Phytochemistry, Vol. 21, No. 6, pp. 1189–1193, 1982.
Printed in Great Britain.

0031-9422/82/061189-05$03.00/0

PROPERTIES OF PURIFIED CHLOROPLASTIC AND CYTOPLASMIC VALYL- AND LEUCYL-tRNA SYNTHETASES FROM *EUGLENA GRACILIS*

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(*Received* 13 *October* 1981)

Key Word Index—*Euglena gracilis*; Euglenales; chloroplast; aminoacyl-tRNA synthetases; catalytic properties; transfer RNA; spermine.

Abstract—The catalytic properties of purified chloroplastic and cytoplasmic valyl- and leucyl-tRNA synthetases from *Euglena gracilis* were studied and compared. The chloroplastic and cytoplasmic aminoacyl-tRNA synthetases differ in their sensitivity to KCl, divalent cations and spermine. The two leucyl-tRNA synthetases appear to differ more than the two valyl-tRNA synthetases in their affinity for their substrates. But the two valyl-tRNA synthetases appear to differ more than the two leucyl-tRNA synthetases in their specificity towards heterologous tRNAs.

INTRODUCTION

Chloroplasts are the sites of intensive protein synthesis in photosynthetic eukaryotes and they contain up to 60% of the total proteins of green cells. It is now well established that chloroplasts, as well as mitochondria, possess their own protein-synthesizing system which differs from the cytoplasmic system. Chloroplast-specific aminoacyl-tRNA synthetases (EC 6.1.1.–) have been shown to differ from their cytoplasmic counterparts mainly in (1) their intracellular localization, (2) their chromatographic behaviour (for instance on hydroxylapatite), (3) their substrate (tRNA) specificity; while the chloroplastic aminoacyl-tRNA synthetases usually recognize, in addition to chloroplastic tRNAs, tRNAs from procaryotes, the cytoplasmic enzymes usually recognize eucaryotic cytoplasmic tRNAs[1–5].

Chloroplastic aminoacyl-tRNA synthetases have not been studied as much as the bacterial and animal enzymes, mainly because of the inherent instability of aminoacyl-tRNA synthetases extracted from green cells: plant extracts contain a large variety, and frequently large quantities of 'secondary products' such as phenolic compounds which have very strong inhibitory effects on the activity of many enzymes and particularly of aminoacyl-tRNA synthetases[6]. Up to now, a few studies only have been performed on cytoplasmic and chloroplastic aminoacyl-tRNA synthetases using partially purified and often unstable enzymes[3, 7]. We have developed a method for the purification to homogeneity of chloroplastic and cytoplasmic valyl- and leucyl-tRNA synthetases from *Euglena gracilis*[8–11] yielding enzymes whose specific activities are comparable to those of the corresponding enzymes from *E. coli*[12, 13] and yeasts[14–16].

We are presenting here the first report on the comparison of the catalytic properties of purified chloroplast valyl- and leucyl-tRNA synthetases with those of their cytoplasmic counterparts.

RESULTS

Optimal pH

Valyl-tRNA synthetases (ValRS) and leucyl-tRNA synthetases (LeuRS) from *Euglena gracilis* show slightly basic optimal pH values as do homologous enzymes from other sources. The chloroplastic and cytoplasmic ValRS show identical optimal pH in the range of 7.5–8 (Tris–HCl or cacodylate buffer). In contrast, a slight difference in the optimal pH has been observed in the case of the chloroplastic LeuRS (optimal pH = 8.5) and the cytoplasmic LeuRS (optimal pH = 8) in the different buffer systems tested (Tris–HCl, Hepes–KOH or cacodylate–HCl).

Mg^{2+}/*ATP ratio*

Euglena chloroplastic and cytoplasmic ValRS have a different optimal Mg^{2+}/ATP ratio: 2.5 for the chloroplastic ValRS and 1 for the cytoplasmic enzyme. Those values are different from those published by Krauspe and Parthier[3] for non-purified *Euglena* ValRS: 1 for chloroplastic ValRS and 5 for cytoplasmic ValRS. On the contrary, the optimal Mg^{2+}/ATP ratios found for the two LeuRS are very similar: 1.2 for the chloroplastic enzyme and 1.1 for the cytoplasmic LeuRS. Krauspe and Parthier[3] found an optimal ratio of 3 for the two non-purified *Euglena* LeuRS.

