Phytochemistry, Vol. 28, No. 11, pp. 2927–2929, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

DIFFERENTIAL INFLUENCE OF STIMULATING FACTORS ON CYTOPLASMIC AND CHLOROPLASTIC AMINOACYL-*t*RNA SYNTHETASE ACTIVITY

MAREK RADLOWSKI and SŁAWOMIR BARTKOWIAK

Institute of Plant Genetics, Polish Academy of Sciences, 60–479 Poznań, Strzeszyńska 30/36 Poland

(*Received in revised form* 3 *April* 1989)

Key Word Index—*Phaseolus vulgaris*; Papilionaceae; bean; *Euglena gracilis*; Euglenales; alga; stimulating factors; cytoplasmic and chloroplastic leucyl-*t*RNA synthetase.

Abstract—The effect of stimulating factors on the activity of aminoacyl-*t*RNA synthetases from bean leaves and *Euglena gracilis* cytoplasm and chloroplasts in the reaction of aminoacylation was investigated. Leucyl-*t*RNA synthetase was chosen as a model system. Stimulating factors isolated from bean, lupin and maize seeds have different effects on the activity of leucyl-*t*RNA synthetase. In all cases the stimulating factor from maize appeared to be active. The stimulating factors I and II from bean in a homologous system stimulate only the cytoplasmic enzyme. The most interesting systems were taken for further examination of the $^{32}PP_i$-ATP exchange reaction, where a weaker effect was found.

INTRODUCTION

Available data prove the existence of significant differences between isospecific aminoacyl-*t*RNA synthetases originating from cytoplasm and chloroplasts [1]. These differences concern not only M_rs, but also migration on chromatographic columns, their reaction with specific *t*RNAs, Michaelis constant values for respective substrates, amino acid composition and immunological data [2–5]. Taking into consideration the facts mentioned above, it is possible to believe that there are some structural differences between these enzymes.

Some time ago low M_r factors stimulating the activity of aminoacyl-*t*RNA synthetases [6], which occur commonly in plants [7] were described. The previous results of the studies on the chemical nature of these compounds isolated from lupin and maize seeds indicate that they have the character of short peptides [8]. A similar effect on these enzymes was found in yeast [9], Drosophila [10] and mammals [11, 12], but it was caused by lipids [9, 11] or proteins [10, 12]. The role of similar low M_r, endogenous compounds in the control of transcription and translation [13] as well as in regulation of the activity of RNA polymerase [14] was investigated.

It seemed interesting to compare the influence of stimulating factors originating from various plant sources on bean leaves and *Euglena gracilis* leucyl-*t*RNA synthetases from cytoplasm and chloroplasts. Significant differences have been revealed between aminoacyl-*t*RNA synthetases in cytoplasm and those originating from cell organelles [1–5, 15–17]. The stimulating factors isolated from bean, maize and lupin seeds have been tested for their effect on the kinetics of the aminoacylation and amino acid activation reactions.

RESULTS AND DISCUSSION

The influence of stimulating factors from bean, maize and lupin seeds on the kinetics of aminoacylation reaction of bean leaves and *Euglena gracilis* leucyl-*t*RNA synthetases from cytoplasm and chloroplasts has been examined. Results for the cytoplasmic enzyme of bean leaves are shown in Fig. 1A. Both stimulating factors from bean seeds increase the activity of cytoplasmic enzyme to the same extent (3.5 times) independently of the fact that they differ from each other with respect to their migration on a Sephadex G-25 column. The faster migrating factor is marked as factor I, the one migrating more slowly as factor II. Similarly, the factor from lupin is one of the two factors (the bigger one) isolated from lupin seeds [7]. The factor from maize manifests significant stimulating properties (the value of stimulation is over twice), while in the case of the lupin factor there was a tiny or even no stimulating effect on the enzyme activity. It is unexpected because lupin is more closely related to bean than corn is.

The influence of stimulating factors from these plant sources on the leucyl-*t*RNA synthetase from bean leaves chloroplasts is different (Fig. 1B). The factors from bean exhibit a small or even no effect on the enzyme activity. It might reflect the lack of such compounds in the chloroplasts or the existence of factors which are different from those isolated from bean seeds. Maize stimulating factor shows an important effect on the activity of the enzyme from chloroplasts ($\times 4$) in comparison to its influence on the cytoplasmic synthetase. Only the factor from lupin seeds behaves in a similar way, as it does in the previous case.

