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STIMULATING FACTORS FOR AMINOACYL-tRNA SYNTHETASES IN SEEDS OF VARIOUS PLANTS

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Key Word Index—Dry seeds; factors stimulating aminoacyl-tRNA synthetases; presence and specificity.

Abstract—Factors stimulating the reaction of aminoacylation tRNAs were isolated from dry seeds of several plants. The factors show similar MWs during chromatography on Sephadex G-25. Differential stimulation in homogeneous and heterogeneous enzyme-factor systems in four lupin varieties and two inbred lines of maize was observed. It is suggested that the factors from various plants do not influence enzymes selectively but have ability to stimulate the aminoacylation reactions regardless of their origin.

INTRODUCTION

The first step in protein synthesis is the activation of amino acids by esterification with tRNAs. These reactions are catalysed by aminoacyl-tRNA synthetases [1]. Aminoacyl-tRNAs are used not only in the process of protein synthesis but their possible role in the regulation of DNA and RNA synthesis and repression of biosynthetic enzymes for the respective amino acids has been suggested [2, 3]. Thus, changes in aminoacyl-tRNA synthetase activities bringing about changes in the production of aminoacyl-tRNAs may play a crucial role in the regulation of some processes in cells. Changes in aminoacyl-tRNA synthetase activities caused by low-MW compounds or proteins have been detected in eucaryotic cells, yeast [4], animal tissues [5], plants [6, 7] and *Tetrahymena pyriformis* [8].

In previous papers we have given evidence that seeds of yellow lupin contain a low-MW factor stimulating the aminoacylation reactions [6] and influencing the synthetase–tRNA complex [9]. In the present communication we attempt to show that stimulating factors similar to those of lupin are present in seeds of several plants.

RESULTS AND DISCUSSION

The supernatants after ultracentrifugation of the enzyme associates (see Experimental) were passed through a Sephadex G-25 column (Fig. 1). For all plants, fractions stimulating the aminoacylation reactions were observed after chromatography. These fraction were collected together and used in further experiments. The factors obtained did not reveal enzymatic activity and could be kept at $-18°$.

Table 1 gives data of the stimulation of aminoacyl-tRNA synthetase activities by the factors isolated from all plants tested. The synthetase activities in the presence of the factors show large increases compared with the enzymes without the factors. The enzymes isolated from pea, bean and maize exhibited a relatively low activity, which, in the presence of the factors, increased several fold. The synthetases from lupin and wheat had relatively high activities even without the factors, but in their presence the rate of aminoacylation increased less compared with peas, bean and maize. We cannot as yet explain the differences observed for individual plants. We may assume that in some plants the enzyme–factor complex is more stable and during isolation complete separation of both molecules does not occur. This assumption may be proved by observing the gradual decrease in activity during purification of isoleucyl- and leucyl-tRNA synthetase from seeds of yellow lupin and the simultaneous increase in the activities of these enzymes after addition of the factors to the assays [Bartkowiak S., Poniatowska, M. and Wojtowicz, B., unpublished results]. The MWs of the factors were determined on the same column, their presence being detected as a peak of synthetase stimulation. The markers used were: vitamin B_{12}, oxygenated glutathione and riboflavin.

Table 2 shows that the MWs of the factors from the plants tested, determined by the gel filtration method on Sephadex G-25, were similar and were in the range 950–1300 daltons. For some plants two stimulation peaks can be observed (Fig. 1, e.g. bean, lupin, rye, maize). The presence of the stimulation peak in soybean at a MW of 400 may be caused by molecules other than those present in the main peak, or by breakdown products, since occasionally we observed a very unstable stimulatory capacity in samples from this part of the column. In the experiments presented in Tables 3–5 we tested the influence of the factors from one plant on the activity of some individual aminoacyl-tRNA synthetases from others. This investigation was carried out on valyl-, tryptophanyl- and arginyl-tRNA synthetase. The use of an amino acid mixture for aminoacylation, as in the experiments presented above, might fail to give an explanation to this question, because in an amino acid mixture one measures the average level of activity of many aminoacyl-tRNA synthetases.

The results from Tables 3–5 show that no specific influence of the factors on the activity of the synthetases