The differences between the values obtained in this work and those obtained with non-purified enzymes are

perhaps due to the presence in crude preparations of other compounds which can bind Mg^{2+} or ATP.

Effects of KCl

It has been shown that KCl sometimes has a stimulating effect on the aminoacylation reaction when it is present at low concentration (< 100 mM) but that it always has an inhibitory effect at high concentration (> 100 mM). In the case of *Euglena* ValRS and LeuRS, Fig. 1 shows that KCl acts differently on the cytoplasmic and the chloroplastic enzymes. At concentrations lower than 30 mM, KCl enhances the activities of the two cytoplasmic enzymes, but has no effect on their chloroplastic homologues. Concentrations of KCl higher than 30 mM, inhibit the reaction catalysed by cytoplasmic ValRS and LeuRS more strongly than the reactions catalysed by the chloroplastic enzymes.

The fact that the inhibitory effect of high KCl concentrations is stronger on cytoplasmic aminoacyl-tRNA synthetases than on chloroplastic ones, has been already observed in non-purified systems [2, 3, 17]. However, the ability of low KCl concentrations to stimulate cytoplasmic but not chloroplastic enzyme has not been seen before.

Effects of divalent cations

Divalent cations are necessary for the aminoacylation reaction as for other ATP-dependent reactions. The reaction mixture generally contains Mg^{2+} ions, but these ions can often be substituted, at least partially, by Mn^{2+}, Ca^{2+} or Co^{2+} ions [18, 19]. Figures 2(A) and (B) show the effects of these ions on chloroplastic and cytoplasmic ValRS activities. The Mg^{2+} ions are the most effective, but Mn^{2+} is able to allow 60% of cytoplasmic and chloroplastic maximal activities. The Co^{2+} ions can partially replace Mg^{2+} ions in the case of chloroplastic ValRS only, and Ca^{2+} ions are unable to promote the aminoacylation reaction catalysed by both ValRS. It is of interest to note that the inhibition caused by suboptimal divalent cation concentrations is stronger in the case of cytoplasmic ValRS than in the case of chloroplastic ValRS.

Figures 2(C) and (D) show that Ca^{2+} ions can only replace Mg^{2+} in the reaction catalysed by cytoplasmic LeuRS. In contrast, all four ions (Mg^{2+}, Ca^{2+}, Mn^{2+} and Co^{2+}) promote the chloroplast LeuRS activity (with 100, 80, 75 and 40% of the maximal activity, respectively). The inhibition by high concentrations of divalent cations is more important for the cytoplasmic LeuRS than for chloroplastic LeuRS as also observed for the two ValRS.

Effects of spermine

It is known from the work of Takeda and Igarashi [20] that polyamines have a stimulatory effect on the aminoacylation reaction. Figure 3(A) shows that spermine has a Mg^{2+} concentration-dependent effect on chloroplastic ValRS activity. Spermine is unable to promote aminoacylation in the absence of Mg^{2+}, but in the presence of suboptimal Mg^{2+} concentration (2 mM), spermine can restore maximal activity (normally obtained with a concentration of 8 mM Mg^{2+}). In the case of the cytoplasmic ValRS (Fig. 3B), spermine is unable to restore the full activity when the Mg^{2+} concentration is lower than the optimal one. As shown in Fig. 3(D), in the absence of Mg^{2+} ions spermine allows a very low level of cytoplasmic LeuRS activity, a phenomenon which is not observed with the two ValRS or with

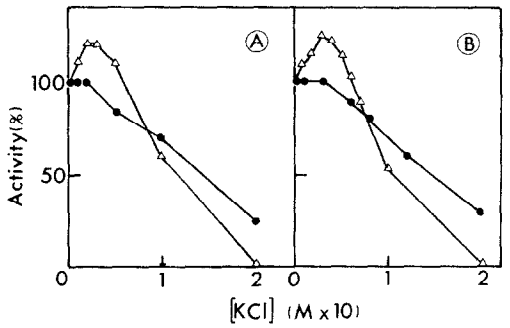

Fig. 1. Effects of KCl concentration on the aminoacylation reaction catalysed by ValRS (A) and LeuRS (B). (●—●) Chloroplastic enzymes; (△—△) cytoplasmic enzymes. The activities are expressed as the percentage of the activity without KCl.