The influence of stimulators on the activity of leucyl-*t*RNA synthetase of *Euglena gracilis* cytoplasm is shown in Fig. 2A. In all cases a considerable increase in the enzymatic activity has been found. Stimulating factor II from bean, factors from maize and lupin all increase the rate of the aminoacylation reaction *ca* 2.5–3 times. Factor I from bean differs from them in its effect, and it stimulates this enzymatic activity four-fold. It is very interesting to compare it with the leucyl-*t*RNA synthetase from the cytoplasm of bean leaves, where both bean factors stimu-

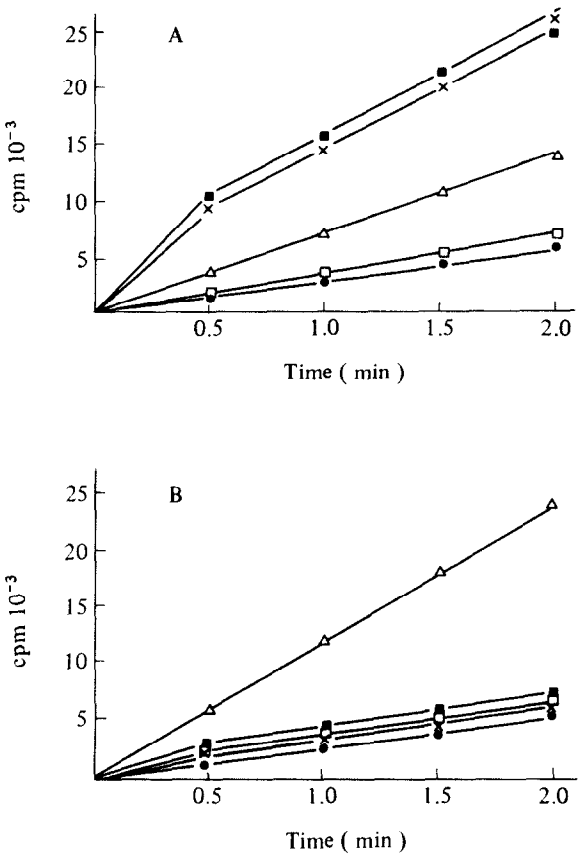

Fig. 1. Kinetics of the aminoacylation reaction leucyl-*t*RNA synthetase from bean leaves cytoplasm (A) and chloroplasts (B). ●——● without factor in the assays; ■——■ factor I from bean in the assays; ×——× factor II from bean in the assays; △——△ factor from maize in the assays; □——□ factor from lupin in the assays.

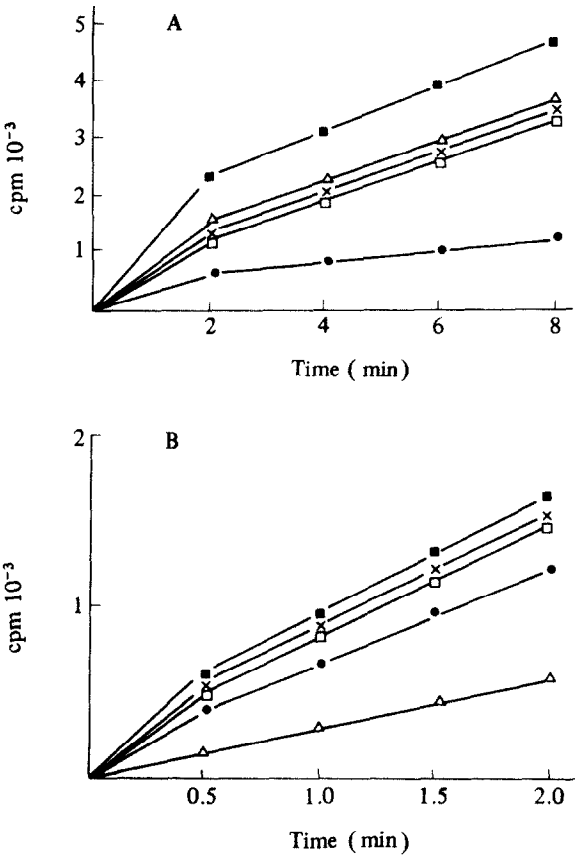

Fig. 2. Kinetics of the aminoacylation reaction leucyl-*t*RNA synthetase from *Euglena gracilis* cytoplasm (A) and chloroplasts (B). ●——● without factor in the assays; ■——■ factor I from bean in the assays; ×——× factor II from bean in the assays; △——△ factor from maize in the assays; □——□ factor from lupin in the assays.

