraction, the equivalent of 0·4 mg RNA per incubation tube; and ribosomes, the equivalent of 0·4 mg *r*RNA per incubation tube. The period of incubation was 40 min.

RESULTS AND DISCUSSION

In the presence of cofactors, energy-donors, regenerating components and supernatant fractions, the ribosomes isolated from figs were clearly able to incorporate labelled phenylalanine into protein *in vitro* (Table 1). Although the essential requirements needed to sustain the cell-free protein synthetic activity were similar to those reported for other plant systems,[11–16] the optimum concentration of each component in the incubation medium varied slightly. Addition of poly U substantially increased the rate of ^{14}C-L-phenylalanine incorporation up to more than 30%. Under our experimental conditions the optimum amount of poly U was found to be 50–60 μg/ml (Fig. 1).

Incorporation of ^{14}C-L-phenylalanine into protein exhibited complete dependence on ribosomes. Ribosomes, equivalent to 400 μg of ribosomal RNA were the optimum quantity for the system. A complete dependence on the Mg^{2+} concentration was also indicated (Table 1) with an optimal Mg^{2+} concentration of *ca.* 12 mmoles (Fig. 2). When the pH 5 fraction was omitted, a low but significant level of incorporation was always observed

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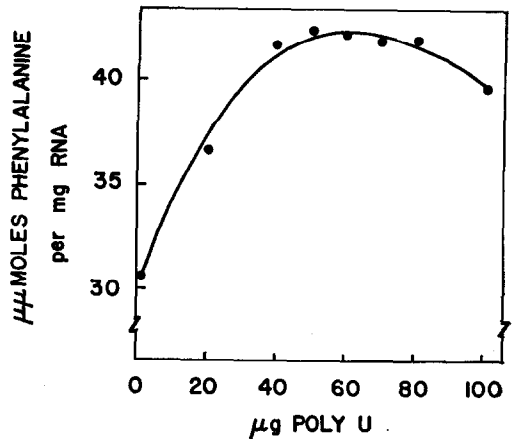

FIG. 1. AMINO ACID INCORPORATION BY ISOLATED FIG FRUIT RIBOSOMES AS A FUNCTION OF ADDED POLY U. THE INCUBATION MEDIUM AND PROCEDURE ARE AS DESCRIBED IN THE EXPERIMENTAL EXCEPT FOR THE ADDITION OF VARIOUS CONCENTRATIONS OF POLY U.

(from 10 to 35 $\mu\mu$moles phenylalanine/mg RNA). The observed increase in polypeptide chain formation with the addition of pH 5 fraction prepared from the same fruit tissue indicates the presence of active *t*RNA and polymerizing enzymes and the absence of inhibitory factors. In some cases, the supernatant fraction prepared from the same plant tissue failed to support protein synthesis activity in a cell-free system. This was attributed to the presence of natural inhibitors of protein synthesis and relatively high activity of nucleases.[14,17] The equivalent of 0·40 mg RNA from the supernatant solution was the optimum quantity for the system under our experimental conditions. *In vitro* protein synthesis by the fig ribosomes increases linearly for the first 10 min and reaches the maximum activity after 40 min (Fig. 3).

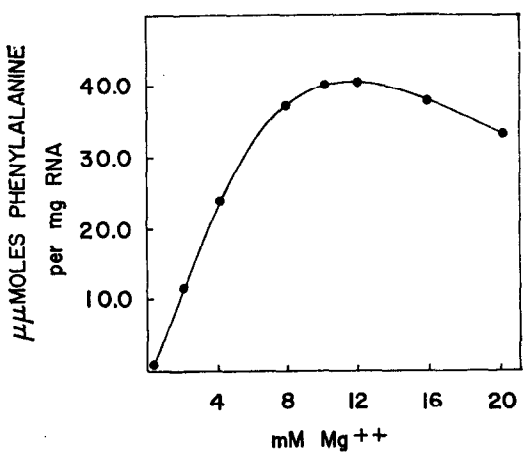

FIG. 2. THE EFFECT OF Mg^{2+} CONCENTRATION CHANGE ON THE INCORPORATION OF ^{14}C-L-PHENYLALANINE INTO PROTEIN BY THE FIG FRUIT SYSTEM. THE INCUBATION MEDIUM IS DESCRIBED IN THE EXPERIMENTAL.

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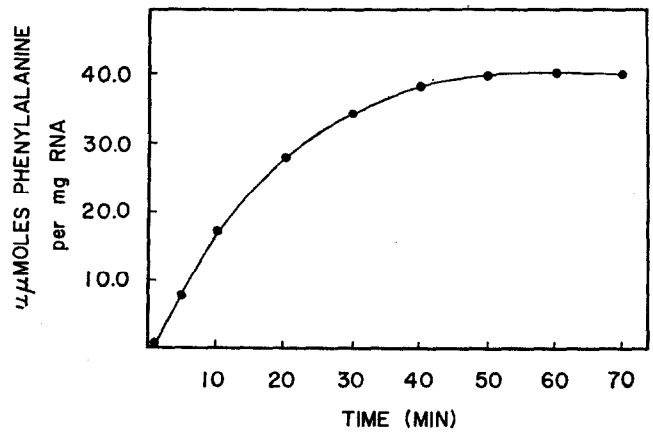

FIG. 3. KINETICS OF ^{14}C-L-PHENYLALANINE INCORPORATION INTO PROTEINS SYNTHESIZED IN A CELL-FREE SYSTEM BY RIBOSOMES AND pH 5 ENZYME FRACTIONS FROM FIG FRUIT. THE INCUBATION WAS CARRIED OUT AS DESCRIBED UNDER EXPERIMENTAL.