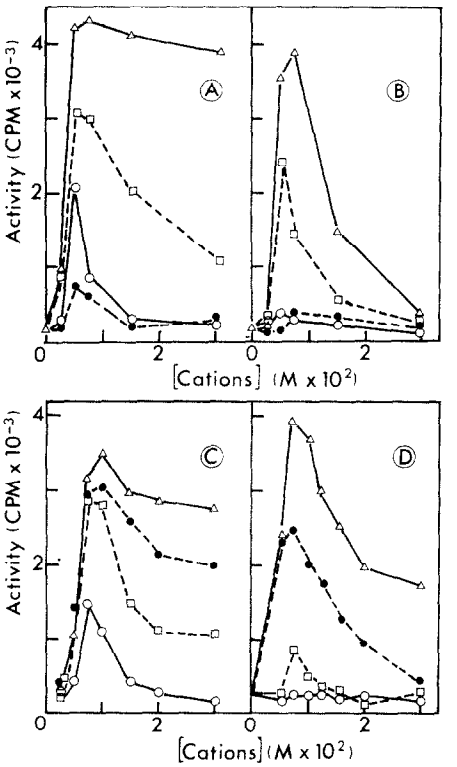

Fig. 2. Effects of divalent cation on the aminoacylation reaction catalysed by chloroplastic (A) and cytoplasmic (B) ValRS and by chloroplastic (C) and cytoplasmic (D) LeuRS. (△—△) Mg^{2+}; (□---□) Mn^{2+}; (○—○) Co^{2+}; (●---●) Ca^{2+}.

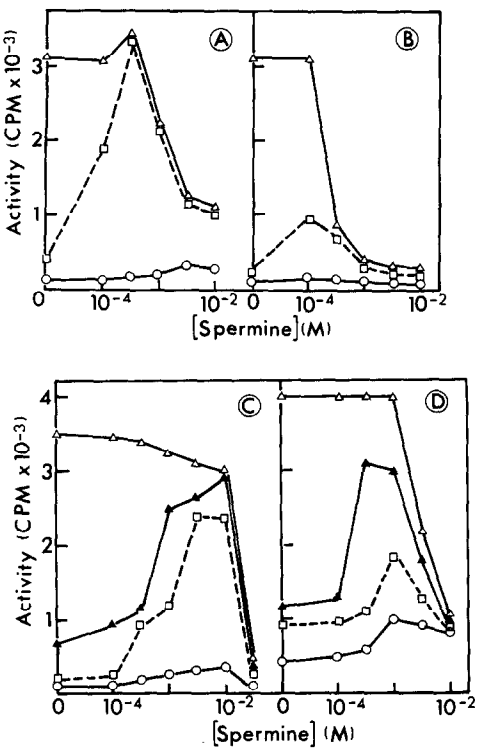

Fig. 3. Effects of spermine concentration on the aminoacylation reaction catalysed by chloroplastic (A) and cytoplasmic (B) ValRS and by chloroplastic (C) and cytoplasmic (D) LeuRS. Reactions were performed in the presence of various Mg^{2+} concentrations: (○—○) 0 mM Mg^{2+}; (□---□) 2 mM Mg^{2+}; (▲—▲) 4 mM Mg^{2+}; (△—△) 8 mM Mg^{2+}.

chloroplastic LeuRS (Fig. 3C). The replacement of Mg^{2+} by spermine has been reported to be effective in the case of yeast [15] and soybean [21] LeuRS. Figures 3(C) and (D) show that in the presence of suboptimal Mg^{2+} concentrations (2 and 4 mM), spermine acts as a strong activator of both LeuRS, but does not restore the aminoacylation activity at its optimal level (which is obtained with 8 mM Mg^{2+}) even in the case of the chloroplastic LeuRS (whereas this is achieved in the case of the chloroplastic ValRS; see Fig. 2A).

The synergism of spermine and Mg^{2+} ions is greater in the case of the chloroplastic aminoacyl-tRNA synthetases than in the case of the cytoplasmic ones. However, as previously shown[2], the inhibitory effect of high spermine concentrations is more important with cytoplasmic enzymes than with chloroplastic enzymes.

