# IMMUNOLOGICAL EVIDENCE FOR STRUCTURAL DIFFERENCES BETWEEN *EUGLENA GRACILIS* CHLOROPLASTIC VALYL- AND LEUCYL-tRNA SYNTHETASES AND THEIR CYTOPLASMIC COUNTERPARTS

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## 1. Introduction

In a plant cell, there are at least two aminoacyl-tRNA synthetases (EC 6.1.1. . . .) specific for the same amino acid, one in the cytoplasm and the other in the chloroplasts. There is indirect evidence that both enzymes are coded for by the nuclear genome in spite of the existence of a protein synthesis machinery inside the chloroplast (for a general review, see [1]). The chloroplastic aminoacyl-tRNA synthetases are known to differ from their cytoplasmic counterparts in their substrate (tRNA) specificity and their chromatographic mobility [1] but so far little was known about their structural similarities or differences.

*Euglena gracilis* chloroplastic and cytoplasmic valyl- and leucyl-tRNA synthetases (ValRS and LeuRS, respectively) have been purified in our laboratory [2–5]. Studies on their catalytic properties [6] and on their main structural features, including amino acid composition and tryptic peptide map [7] have shown that chloroplastic ValRS and LeuRS are different from their cytoplasmic counterparts. Immunological studies allow another approach to detect homologies between related proteins. In this report, we show that there is no immunological cross-reaction between chloroplastic ValRS and LeuRS and their cytoplasmic homologues. Taken together these results are in favor of the idea that a chloroplastic aminoacyl-tRNA synthetase and its cytoplasmic counterpart are coded for by different genes.

*Abbreviations:* ValRS, valyl-tRNA synthetase; LeuRS, leucyl-tRNA synthetase; PBS, phosphate-buffered saline; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate

## 2. Materials and methods

### 2.1. *Enzymes*

Chloroplastic and cytoplasmic ValRS and LeuRS were purified from green cells of *Euglena gracilis* Z as described in [2–5]. One enzyme unit is defined as the amount of enzyme which catalyzes the aminoacylation of 1 nmol of tRNA in 1 min at 30°C.

### 2.2. *Immunization*

Antibodies against each of the four purified aminoacyl-tRNA synthetases were raised in rabbits by subcutaneous dorsal injections (~10 shots into the back of the chipped animal). Each rabbit received an emulsion containing 150 μl of complete Freund's adjuvant (Difco), half a dose of adsorbed anti-whooping cough vaccine (Mérieux, France) and 150 μl of enzyme solution (100 μg) extensively dialyzed against phosphate-buffered saline (PBS). Two booster injections were performed at a four week interval. Ten days after the third injection, the blood was taken via the ear vein and was allowed to clot 8 h at room temperature. The serum was collected by centrifugation at 18 000 rev./min for 15 min and 500 μl aliquots were frozen in liquid nitrogen and stored at −40°C. Control serum (pre-immune serum) was also prepared one day prior to the first injection.

### 2.3. *Immuno-inactivation of the enzymes*

Aliquots of purified enzyme (about $3.10^{-2}$ units) were incubated at 4°C for 2 h with increasing amounts of immune-serum in the aminoacylation medium [6] deprived of tRNA. As LeuRS and ValRS are sensitive to dilution, the protein concentration of the reaction

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mixture was maintained constant by addition of pre-immune serum. The residual activity was determined by measuring the aminoacylation of tRNA added to the reaction mixture. Control experiments performed using only pre-immune serum showed no immuno-inactivation of the aminoacyl-tRNA synthetases.

2.4. *Immuno-electrophoresis of the enzymes*

Rocket immuno-electrophoresis was performed as in [8] in a 37.5 mM Tris–100 mM glycine buffer (pH 8.6) on 1 mm thick agarose gels (1%, w/v) containing 0.8% (v/v) emulfogen (GAF, France). The electrophoresis was carried out at 1 V/cm for 18 h and the immuno-plates were then washed in PBS for 24 h and in distilled water for another 24 h. Finally the gels were dried, stained with a 0.25% (w/v) Coomassie blue solution for 5 min and destained with a mixture containing methanol/acetic acid/water (4:1:5, by vol.)