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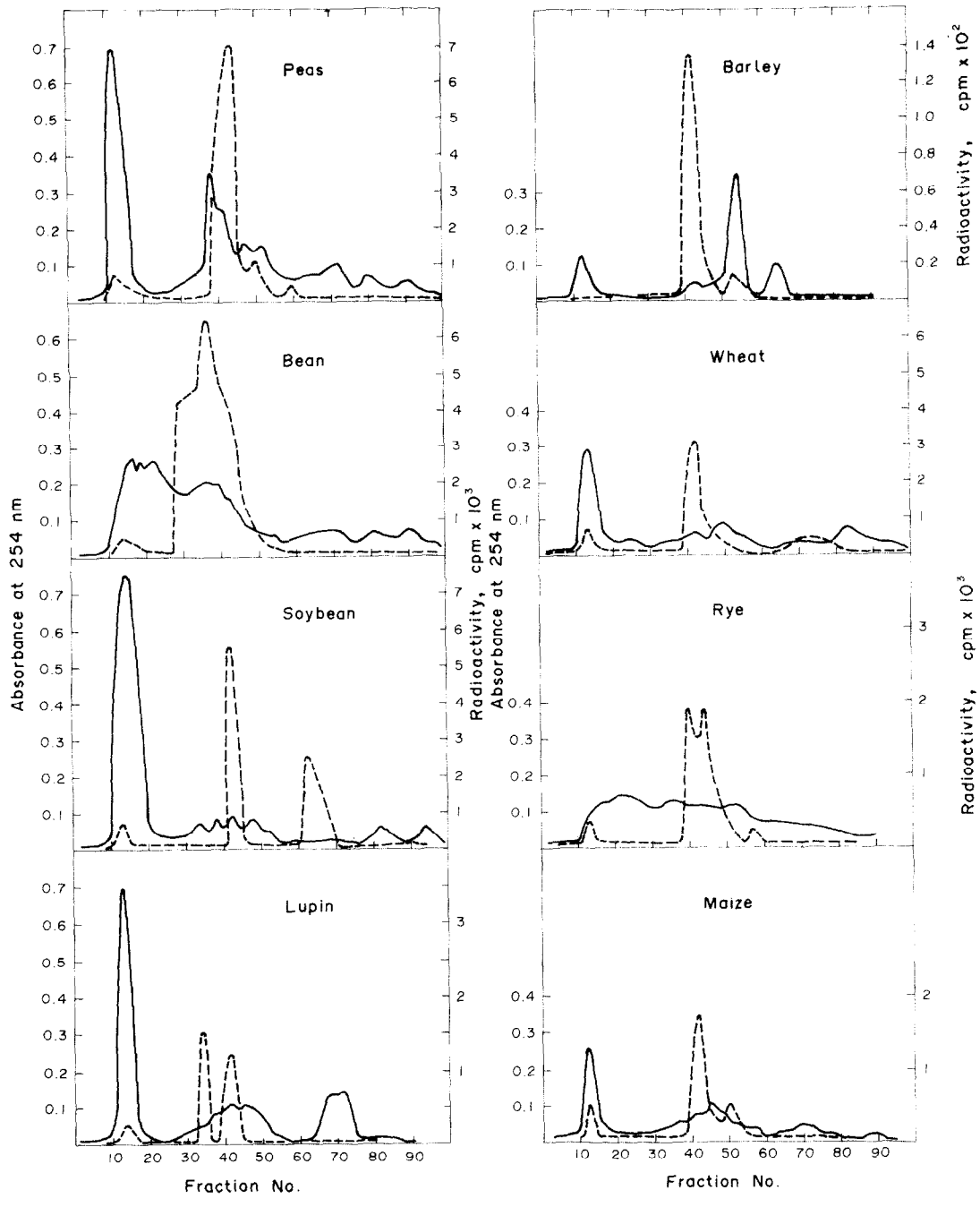

Fig. 1. Chromatography on a Sephadex G-25 column (1 × 90 cm) of the stimulating factors isolated from peas, bean, soybean, lupin, barley, wheat, rye, and maize. Portions (10 μl) of obtained fractions were assayed with 40 μl ^{14}C-labelled protein hydrolysate (Amersham CFB.25) and appropriate amounts of enzymes. Stimulation was obtained by subtraction of the results of the assays of the enzyme from the assays containing the fraction from Sephadex G-25 CC. (———) $A_{254\,nm}$ of eluate obtained in Uvicord; (– – –) stimulation.

can be observed regardless of their origin. The differences in activity of the synthetases in the presence of individual factors isolated from tested plants are caused by different amounts of the factors in the individual preparations, and are not due to their properties as specific stimulators. However, we observed greater stimulation in some heterogeneous enzyme–factor systems than in homogeneous ones (e.g. valyl-tRNA synthetase in the system soybean–soybean, soybean–wheat, wheat–wheat). This problem requires further detailed investigation.

We also attempted to determine the magnitude of stimulation of aminoacyl-tRNA synthetase activities by the factors for plants closely allied to each other. For these experiments four varieties of yellow lupin and two

Table 1. Stimulation of aminoacyl-tRNA synthetase activities by the factors

Plant (dry seeds)	Activity of aminoacyl-tRNA synthetases without the factor (cpm)	Activity of aminoacyl-tRNA synthetases in the presence of the factor (cpm)	Stimulation (fold)
1. Bean	420	6640	15.8
2. Peas	650	7660	11.8
3. Maize*	320	2020	6.3
4. Soybean	470	1020	2.2
5. Rye	1100	3020	2.8
6. Wheat	1950	4990	2.6
7. Barley	600	2020	3.4
8. Lupin†	2400	3900	1.6

The data represent the mean of three assays. Assays were performed with C-14 protein hydrolysate (Amersham CFB.25) and 10 μl of the factors obtained after Sephadex G-25 CC.

* Inbred line S-61.

† Jantar variety.