Specificity towards tRNAs

The specificity of chloroplastic ValRS for tRNAs appears to be very different from that of the cytoplasmic ValRS. Krauspe and Parthier[3] have shown that *Euglena* chloroplastic ValRS is able to aminoacylate the tRNA from *Anacystis nidulans* (a blue-green alga) but not the cytoplasmic tRNA from a bleached *Euglena gracilis* mutant. In contrast, *Euglena* cytoplasmic ValRS can charge the tRNA from the mutant but not that from *Anacystis*. We have also shown that *Euglena* chloroplastic ValRS charges *E. coli* tRNA and not yeast tRNA, whereas the cytoplasmic ValRS is able to aminoacylate yeast tRNA but not tRNAs from *E. coli*. Furthermore, we have shown that the RNA from Eggplant Mosaic Virus, which has at its 3′ end a tRNA-like structure able to accept L-valine[22], can be aminoacylated by *Euglena* cytoplasmic ValRS while chloroplastic ValRS is not able to aminoacylate this RNA.

Chloroplastic LeuRS exhibits a high level of specificity towards tRNAs, just as the two ValRS: this enzyme recognizes procaryotic tRNAs such as those from *Anacystis nidulans* or from *E. coli* very well, but is unable to charge tRNAs from yeast or from the *Euglena* bleached mutant. In contrast, cytoplasmic LeuRS recognizes tRNAs from the bleached mutant and from yeast, but also some $tRNA^{Leu}$ isoacceptors from *E. coli*. It therefore does not have as strict a tRNA specificity as the chloroplastic LeuRS.

Apparent K_m values

The K_m values of *Euglena* chloroplastic and cytoplasmic ValRS and LeuRS for their substrates are presented in Table 1.

The most important difference between chloroplastic and cytoplasmic ValRS is the affinity for L-valine which is 3.3 times greater for the chloroplastic enzyme. The K_m values of the two *Euglena* ValRS are of the same order of magnitude as those of the ValRS purified from other organisms[14, 23, 24].

Chloroplastic and cytoplasmic LeuRS exhibit greater differences than the two ValRS in their affinities for their substrates. The chloroplastic LeuRS shows a 3 times greater affinity for L-leucine than the cytoplasmic LeuRS, but the cytoplasmic LeuRS exhibits a 12 times greater affinity for ATP than its chloroplastic counterpart. The affinity of cytoplasmic LeuRS for ATP is also greater than that of LeuRS purified from other sources[13, 15, 16, 25].

It is of interest to note that *Euglena* cytoplasmic ValRS and LeuRS have a similar affinity for *Euglena* and yeast tRNAs, while *Euglena* chloroplastic ValRS and LeuRS have a similar affinity for *Euglena* and *E. coli* tRNAs. This feature has been very helpful in the purification of *Euglena* enzymes, as it has allowed the use of commercial yeast or *E. coli* tRNAs, respectively, for routine tests of column fractions and for affinity chromatography[8–11].

DISCUSSION

In this report we are presenting the results of the first comparative study on the catalytic properties of highly purified chloroplastic and cytoplasmic aminoacyl-tRNA synthetases. These results reveal a certain number of differences between the *Euglena* chloroplastic ValRS and LeuRS and their cytoplasmic counterparts.

In general, the concentration and the nature of salt present in the reaction mixture seem to affect more strongly the activities of cytoplasmic ValRS and LeuRS than the activities of their chloroplastic counterparts. Low KCl concentrations (< 30 mM) are able to enhance cytoplasmic enzyme activities but do not affect chloroplastic enzyme activities. High concentrations of KCl, or divalent cations or spermine, have

Table 1. Apparent K_m values determined for chloroplastic and cytoplasmic ValRS and LeuRS

Substrate	Chloroplastic ValRS	Cytoplasmic ValRS	Chloroplastic LeuRS	Cytoplasmic LeuRS
L-Valine	1.5×10^{-5} M	5×10^{-5} M	—	—
L-Leucine	—	—	8×10^{-6} M	2.4×10^{-5} M
ATP	5×10^{-5} M	7×10^{-5} M	1.3×10^{-4} M	1.1×10^{-5} M
Euglena tRNA*	6×10^{-8} M	5×10^{-8} M	1.3×10^{-6} M	1.6×10^{-6} M
E. coli tRNA*	8×10^{-8} M	—	8.8×10^{-7} M	—
Yeast tRNA†	—	8×10^{-8} M	—	2×10^{-7} M

*In these experiments, total (*Euglena* or *E. coli*) tRNA was used. The calculation of the apparent K_m values is based on the capacity of total tRNA to be charged by the enzyme tested (as measured from the aminoacylation plateau).