The amino acid incorporating system was highly sensitive to the addition of pancreatic ribonuclease (Table 1). Ten μg of ribonuclease resulted in 92–95% inhibition. Similar inhibition by ribonuclease for various systems has been reported.[18,19] Puromycin (0·5 μmole) almost completely inhibited activity of the ribosomes (Table 1). Lack of inhibition, by deoxyribonuclease, indicates that the system is not dependent on DNA. Table 1 also shows the requirement of the system for ATP and GTP.

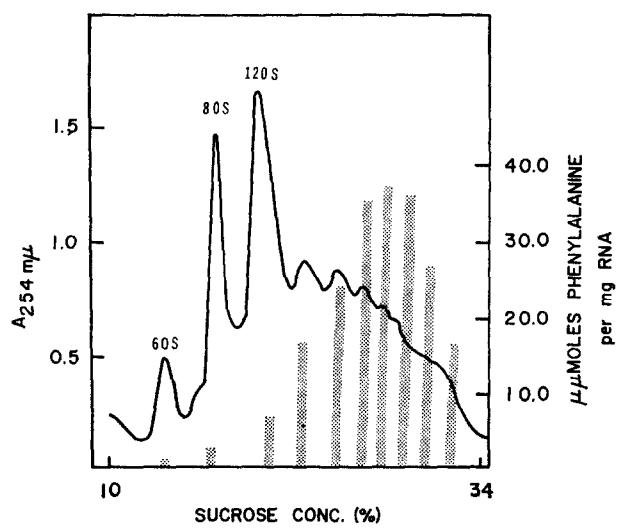

FIG. 4. PROTEIN SYNTHESIS IN A CELL-FREE SYSTEM UTILIZING FIG FRUIT RIBOSOMAL UNITS FROM EACH DEFINED PEAK AREA AFTER SUCROSE DENSITY GRADIENT SEPARATION. THE INCUBATION CONDITIONS ARE OUTLINED IN THE TEXT. POLY U WAS NOT INCLUDED.
(▦ μμmoles phenylalanine/mg RNA).

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It has been found that active protein synthesis and a relatively high polysome/monomer ratio were associated with periods of rapid growth of the fig fruit.[10] Therefore, experiments were designed to assess the active involvement of fig polysomes in the process of polypeptide chain synthesis with the fig fruit system and also examine the relative activity of each of the polysomal fractions. For this purpose, ribosomal preparations were resolved by sucrose density gradient centrifugation and individual fractions from the well-defined peaks were collected and used separately as a source of ribosomes in the cell-free system. As illustrated in Fig. 4, fractions corresponding to hexamers and heptamers were the most active aggregates in incorporating ^{14}C-L-phenylalanine into polypeptide chains. A gradual increase in protein synthetic activity starting with the monomers was noted. The slight activity noted with the monomer fraction indicates that single ribosomes may have retained fragments of *m*RNA.

It is of interest that the general response of the fig fruit cell-free system to several parameters (Mg^{2+} concentration, period of incubation, and presence of poly U) was of a similar pattern not only to other plant systems, but also to those prepared from bacteria,[19] insects[20] and higher animals.[21]

The presence of acidic vacuolar contents, protein binding constituents, enzyme inactivating factors and phenolics have hindered the isolation of active sub-cellular fractions and organelles from fruit tissues.[22,23] However, the above finding combined with recently published results[10] indicates the feasibility of isolating an active system from fruit tissue capable of synthesizing protein *in vitro*.

EXPERIMENTAL

Isolation of ribosomes. Figs (*Ficus carica* L., cv. Mission) were harvested during growth Period II (the intermediate slow-growing phase of the double sigmoid growth curve[24]), placed immediately in ice, and brought to the laboratory where the fruit were either used immediately or stored at −60°. The procedure described by Marei and Romani[10] was used to isolate ribosomes.

The ribosomal fractions were collected from sucrose density gradients as previously described.[10] The pH enzyme fraction was prepared following the method of Gadallah *et al.*[20] The incubation medium for amino acid incorporation by ribosomes was that used by Campagnoni and Mahler[21] and contained, in mmoles: Tris buffer (pH 7·3) 50; KCl, 20; $MgCl_2$ 10; 2-mercaptoethanol 10; GTP, 1·0; ATP, 1·0; phosphenol pyruvic acid, 10; thioglycerol, 10; sucrose, 300, and each of 19 amino acids (lacking phenylalanine) 0·1. In addition the medium contained 25 μg pyruvate kinase and the equivalent of 0·4 mg RNA from the pH 5 enzyme fraction and the equivalent of 0·4 mg ribosomal RNA from the ribosomal suspension. One μC of ^{14}C-L-phenylalanine (50 mC/m-mole) was also added to the incubation medium. The final volume of each incubation medium was 1·0 ml. When poly U was used, 50 μg/ml was added to the medium. For zero-time controls, the reaction was stopped by addition of trichloroacetic acid (TCA) to a final concentration of 10%. After standing for 40 min at 37°, the samples were centrifuged at 9000 *g* for 10 min. Pellets were collected and mixed in 10% TCA, covered and then heated in a 90° bath for 30 min. They were then chilled at 0° for 30 min, and the resultant precipitates were collected on millipore membrane filters (HA 0·45μ, 22 mm dia.). Each filter was washed with 100 ml of 10% TCA, dried and the radioactivity measured by liquid scintillation spectrophotometry. RNA was determined by the orcinol reaction.[25]

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Key Word Index—Ficus carica; Moraceae; protein synthesis; ribosomes; cell-free systems.