Table 2. MWs of the factors obtained by gel filtration on Sephadex G-25 from various plants

	Bean	Peas	Maize	Soybean	Rye	Wheat	Barley	Lupin
MW	(a) 1360 (b) 1050	950	(a) 1050 (b) 950	(a) 950 (b) 400	950	950	950	(a) 1300 (b) 950

Markers used were: vitamin B_{12}, oxidized glutathione and riboflavin.

Table 3. Stimulation of valyl-tRNA synthetase activity by the factors for soybean, rye, wheat, barley and lupin in the homogeneous and heterogeneous enzyme–factor systems

Source of the enzyme	Activity of the enzyme without the factor	Source of the factor				
		Soybean	Rye	Wheat	Barley	Lupin
Soybean	5.6	88.1	13.7	39.0	14.6	42.3
Rye	7.4	78.0	9.8	28.0	10.7	38.1
Wheat	7.3	100.4	12.0	35.4	12.0	45.0
Barley	7.7	77.6	11.2	28.6	12.5	33.0
Lupin	4.4	58.2	11.2	22.8	11.3	27.5

Data represent the mean of three experiments. The enzyme activities were expressed as counts/10 min/mg protein $\times 10^{-3}$. Assays were performed with 10 μl of the factors obtained after Sephadex G-25 CC.

Table 4. Stimulation of arginyl-tRNA synthetase activity by the factors for soybean, rye, wheat, barley and lupin in homogeneous and heterogeneous enzyme–factor systems

Source of the enzyme	Activity of the enzyme without the factor	Source of the factor				
		Soybean	Rye	Wheat	Barley	Lupin
Soybean	1.2	10.0	5.1	10.0	9.0	9.1
Wheat	1.7	12.1	6.5	32.1	15.1	17.2
Rye	1.4	10.3	7.3	20.0	10.2	8.7
Barley	1.4	12.0	7.2	25.2	11.3	10.3
Lupin	2.5	9.1	7.0	17.1	9.6	6.7

Data represent the mean of three experiments. Enzyme activities and amount of the factor in the assays as in Table 3.

Table 5. Stimulation of tryptophanyl-tRNA synthetase activity by the factors for soybean, rye, wheat, barley and lupin in homogeneous and heterogeneous enzyme–factor systems

Source of the enzyme	Activity of the enzyme without the factor	Source of the factor				
		Soybean	Rye	Wheat	Barley	Lupin
Soybean	7.7	22.0	10.3	14.5	12.0	19.5
Rye	7.0	22.0	10.0	13.0	10.0	15.2
Wheat	6.0	21.5	9.7	9.3	15.1	16.0
Barley	6.1	20.1	10.0	13.0	10.5	16.0
Lupin	8.6	19.5	12.6	15.1	10.0	15.1

Data represent the mean of three experiments. The concentration of protein in the assays was established in order to standardize the activities of the enzymes from various plants in the absence of the factor. Amount of the factor in assays as in Table 3.

inbred lines of maize were chosen (Table 6). In the case of closely allied plants we observed differential activity of the synthetases when the stimulating factor originated in a different variety. For inbred lines of maize we obtained greater activity of the synthetases in a heterogeneous enzyme–factor system than in a homogeneous system. The increases in stimulation in the heterogeneous system of some lupin varieties (Bas–Afus), and in the heterogeneous system of maize, were correlated with the vigour of growth of the hybrids [Tomaszewski, Z. and Krolikowski, Z. personal communication], which may point to the importance of stimulation of the activity of aminoacyl-tRNA synthetases in the growth cycle of plant cells.

EXPERIMENTAL

Plant materials. Dry seeds of soybean, green pea, bean, wheat, barley, rye, maize and lupin, purchased from the Plant Breeding Stations at Wiatrowo, Przebedowo and Smolice were used.

Reagents. ^{14}C-labelled amino acids (110–342 mCi/mmol) were purchased from the Radiochemical Centre, Amersham and Sephadex G-25 from Pharmacia.

Isolation of aminoacyl-tRNA synthetases. Aminoacyl-tRNA synthetases were obtained during ultracentrifugation as described earlier. [6].

Isolation of the factors. Isolated from dry seeds as described in ref. [6] for lupin.

Isolation of tRNAs. tRNAs were isolated from yellow lupin seeds according to the method of ref. [10].

Protein determination. Carried out according to the method of ref. [11].

Assays. Performed as described earlier [6] with tRNAs from lupin seeds being used with aminoacyl-tRNA synthetases from all tested plants.

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Table 6. Effects of the factors on aminoacyl-tRNA synthetase activities determined for four lupin varieties and two inbred lines of maize

Source of the enzymes	Source of the factor					
	Baltyk	Bas	Jantar	Afus	S-61	S-10
Lupin varieties						
Baltyk	100	106	150	156	—	—
Bas	84	100	98	201	—	—
Jantar	80	80	100	23	—	—
Afus	200	103	107	100	—	—
Inbred lines of maize						
S-61	—	—	—	—	100	130
S-10	—	—	—	—	125	100

Changes in activity of the synthetases were expressed as a percentage. Stimulation in the homogeneous enzyme–factor system was determined as 100%. Aminoacylation was performed as given in Table 1.

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