†In these experiments purified yeast $tRNA_2^{Val}$ and $tRNA_3^{Leu}$ were used.

stronger inhibitory effects on the activities of cytoplasmic ValRS and LeuRS than on those of their chloroplastic counterparts. Furthermore, Mg^{2+} ions can be replaced by other divalent cations or by spermine with a higher efficiency in the reaction catalysed by chloroplastic enzymes than in that catalysed by cytoplasmic enzymes.

If one considers the affinity towards their substrates, the two LeuRS appear to differ more than the two ValRS. But if one considers the specificity towards tRNAs, the two ValRS appear to have larger differences than the two LeuRS. Cytoplasmic and chloroplastic ValRS show a rather strict specificity for tRNAs of eucaryotic and procaryotic origin, respectively. In the case of the LeuRS, in contrast, the cytoplasmic enzyme recognizes tRNAs from eucaryotic cytoplasm, but also some tRNAs of procaryotic origin, especially *E. coli*. A similar situation was observed in the case of the LeuRS from *Phaseolus vulgaris* [26]; the cytoplasmic LeuRS of this plant was shown to be able to aminoacylate one of the $tRNA^{Leu}$ isoacceptors of *E. coli*.

EXPERIMENTAL

Chemicals. [^{3}H]- and [^{14}C]amino acids were obtained from the Commissariat à l'Energie Atomique (Saclay, France). All other chemicals were of analytical grade obtained from Merck.

Euglena tRNA was prepared according to [27]. Yeast tRNA was from Boehringer and *E. coli* tRNA was from Schwartz-Mann.

Purification of aminoacyl-tRNA synthetases. Purified chloroplastic and cytoplasmic ValRS and LeuRS were prepared as previously described [8–11]. They exhibit the following sp. act. (units/mg protein): 1100 for the chloroplastic ValRS, 1200 for chloroplastic LeuRS, 400 for cytoplasmic ValRS and 1300 for cytoplasmic LeuRS. One unit is defined as the amount of enzyme catalysing the aminoacylation of 1 nmol of tRNA in 1 min.

Aminoacyl-tRNA synthetase activity was measured by the esterification of radioactively labeled amino acids to tRNA was from Boehringer and *E. coli* tRNA was from wise stated: *Chloroplastic ValRS*: Tris–HCl pH 8 20 mM, $MgCl_2$ 5 mM, β-mercaptoethanol 5 mM, ATP 2 mM, bovine serum albumin 0.1 mg/ml, L-[^{14}C]valine (20 μCi/μM) 5.7×10^{-5} M, total *Euglena* or *E. coli* tRNA 0.4 mg/ml. *Cytoplasmic ValRS*: The same mixture was used as in the case of chloroplastic ValRS, except that ATP was 5 mM and that total *Euglena* or yeast tRNA (0.4 mg/ml) was used. *Chloroplastic LeuRS*: Tris–HCl pH 8, 20 mM, $MgCl_2$ 7.5 mM, β-mercaptoethanol 5 mM, ATP 7.5 mM, bovine serum albumin 0.1 mg/ml, L-[^{14}C]leucine (20 μCi/mM) 5.7×10^{-5} M, total *Euglena* or *E. coli* tRNA 0.9 mg/ml. *Cytoplasmic LeuRS*: The same reaction mixture was used as in the case of chloroplastic LeuRS, except that total *Euglena* or yeast tRNA (0.9 mg/ml) was used. Upon addition of the enzyme, the reaction mixture was incubated at 30° for 2 min. Aliquots of 80 μl were put on a Whatman 3 MM paper disc, which was washed according to ref. [28] and counted in a liquid scintillation counter.

Acknowledgements—Dr. M. Pinck provided us with a generous gift of Eggplant Mosaic Virus RNA. We are grateful to Mrs A. Klein for her excellent technical assistance and to Mrs G. Issler for typing the manuscript. This work was supported by a grant 79-7-1050 from the Délégation à la Recherche Scientifique et Technique.

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