QUANTITATIVE IMMUNOENZYMIC METHOD OF DETERMINATION OF EMBRYONIC PREALBUMIN (EPA-1) IN BIOLOGICAL FLUIDS

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Embryonic prealbumin, which we have found [1, 5, 7] both in biological fluids and tissues from human embryos and fetuses and in extracts of tumor tissues, is a typical glycoprotein. Since another embryonic prealbumin has been found in human amniotic fluid [2], we shall refer to the embryonic prealbumin described previously [1] as EPA-1.

No EPA-1 was found by immunodiffusion with a standard test system in blood sera of donors, pregnant women, and cancer patients [1], except in certain samples of blood serum from patients with connective tissue tumors [6].

The object of this investigation was to develop a quantitative immunoenzymic method (IEM) for the determination of EPA-1, which was suggested previously for the determination of other antigens [9, 11, 12].

To obtain antisera against EPA-1 rabbits were immunized with a semipurified preparation of that protein [2]. The resulting antisera were exhausted with lyophilized human plasma under the control of immunodiffusion analysis.

Antibodies against EPA-1 were isolated from monospecific antisera on an immunosorbent prepared on the basis of Ultragel, on which EPA-1 was immobilized with glutaraldehyde [10]. For "planting" on the immunosorbent, the EPA-1 preparation used for immunization was applied. The isolated antibodies were concentrated by ultrafiltration, dialyzed against buffered physiological saline (BPS), pH 7.4, and lyophilized.

To prepare the conjugate, horseradish peroxidase type VI, from Sigma, was used. The antibodies were conjugated with the enzyme by a one-stage method, using glutaraldehyde from Merck [8]. To 0.5 mg of antibodies against EPA-1 in 1 ml of BPS, pH 7.4, 2 mg peroxidase was added, and the resulting mixture was dialyzed against two changes of BPS in the course of 18 h at 4°C. After dialysis, a 25% solution of glutaraldehyde was added to the resulting mixture to a final concentration of 0.1% and the mixture was then incubated for 1-2 h at room temperature and dialyzed against BPS overnight. The conjugate was then transferred into 0.05 M Tris-HCl buffer, pH 8.0, and dialyzed against two changes of buffer. After dialysis the contents were diluted to 4 ml with 0.05 M Tris-HCl buffer, pH 8.0, 1% bovine serum albumin was added for stabilization, and 0.02% sodium azide was added as preservative. The conjugate thus prepared was kept at 4°C.

As the solid phase, polystyrene tissue culture dishes of the 3040-11 TM type (from Falcon) were used [3]. The antibodies were "planted" on the solid phase in carbonate buffer, pH 9.6, in a volume of 0.2 ml into each compartment. The planted antibodies were incubated overnight at 4°C. The technique of conduct and method of reading the results of IEM were described previously [12].

To plot a calibration curve an EPA-1 preparation made from the precipitate by the following method was used. The precipitate obtained by mixing monospecific antiserum against

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Samples of blood serum from	No. of tests	EPA-1 concentration, ng/ml						
		4,0	8,0	16,0	32,0	64,0	128,0	
Blood donors (men and women)	84							
Pregnant women (3-40 weeks)	84							
Patients with tumors of bone, cartilage, and soft tissues	27							
Patients with tumors of the gastrointestinal tract	41							
Patients with brain tumors	51							
Patients with tumors of the breast	13							
Patients with tumors of the respiratory organs	5							
Patients with other tumors	18							

Fig. 1. Immunoenzymic determination of EPA-1 in serum of blood donors, pregnant women, and cancer patients.

EPA-1 with a semipurified EPA-1 preparation was carefully washed with physiological saline and dissociated in glycine–HCl buffer, pH 2.2. Antibodies were precipitated from the solution with 0.6 M sulfosalicylic acid, and the EPA-1 preparation which remained in the supernatant was dialyzed against distilled water and lyophilized. With the aid of the preparation thus obtained the sensitivity of the standard test system to EPA-1 was determined. The minimal amount of EPA-1 capable of "bending" the precipitation arc of that protein was 1 μg/ml. Next, to plot a calibration curve, we successfully used not only a purified preparation of EPA-1, but also a semipurified preparation of this protein, titrated with the aid of the standard test system.

Immunochemical analysis of the resulting conjugate showed that about 10% of the antibodies preserved their immunochemical activity. It was thus possible to work with the conjugate in a dilution of 1:100, when the concentration of labeled antibodies in the solution was about 125 ng/ml. The optimal concentration of planting antibodies was 5-10 μg/ml carbonate buffer, pH 9.6. The conditions chosen enabled from 4 to 100 ng/ml of EPA-1 to be determined in various biological fluids.

The results of the use of the IEM to determine EPA-1 in the blood serum of donors, pregnant women, and patients with various tumors are shown in Fig. 1.

