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Immuno-chemical non-cross-reactivity between eukaryotic and prokaryotic seryl-tRNA synthetases

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Monospecific polyclonal antibodies (pAbs) against highly purified bovine seryl-tRNA synthetase (SerRS, EC 6.1.1.1) were prepared and their specificity tested. The interactions of pAbs with SerRS from different organisms were investigated by protein immunoblotting and ELISA methods. pAbs inhibit eukaryotic SerRS aminoacylating activity and exert no effect on SerRS activity from prokaryotes. It is proposed that prokaryotic and eukaryotic SerRS evolve from different ancestor genes.

Seryl-tRNA synthetase; Monospecific polyclonal antibody; Immunochemical cross-reaction

1. INTRODUCTION

Aminoacyl-tRNA synthetases play a key role in protein synthesis by catalysing the highly specific formation of aminoacyl-tRNA [1]. A great success in studying structural and functional properties of prokaryotic aminoacyl-tRNA synthetases has been achieved; however, eukaryotic enzymes of this group were studied insufficiently. They are characterized by a more complicated molecular organization; the new domains in the structure of eukaryotic aminoacyl-tRNA synthetases are elucidated by comparison with the prokaryotic enzymes [2]; some of the eukaryotic aminoacyl-tRNA synthetases form high molecular weight multienzyme complexes [3].

The investigation of eukaryotic aminoacyl-tRNA synthetases is complicated by low levels of these enzymes in cells and tissues and by the imperfectness of existing methods of their purification.

Recently, we have developped a convenient method for purification of SerRS from mammalian liver [3]. This method provides sufficient amounts of the enzyme needed to prepare the pAbs and to further investigate its properties.

SerRS from bovine liver is composed of two identical subunits, each with molecular mass of 64 kDa [4] and presenting a structural dimer of the α_2 type.

Recent evidence indicates that pAbs make it possible to effectively combine molecular and cellular studies of the same proteins. The most characterized mammalian aminoacyl-tRNA synthetase by immunochemical methods is the tryptophanyl-tRNA synthetase from beef pancreas [5–7].

The present work is concerned with the use of pAbs against SerRS from bovine liver for the investigation of immuno-chemical cross-reactivity between SerRS from different eukaryotic and prokaryotic organisms.

2. MATERIALS AND METHODS

2.1. *Materials*

Bovine and rabbit livers were used fresh or frozen at −70°C. Tissue extracts were obtained by homogenization in 50 mM Tris-HCl buffer, pH 7.5, containing 5 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 2.5 mM PMSF. The homogenate was spun at 30 000 × *g* for 1 h.

2.2. *Purification of SerRS*

SerRS from bovine liver was purified to at least 95% purity as described [4]. The enzyme activity was assayed in the reaction of $tRNA^{Ser}$ aminoacylation. SerRS from rabbit liver was purified by the same method [3].

2.3. *Preparation of monospecific polyclonal antibodies*

Rabbits of 2–3 kg weight were immunized with 25–50 mg of highly purified native bovine SerRS in complete Freund's adjuvant (Flow Lab). This immunization was repeated after 8–10 weeks in uncomplete Freund's adjuvant. After 24 days rabbits were boosted by injection of 100 mg of enzyme without the adjuvant in buffer containing 5 mM sodium phosphate and 0.15 M NaCl, pH 7.5 (PBS). Seven days after the last immunization, the blood from rabbits was collected, the serum was tested for content of antibodies against SerRS using ELISA method. Immunoglobulins were isolated from the serum by precipitation with sodium sulphate (up to a final concentration 1.2 M). Mono-

Abbreviations: SerRS, seryl-tRNA synthetase; *E. coli*, *Escherichia coli*; PMSF, phenylmethylsulphonyl fluoride; SDS, sodium dodecylsulphate; PBS, sodium phosphate buffer; pAbs, rabbit monospecific polyclonal antibodies against bovine SerRS; BSA, bovine serum albumin.