late the activity of the enzyme more or less to the same extent. In contrast to its effect on the cytoplasmic bean leaves synthetase, the lupin factor stimulates three-fold activity of the enzyme from the cytoplasm of *Euglena gracilis*. The kinetics of the aminoacylation reaction for leucyl-*t*RNA synthetase from *Euglena gracilis* chloroplasts are shown in Fig. 2B. The lupin factor and both bean factors stimulate the activity of this enzyme to a small extent, just as in the case of leucyl-*t*RNA synthetase of bean leaves chloroplasts. The effect of the factor from maize seeds is in this experiment dramatically different from all other cases presented in the paper. It is emphasized that, while a four-fold stimulation of the activity of the chloroplastic enzyme from bean leaves appeared, in the last case significant inhibition was found.

A two-step mechanism of the aminoacylation reaction is generally proposed [18]. The first step, amino acid activation, can be measured by the $^{32}PP_i$-ATP exchange reaction. The stimulating factor from maize seeds showed a different influence on the aminoacylation reaction of chloroplastic enzymes isolated from bean leaves and *Euglena gracilis*. Influence of this effector on the $^{32}PP_i$-ATP exchange reaction was also investigated. Results are shown in Fig. 3A and 3B. There is no inhibition in the case of the chloroplastic enzyme from *E. gracilis*. The activity of the bean leaf chloroplastic enzyme is stimulated in the presence of the maize factor, but not as much as in the case of the amino-acylation reaction. The $^{32}PP_i$-ATP exchange reaction was performed in the presence of *t*RNA. *t*RNA plays a role in the stabilization of the ternary complex enzyme:*t*RNA:stimulating factor [19]. However, the lack of inhibition of the activity for the enzyme from *Euglena gracilis* and the smaller stimulation for the bean leaf enzyme leads to the conclusion that the main influence of the stimulating factor is on the aminoacylation reaction.

Interpretation of the data was possible thanks to the use of the purified homogeneous preparations of leucyl-*t*RNA synthetases [3, 20–22]. Due to this fact there was no danger of contamination of the cytoplasmic enzyme by a chloroplastic one and vice versa, which could essentially alter the results. The stimulating properties of the bean factors on the cytoplasmic enzymes and the lack of such an effect on the enzymes originating from chloroplasts in a homologous system is especially interesting. The inhibition of aminoacyl-*t*RNA synthetases activities

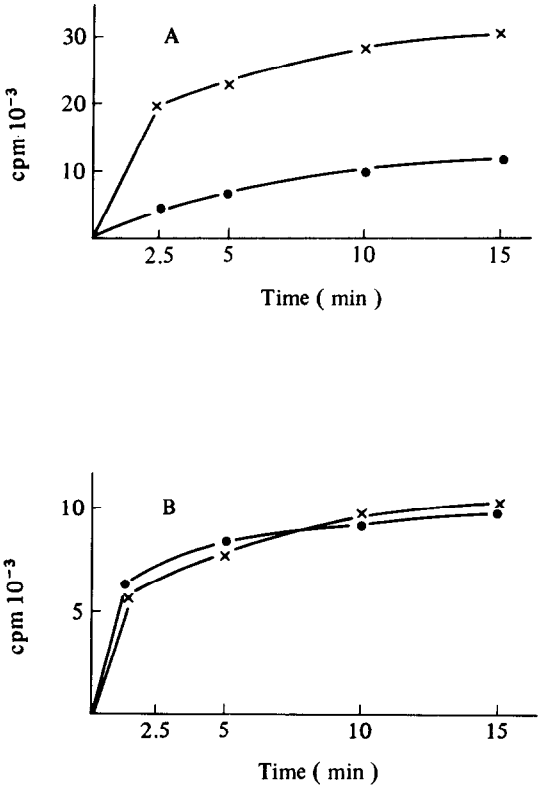

Fig. 3. Kinetics of the $^{32}PP_i$-ATP exchange reaction chloroplastic leucyl-tRNA synthetase from bean leaves (A) and *Euglena gracilis* (B). ●——● without factor in the assays; ×——× factor from maize in the assays.

by a stimulating factor was already described [6], but it involved enzymes from yeast and *E. coli*. Thus, properties of the chloroplastic synthetase from *Euglena gracilis* are similar to those of synthetases of bacterial and yeast origin [15], but this is not the case for the bean leaf chloroplastic enzymes.

