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ISOLATION FROM CALF BRAIN OF A POLYPEPTIDE FRACTION AFFECTING AMINOACYLATION OF tRNA

The preparation of tRNA obtained from calf brain by three conventional methods exhibits the presence of a slow-migrating fraction in polyacrylamide-gel electrophoresis. This fraction constitutes 2.2.5% of the isolated tRNA and has been identified as a polypeptide of molecular weight of 6000. The aminoacylation with glutamic acid, glycine, leucine and phenylalanine of tRNA devoid of this polypeptide is reduced by half as compared with the initial preparation.

Intracellular and molecular organization of aminoacyl-tRNA synthetases and tRNAs in mammalian cells is unknown. During the last few years several reports appeared suggesting that aminoacyl-tRNA synthetases could be associated with other macromolecules in mammalian cells. Dickman & Boll (1976) have shown that aminoacylation of liver tRNA is increased in the presence of a protein. Hildeman (1977) confirmed the existence in mammalian cells of an activator protein of molecular weight of 25 000 - 35 000. In 1977 Bartkowiak and Radlowski reported on a low-molecular factor affecting aminoacylation in plants.

In the present paper we describe isolation from calf brain of a polypeptide fraction which affects the acceptor activity of aminoacyl-tRNA synthetases.

MATERIALS AND METHODS

The experiments were carried out on the calf brain freshly obtained from the municipal slaughterhouse.

Transfer RNA from brain cortex was obtained by the method of Zubay (1962) modified by Sein et al. (1969). In some experiments the crude tRNA preparations were fractionated in RPCs chromatography system (Gillam et al., 1967) or on BD-cellulose column (Weiss & Kelmers, 1967). The concentration of tRNA was determined at 260 nm, 1 mg of tRNA corresponding to 20 absorbance units (Rubin et al., 1967).
The tRNA preparations were analysed by polyacrylamide-gel electrophoresis according to Peacock & Dingman (1967) with some modifications. The stock buffer solution, pH 7.2, contained 120 mm-Tris/HCl, 60 mm-CH₃COONa and 3 mm-EDTANa₂. Electrophoresis was carried out in 5% gel. Nucleic acid was detected by staining with "stainsall".

The crude preparation of aminoacyl-tRNA synthetases was isolated from the postmitochondrial fraction of the brain homogenate by the method described by Chareziński & Borkowski (1981). The concentration of protein was determined by the method of Lowry et al. (1951).

Aminoacylation of tRNA was measured according to Borkowski & Bruszskiewicz-Żarnowska (1975).

Molecular weight of the isolated polypeptide was determined by thin-layer and column gel filtration on Sephadex G-100 (Andrews, 1964).

RESULTS AND DISCUSSION

Calf brain tRNA was prepared by three conventional methods: by the method of Scin et al. (1969), RPC₅ chromatography and BD-cellulose chromatography. Each preparation gave on polyacrylamide-gel electrophoresis a single band of a metachromatic coloured substance (Plate 1).

The presence of the substance accompanying tRNA was also demonstrated in different preparations of tRNA from rabbit brain and liver separated by means of BD-cellulose chromatography (Gillam et al., 1967) or in the RPC₅ system (Weiss & Kelmers, 1967).

Fig. 1. Separation on Sephadex G-100 column of the fractions obtained from gel electrophoresis. ○, tRNA; ●, polypeptide fraction.
Plate I. Polyacrylamide-gel electrophoresis of total tRNA from calf brain. P. Polypeptide
For separation of tRNA and the unknown substance preparative polyacrylamide-gel electrophoresis was applied. From each slab the regions containing this substance and those containing pure tRNA (Plate I) were cut out and homogenized in a Potter-Elvehjem homogenizer with a small volume of electrophoretic buffer, pH 7.2. After centrifugation the pellets were washed twice with the same buffer. The extracts were loaded onto two separate Sephadex G-100 columns. The elution profiles of both preparations are presented in Fig. 1. On molecular filtration the solution containing the unknown substance gave two peaks. The second peak (II) was collected and after lyophilization it was analysed. The molecular weight was calculated from gel filtration on Sephadex G-100 and thin-layer chromatography. The substance turned out to be a polypeptide of $M_r$ about 6000, and it has been calculated that it constituted 2 - 2.5% of crude tRNA.

Our preliminary results showed that the purified tRNA stripped of the polypeptide lost about 50% of its amino acid acceptor activity with glutamic acid, glycine, leucine and phenylalanine. When the polypeptide fraction was added to the incubation medium, 20% restitution of the acceptor activity of tRNA was observed. tRNA eluted from the polyacrylamide gel incubated with the $^{14}$C-amino acids and the polypeptide fraction was always charged up to 40% of the radioactivity bound by tRNA containing the polypeptide fraction, and approached the radioactivity value of the electrophoretically purified tRNA in the presence of the enzyme without an activator. However, there was no relation between the tRNA charging level and concentration of the polypeptide.

The separation of the polypeptide from tRNA by means of electrophoresis suggests the electrostatic character of the bond between tRNA and the polypeptide. Experimental evidence has been presented for the significance of electrostatic effects of mono- and bivalent cations on tRNA conformation (Jack et al., 1977; Quigley et al., 1978; Sussman et al., 1978). It is possible that the polypeptide fraction strongly linked with tRNA might stabilize conformation of tRNA molecule.

REFERENCES


OTRZYMYWANIE POLIPEPTYDOWEJ FRAKCJI Z MÓZGU CIEŁĄT
UCZESTNICZĄCEJ W AMINOACYLCJI tRNA

Streszczenie

Preparaty tRNA otrzymane z mózgu ciełat trzema konwencjonalnymi metodami wykazywały obecność wolnomigrującej frakcji w elektroforezie w żelu poliakrylamidowym. Frakcja ta stanowiąca 2-2.5% izolowanego tRNA została zidentyfikowana jako polipeptyd o masie cząsteczkowej 6000. Aminoacylacja kwasem glutaminowym, glicyną, leucyną i fenyloalaniną tRNA pozbawionego tego peptydu jest zredukowana o połowę w stosunku do wyższego preparatu tRNA.