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specific antibodies were obtained by chromatography on affinity columns prepared by coupling the highly purified SerRS to the CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals), as described [8]. Affinity columns with immobilized BSA or immunoglobulins of non-immune rabbits were prepared analogously.

The concentration of pAbs was assayed spectrophotometrically using the extinction coefficient at 280 nm $E = 1.35$ $cm^{-1} \cdot mg^{-1}$.ml and M_r 150 000.

2.4. *Enzyme-linked immunosorbent assay (ELISA)*

In the ELISA reaction, bovine SerRS or other purified synthetases (10 mg/ml in 0.1 ml PBS, pH 7.5) were adsorbed to the wells of a 96-well plate (Flow Lab) either at 4°C overnight or at 37°C for 2–3 h. The remaining adsorption sites were blocked with PBS, containing 0.1% Tween-20 (PBS-T) and 0.5% BSA (Sigma) and various amounts of antibodies were added in 0.1 ml PBS-T to the wells; incubation with antibodies for 1 h at 20°C. All further procedures were carried out at 20°C. Then, wells were washed with PBS-T and biotinylated antibodies of goat against whole molecules of rabbit IgG (DiaGene, Moscow) were added to the wells and incubated for 1 h. The wells were washed with PBS-T and then peroxidase-conjugated avidin was added to the wells and incubated for 20 min. After washing with PBS-T the reaction product was visualized by adding ABTS (0.5 mg/ml, Sigma) in 50 mM citrate buffer, pH 7.5 and 0.05% H_2O_2. The reaction was stopped by adding 0.05 ml citrate buffer containing 0.3% NaN_3 and the result of reaction was assayed quantitatively on Multiscan.

2.5. *Protein immunoblotting*

The *E. coli*, yeast and human erythtoblastoma K-562 cells were lyzed in RIPA buffer (20 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 1 mM EDTA, 1% Nonidet P-40, 1% sodium deoxycholate and 0.1% SDS). The 30 000 × *g* supernatant proteins of cell and tissue extracts were separated by 7–22% gradient SDS-PAGE [9] (9 × 12 cm plates, 1 mm gel thickness), then transferred to membrane filters BA-85 (Schleicher and Schull) under the pressure. One of two filters was washed with PBS-T and incubated with pAbs (10 mg/ml) in PBS-T for 1 h. After washing with PBS-T the filter was incubated with biotinylated goat antibodies against the whole molecule of rabbit IgG for 1 h, was washed five times with PBS-T and then incubated with peroxidase-conjugated avidine for 20 min. After washing the filter was developped with 4-chloro-1-naphtol (0.5 mg/ml) and H_2O_2 (0.05%) in 50 mM Tris-HCl buffer, pH 7.5. The second filter was stained with 1% Amidoblack.

3. RESULTS AND DISCUSSION

The results presented in Fig. 1 prove that the pAbs obtained are highly specific for SerRS: after the incubation of SerRS solution with pAbs coupled to CNBr-activated Sepharose 4B (Fig. 1, curve 1) the supernatant loses the ability to catalyze aminoacylation of $tRNA^{Ser}$. The incubation of SerRS with matrix coupled with the immunoglobulins of non-immune rabbits or with BSA hardly affects the SerRS acylating activity of supernatant (Fig. 1, curves 2 and 3).

One can see that pAbs inhibit the SerRS acylating activity (Fig. 2, curve 1), whereas the immunoglobulins of non-immune rabbits (Fig. 2, curve 2) or monoclonal antibody Am2 which strongly inhibit acylating activity of bovine tryptophanyl-tRNA synthetase [5] (Fig. 2, curve 3) do not inactivate SerRS. Incomplete inhibition of SerRS activity by pAbs has not been further analyzed. In principle, it could be caused by the presence in the pool of pAbs not only to enzyme-inhibiting molecules but also to stabilizing and activating molecules.