The studies did not cover enzymes originating from mitochondria, which differ from cytoplasmic and chloroplastic synthetases [5, 15–17]. It is, therefore unknown whether the studied compounds have an influence on the activity of mitochondrial enzymes.

EXPERIMENTAL

Reagents. L-[^{3}H] Leucine (40 Ci/mmol) and L-[U^{14}C] leucine (300 mCi/mmol) were purchased from the Commissariat a l'Energie Atomique (Saclay); $Na^{32}PP_i$ was purchased from Amersham; ATP was from Sigma; *E. coli* and yeast tRNA were from Schwarz/Mann and Boehringer respectively.

Enzymes. Cytoplasmic and chloroplastic leucyl-tRNA synthetases from bean leaves and *Euglena gracilis*, purified as described [3, 20–22] respectively, were a gift from Prof. J. H. Weil's laboratory (IBMC, Strasbourg).

Factors. The stimulating factors were isolated as described previously [6].

Assays. The assays of aminoacylation reaction were performed as refs [3, 20–22] respectively; the assays of $^{32}PP_i$-ATP exchange reaction were performed as ref. [23]. The amounts of the factors in the assays were determined in a preliminary experiment.

REFERENCES

1. Weil, J. H. and Parthier, B. (1982) in *Nucleic Acids and Proteins in Plants I, Encyclopedia of Plant Physiology, New Series*, Vol. 14 A (Boulter, D. and Parthier, B., eds).
2. Locy, R. D. and Cherry, J. H. (1978) *Phytochemistry* **17**, 19.
3. Dietrich, A., Souciet, G., Colas, B. and Weil, J. H. (1983) *J. Biol. Chem.* **258**, 12386.
4. Colas, B., Imbault, P., Sarantoglou, V. and Weil, J. H. (1982) *FEBS Letters* **141**, 213.
5. Jeannin, G., Burkard, G. and Weil, J. H. (1976) *Biochim. Biophys. Acta* **442**, 24.
6. Bartkowiak, S. and Radłowski, M. (1977) *Biochim. Biophys. Acta* **474**, 619.
7. Bartkowiak, S., Poniatowska, M. and Radłowski, M. (1983) *Phytochemistry* **22**. 413.
8. Bartkowiak, S. (1982) *Acta Biol. Pol.* **10**, 177 (English summary).
9. Dimitrijevic, L. and Godefroy-Colburn, Th. (1974) *FEBS Letters* **45**, 194.
10. Hiatt, V. and Hillman, R. (1980) *Biochim. Biophys. Acta* **609**, 400.
11. Hradec, J. and Dusek, Z. (1968) *Biochem. J.* **110**, 1.
12. Davies, M. R. and Marshall, R. D. (1975) *Biochim. Biophys. Acta* **390**, 94.
13. Hillar, M. and Przyjemski, J. (1979) *Biochim. Biophys. Acta* **564**, 246.
14. Andersen, H. A. and Islin, H. (1987) *Biochim. Biophys. Acta* **908**, 1.
15. Sinclair, D. and Pillay, D. T. N. (1981) *Z. Pflanzenphysiol.* **104**, 299.
16. Kunugi, S., Uehara-Kunugi, Y., von der Haar, F., Schischkoff, J., Freist, W., Englisch, U. and Cramer, F., (1986) *Eur. J. Biochem.* **158**, 43.
17. Gabius, H. J., Freist, W. and Cramer, F., (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 1241.
18. Ofengang, J. (1977) in *Molecular Mechanisms of Protein Biosynthesis* (Weissbach, H. and Pestka, S., eds), pp. 7–79. Academic Press, New York.
19. Bartkowiak, S. and Radłowski, M., (1984) *Acta Biol. Pol.* **16**, 208.
20. Souciet, G., Dietrich, A., Colas, B., Razafimahatratra, P. and Weil, J. H., (1982) *J. Biol. Chem.* **257**, 9598.
21. Sarantoglou, V., Imbault, P. and Weil, J. H., (1981) *Plant Sci. Letters* **22**, 291.
22. Imbault, P., Colas, B., Sarantoglou, V., Boulanger, Y. and Weil, J. H. (1981) *Biochemistry* **20**, 5855.
23. Kern, D. and Giege, R. (1979) *FEBS Letters* **103**, 274.