## 3. Results

3.1. *Immuno-inactivation*

The ability of antibodies, directed against each aminoacyl-tRNA synthetase, to inhibit the corresponding antigen and the three other aminoacyl-tRNA synthetases was tested. The antibodies against chloroplastic ValRS are able to inactivate chloroplastic ValRS, but neither cytoplasmic ValRS (fig.1A) nor cytoplasmic and chloroplastic LeuRS. Conversely, the antibodies against cytoplasmic ValRS only inhibit cytoplasmic ValRS (fig.1B). The antibodies raised against chloroplastic or cytoplasmic LeuRS also inhibit only the corresponding antigen (not shown).

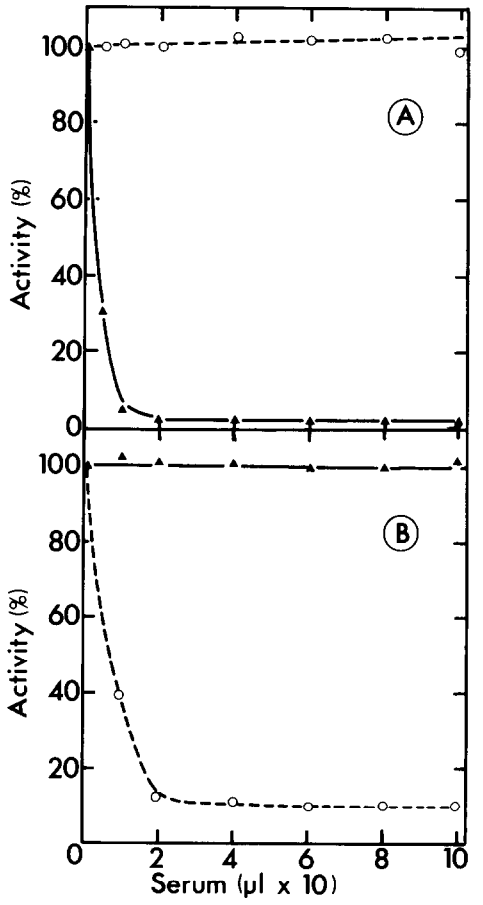


Fig.1. Immuno-inactivation of chloroplastic ValRS (▲——▲) and cytoplasmic ValRS (o– – –o) by antibodies raised against chloroplastic ValRS (A) and cytoplasmic ValRS (B), respectively. Data are expressed as percentages of the activity determined under the same conditions but using pre-immune serum only.

3.2. *Immuno-electrophoresis*

The antisera, tested by immuno-electrophoresis, show a very high specificity. The antibodies raised against cytoplasmic ValRS display, by the rocket technique, a peak of immuno-precipitation with cytoplasmic ValRS but they are unable to precipitate chloroplastic ValRS (fig.2A) or any of the two LeuRS. The antibodies raised against cytoplasmic LeuRS also precipitate selectively cytoplasmic LeuRS (fig.2C). On the other hand, the antibodies against chloroplastic ValRS or LeuRS only precipitate the corresponding enzyme (fig.2B,D). But several peaks of precipitation are observed when a chloroplastic aminoacyl-tRNA synthetase (ValRS or LeuRS) is allowed to react with the corresponding antibodies. The weaker immuno-precipitation peaks are probably due to various aggregated forms of the purified enzyme which can appear upon dialysis against PBS in the absence of the usual stabilizing agent (propane-1,2-diol). Indeed, as shown in fig.3, it is possible to obtain a single immuno-precipitation peak by heating the enzyme sample at 90°C in the presence of emulfogen prior to the immuno-electrophoresis.