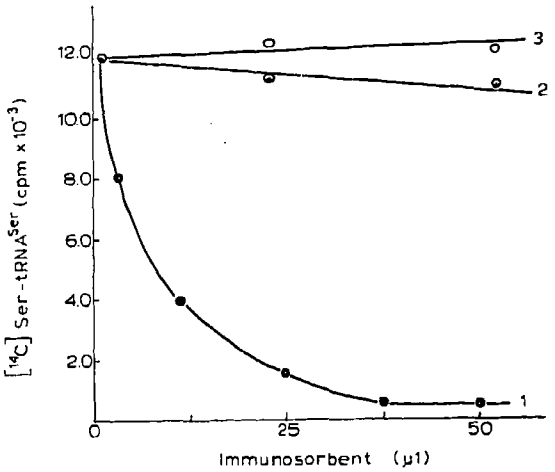

Fig. 1. The effect of preincubation of bovine SerRS with immobilized pAbs on the supernatant aminoacylation activity. pAbs (1), BSA (2) and the IgG fraction of non-immunized rabbits (3) were immobilized on BrCN-activated agarose. The indicated amounts of 2% matrix suspension were incubated at 4°C for 1 h with 1 mg SerRS dissolved in PBS; the matrices were removed by centrifugation. To assay the enzyme activity 0.05 ml supernatant was used.

The properties of pAbs mentioned above open the way to use them for the evaluation of the possible existence of structural and evolutional similarity or diversity of SerRS molecules from different organisms. Fig. 3 illustrates the interaction of pAbs with the SerRS from different organisms by protein immunoblots. It can be seen that only enzymes from eukaryotes (tracks 1, 2 and 6) cross-react with antibodies against bovine SerRS. It has an electrophoretic mobility similar to the bovine SerRS subunit (Fig. 3, track 2). It seems reasonable to assume that the single immunoreactive protein band revealed in various mammalian extracts represents the SerRS subunits of molecular mass close to 64 kDa.

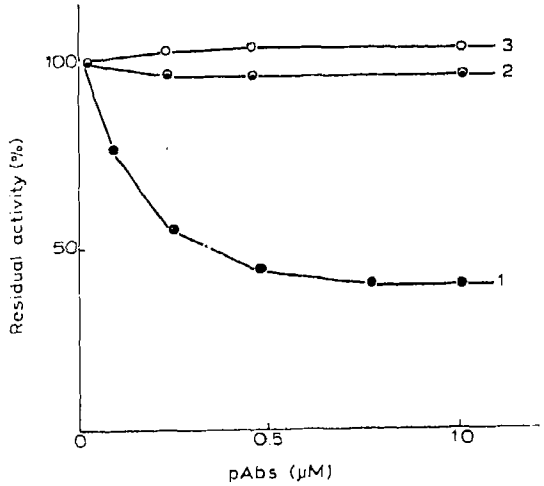

Fig. 2. The dependence of bovine SerRS activity on the pAbs concentration. The reaction mixture (0.1 ml) contained pAbs (1) or monoclonal antibody Am2 against bovine tryptophanyl-tRNA synthetase (3) or IgG fraction of non-immune rabbits (2). The reaction was initiated by adding the enzyme. 2 nM of SerRS was incubated at 37°C for 3 min.