The EPA-1 level in the serum of the blood donors and pregnant women as a rule did not exceed 4 ng/ml, except three sera from blood donors and two from pregnant women, in which the EPA-1 level was between 4 and 12 ng/ml.

An increase in the EPA-1 concentration was observed in patients with tumors, especially tumors of connective tissue (bone, cartilage, soft tissues), and of the brain and gastrointestinal tract. The highest blood concentration of EPA-1 was observed in patients with connective tissue tumors, in agreement with results obtained previously [7]. Detection of EPA-1 in the blood serum of patients with tumors of the breast and respiratory organs confirms reports that this protein is present in tissue extracts of these tumors.

The raised serum EPA-1 level of patients with tumors of the brain and gastrointestinal tract does not correspond with the frequency of detection of this antigen in tissue extracts of these tumors; the probable explanation is that EPA-1 is synthesized, not by the tumor tissue itself, but by the surrounding connective tissue [4].

The immunoenzymic method, as developed by the writers, can thus be recommended for quantitative estimation of EPA-1 in blood serum and in biological fluids.

LITERATURE CITED

1. V. V. Kalashnikov, S. A. Borisenko, and Yu. S. Tatarinov, Byull. Éksp. Biol. Med., No. 6, 725 (1976).
2. V. V. Kalashnikov, M. Yu. Vasil'ev, S. G. Voloshchuk, et al., Ontogenez, No. 4, 389 (1979).

3. V. V. Kalashnikov, D. M. Falaleeva, and Yu. S. Tatarinov, Byull. Éksp. Biol. Med., No. 5, 444 (1979).
4. V. V. Kalashnikov, M. Yu. Vasil'ev, D. M. Falaleeva, et al., Byull. Éksp. Biol. Med., No. 11, 542 (1979).
5. Yu. S. Tatarinov and V. V. Kalashnikov, Nature, 265, 638 (1977).
6. Yu. S. Tatarinov, V. V. Kalashnikov, M. Yu. Vasil'ev, et al., Lancet, 2, 1122 (1978).
7. Yu. S. Tatarinov, V. V. Kalashnikov, and S. G. Voloshchuk, in: Carcino-Embryonic Proteins, Vol. 1, Amsterdam (1979), pp. 351-355.
8. S. Avrameas and T. Ternynck, Immunochemistry, 8, 1175 (1971).
9. E. Engval and P. Perlman, Immunochemistry, 8, 1175 (1971).
10. I. L. Guesdon and S. Avrameas, J. Immunol. Methods, 11, 129 (1976).
11. B. K. Van Weemen and A. H. W. M. Schuurs, FEBS Lett., 15, 232 (1971).
12. A. Voller, D. E. Bidwell, and A. Bartlett, Bull. W. H. O., 53, 55 (1976).

ONE-STAGE METHOD OF OBTAINING ENRICHED FRACTIONS OF RAT BRAIN NEURONS AND GLIAL CELLS

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The object of this investigation was to devise a simple, rapid, and nontraumatic method of isolating fractions of neurons (NF) and glial cells (GF) from the brain of one small animal. Existing methods are lengthy (several hours), laborious, traumatic [6-8, 10-12, 15], and require several small animals; in addition, the fractions are contaminated with organoids [9]. In the method now suggested these disadvantages are reduced to a minimum: the NF and GF can be obtained in the course of 7-10 min.

EXPERIMENTAL METHOD

Hydrodynamic Method of Preparation of the Initial Cell Suspension. The brain of noninbred albino rats weighing 200-250 g, without the cerebellum, was placed in the receiver of a microblender (MRTU-2; Odessa Experimental Factory) and treated with Krebs–Ringer solution (KRS), pH 7.4, in a ratio of tissue to solution of 1:25-1:100. The receiver was placed in the microblender, the revolving shaft of which was immersed in the liquid. The blades of the shaft were replaced by paddles of the same size. The electric motor was switched on and the contents of the receiver mixed at a speed of 200-300 rpm for 30-50 sec. The use of a 0.28 M solution of sucrose gave a smaller yield (for example, that of NF was reduced by 33-50%).

Filtration Method of Obtaining Cell Fractions. The initial suspension, in a volume of 100 ml, was passed through a column (the fractioner) consisting of Plexiglas rings between which screen filters with gaskets were fixed (Fig. 1). The suspension first passed through a screen with pore size of 400-600 μ (the first filter), then one of 200 μ (second filter), and so on. After the suspension, 100 ml of KRS was passed through the column. The fractioner was dismantled and the material removed from the screens with the aid of a small brush, the filters having been immersed in beakers containing cold KRS.

Tissue respiration was studied polarographically [5], the K^+ and Na^+ concentrations on a flame photometer (FPF-58), and protein as described in [13]. The solid residues of the

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