## 4. Discussion

This work is the first study performed with antibodies raised against purified aminoacyl-tRNA syn-

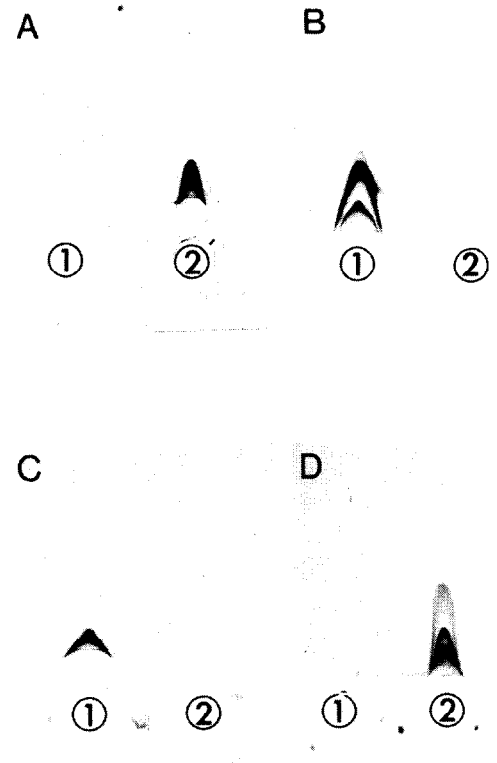


Fig.2. Rocket immuno-electrophoresis. (A) cytoplasmic ValRS antiserum with chloroplastic ValRS (well 1) and cytoplasmic ValRS (well 2). (B) chloroplastic ValRS antiserum with chloroplastic ValRS (well 1) and cytoplasmic ValRS (well 2). (C) cytoplasmic LeuRS antiserum with cytoplasmic LeuRS (well 1) and chloroplastic LeuRS (well 2). (D) chloroplastic LeuRS antiserum with cytoplasmic LeuRS (well 1) and chloroplastic LeuRS (well 2).

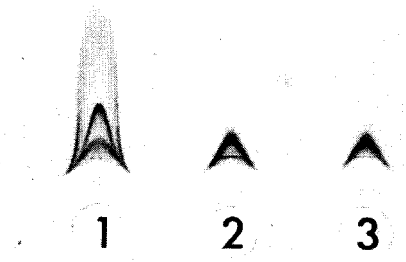


Fig.3. Rocket immuno-electrophoresis of chloroplastic LeuRS against the corresponding antibodies. Well 1, antigen not treated; well 2 and well 3, antigen heated at 90°C in the presence of 2% (v/v) emulfogen for 3 min and 5 min, respectively.

thetases from plant cytoplasm and chloroplasts. The most important feature is the absence of cross-immuno-inactivation and of cross-immuno-precipitation between chloroplastic ValRS and LeuRS and their cytoplasmic counterparts. It therefore appears that the degree of structural homology between a chloroplastic aminoacyl-tRNA synthetase and its cytoplasmic counterpart is not sufficient to generate common antigenic determinants. The results of these immunological studies and those concerning the catalytic [6] and structural [7] properties of the four enzymes indicate that a chloroplastic aminoacyl-tRNA synthetase and its cytoplasmic counterpart are probably coded for by distinct genes.

As chloroplasts and mitochondria have a number of common features, it is interesting to compare our results to those obtained by several authors who have studied mitochondrial and cytoplasmic aminoacyl-tRNA synthetases. Whereas mitochondrial and cytoplasmic ValRS from *Tetrahymena pyriformis* [10] and *Saccharomyces cerevisiae* [11] could not be distinguished, the two LeuRS from *Tetrahymena pyriformis* were shown to be quite different [12] and the antibodies raised against mitochondrial LeuRS did not cross-react with the cytoplasmic enzyme [13]. The fact that *Saccharomyces cerevisiae* mitochondrial methionyl-tRNA synthetase is normal in a mutant which has an altered cytoplasmic methionyl-tRNA synthetase and the fact that the antigenic determinants of the two enzymes are different, has led to the conclusion that the two enzymes are coded for by two distinct nuclear genes [14]. A similar conclusion has been reached in the case of *Neurospora crassa* cytoplasmic and mitochondrial LeuRS, on the basis of structural and immunological studies [15].

The fact that we have specific antibodies against *Euglena gracilis* cytoplasmic and chloroplastic ValRS and LeuRS allows us to selectively precipitate the corresponding enzyme synthesized in an in vitro translation system, and we are presently taking advantage

of this specific reaction to purify the mRNAs corresponding to each of the four enzymes. This should enable us to study the control of the expression of the genes coding for cytoplasmic and chloroplastic aminoacyl-tRNA synthetases.

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