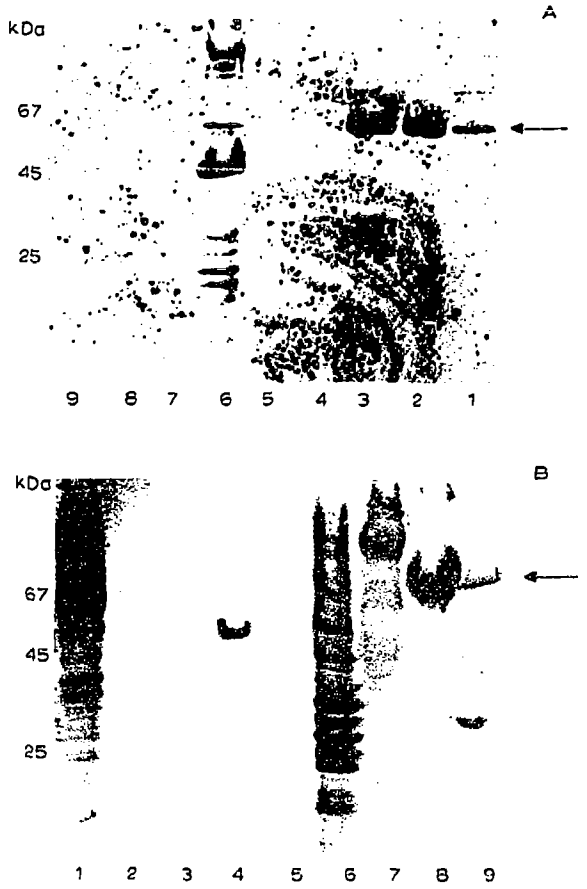

Fig. 3. Immunoreactivity of pAbs with Ser-tRNA synthetases from different eukaryotic and prokaryotic organisms. (A) Enzyme-linked immunological staining; (B) staining with Amidoblack. Immunoblot analysis of supernatants (0.1–0.2 mg) after centrifugation of cell lyzates at 30 000 × *g*: human erythroblastoma K-562 cells (lane 1), yeast (lane 6), *E. coli* (lane 7) and highly purified enzymes: bovine SerRS (lane 2), rabbit SerRS (lane 3), SerRS from *Thermus thermophilus* (lane 4), bovine TyrRS (lane 5). Lane 8, BSA; lane 9, markers. The positions of the SerRS subunit are indicated by arrows.

A band with M_r 47 kDa for yeast (Fig. 3, track 6) may be the product of endogenous proteolysis: some bands with a molecular mass lower than 25 kDa can serve in favour of this supposition. The same difficulties in the investigation of tryptophanyl-tRNA synthetase in cell lyzates of *Neurospora crassa* and *Drosophila* were noted earlier [7].

pAbs against bovine SerRS do not interact with prokaryotic SerRS. These are confirmed by the ELISA reaction for the immunological titration of pAbs obtained on different prokaryotic and eukaryotic antigenes (Fig. 4). Recently, the evaluation of a common antigenic determinant in eukaryotic, prokaryotic and archaebacterial tryptophanyl-tRNA synthetases was described, indicating that a common ancestor enzyme was presumably present in the organisms mentioned above [5].

These data and our results allow for the expression

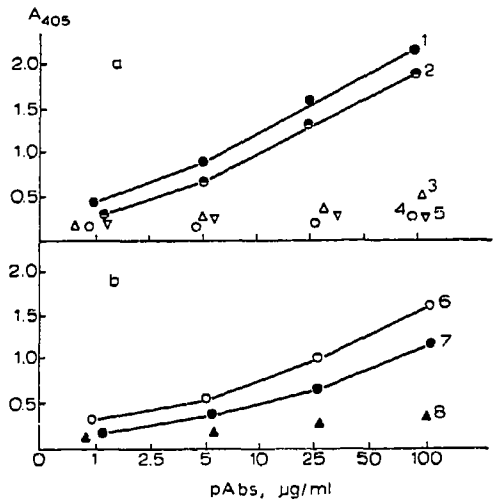

Fig. 4. Immunoreactivity of pAbs with aminoacyl-tRNA synthetases from different organisms; a, interaction of antibodies with purified enzymes; b, interaction of antibodies with cell lyzates: bovine SerRS (1), rabbit SerRS (2), bovine tyrosyl-tRNA synthetase (3), bovine tryptophanyl-tRNA synthetase (4), SerRS from *Thermus thermophilus* (5), lyzates of human K-562 cells (6), yeast (7) and *E. coli* (8).

of a working hypothesis about the evolution of the aminoacyl-tRNA synthetases of various amino acid specificities which may proceed by different ways. For the further development of this working hypothesis, detailed structural and functional investigations of seryl-tRNA synthetases from different organisms are necessary applying a panel of monoclonal antibodies against eukaryotic SerRS. At present, the work on obtaining hybridomata against SerRS from bovine liver is initiated at our laboratory.

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