IMMUNOENZYME ASSAY OF NEUROSPECIFIC ANTIGEN 10-40-4 IN HUMAN AND ANIMAL TISSUE AND ORGAN EXTRACTS

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KEY WORDS: immunoenzyme assay; neurospecific antigen; species specificity.

Neurospecific protein (NSP) 10-40-4, first isolated in the writers' laboratory, has a molecular weight of 76,000 ± 2000 daltons and an isoelectric point of 4.7. The antigen contains no carbohydrate or lipid components, is precipitated by ammonium sulfate at 40-60% saturation, and possesses the immunoelectrophoretic mobility of α-globulin. It has been shown by the immunodiffusion method that NSP 10-40-4 possesses species specificity [1].

The aim of this investigation was to develop a quantitative method of immunoenzyme assay of NSP 10-40-4 and to use this method to measure the concentration of this protein in extracts of human organs and tissues and also for quantitative analyis of the degree of crossing of the antigenic determinants of NSP 10-40-4 in the brain of different species of mammals.

EXPERIMENTAL METHOD

Human, guenon, bovine, dog, rabbit, rat, and guinea pig brain tissue and also human liver, kidney, myocardial, skeletal muscle, lung, skin, and spleen tissue were used for the investigations. The human brain and organs were obtained at autopsy on persons dying from accidents, not later than 3-4 h after death. The animals' brains were obtained immediately after slaughter and received primary treatment within 2-3 h. The method of preparation of the organ extracts, isolation of protein 10-40-4 from brain tissue, and also the scheme of immunization in order to obtain antisera against it was described previously [1].

Antibodies against NSP 10-40-4 were isolated from monospecific antisera, using immunosorbent prepared on the basis of Sepharose 4B (from "Pharmacia," Sweden), activated with BrCN, and a purified preparation of protein 10-40-4. To prepare 10 ml of immunosorbent 7 mg of individual NSP 10-40-4 was used. Activation of Sepharose 4B by cyanogen bromide and its binding with the protein preparation were carried out by the method in [5].

A chromatographic column measuring 9 × 15 (from Pharmacia) was packed with the prepared immunosorbent and 5 ml of monospecific antiserum against NSP 10-40-4 with an original titer of 1:64, mixed with an equal volume of 0.05 M phosphate buffer, pH 7.0, containing 0.5 M NaCl, was passed through it. Adsorbed antibodies against NSP 10-40-4 were eluted with 0.2 M glycine-HCl buffer containing 0.5 M NaCl, pH 2.2. The isolated antibodies were concentrated and dialyzed against deionized water on XM 100A ultrafilters ("Amicon," USA), and then lyophilized.

The immunoglobulin concentration in the fraction of isolated antibodies was determined with the aid of goat antiserum against rabbit γ-globulins (from "Miles," USA). The concentration of specific antibodies was determined by the immunodiffusion method in the modification in [2], using a test system for NSP 10-40-4.

Immunoenzyme assay was carried out in polystyrene plates (from "Linbro," England). The plate was activated with antibodies in carbonate buffer, pH 9.6, overnight at 4°C. Antibodies against NSP 10-40-4 were conjugated with horseradish peroxidase (type IV, from "Sigma," USA) by the periodate method [9]. A 0.08% solution of 5-aminosalicylic acid (from "Serva," West Germany) was used as the substrate.

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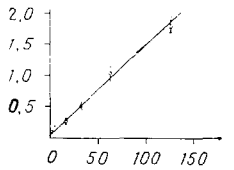

Fig. 1. Calibration curve for immunoenzyme assay of NSP 10-40-4. Abscissa, concentration of antigen 10-40-4 (in mg/ml); ordinate, optical density at 450 nm.

TABLE 1. Results of Immunoenzyme Assay of NSP 10-40-4 in Extracts of Human Organs and Tissues

Organ or tissue	Number of specimens tested	Mean concentration of NSP 10-40-4 in extract, ng/ml
Brain	5	800 000±9 000
Liver	5	18,17±3,5
Kidney	5	36±6
Myocardium	5	38,1±5,9
Lung	5	34±4,8
Spleen	3	22±1,6
Skin	3	31,5±2

TABLE 2. Results of Immunoenzyme Assay of Percentage Crossing of Antigenic Determinants of NSP 10-40-4 of Several Species of Mammals

Species of source of brain extract	% of crossing of antigenic determinants
Human	100
Guenon	6
Dog	3
Rabbit	1,2
Bovine	0,9
Guinea pig	0,25
Rat	0,22

To plot a calibration curve purified NSP 10-40-4 was used. Later, to construct the calibration curve, we used not only the purified antigen, but also a semipurified preparation of the protein, the content of NSP 10-40-4 in which was determined with the aid of the test system. To estimate nonspecific adsorption bovine serum albumin was used. Immunoenzyme assay was carried out by the method in [8]. The results were recorded on a Titerteck Multiscan MC multichannel spectrophotometer (from "FlowLaboratories," England) at 450 nm.

From 5 ml of monospecific antiserum 2-4 mg of antibodies was obtained by immunoaffinity chromatography. Immunodiffusion analysis of the isolated antibodies, using the test system against NSP 10-40-4 and goat antibodies against rabbit immunoglobulins showed that about 90% of the antibodies preserved their specific activity.

Maximal sensitivity of the immunoenzyme method, when the antibodies obtained as described above were used, was achieved with the antibodies in a concentration of 25 μg/ml and dilution of the conjugate of 1:50 for activation of the plate. The conditions chosen enabled neurospecific antigen 10-40-4 to be determined in concentrations of between 1 and 125 ng/ml per sample.

An averaged calibration curve obtained from five experiments is shown in Fig. 1. The standard deviation of the points is ±2%. The results of immunoenzyme assay of NSP 10-40-4 in extracts of the human organs and tissues tested are given in Table 1.

It will be clear from Table 1 that NSP 10-40-4 accounted for 8-9% of water-soluble human brain proteins. NSP 10-40-4 was found in extracts of all the human tissues studied. However,

its content did not exceed 0.5-1% of the quantity of this antigen in the brain extract. A similar situation was observed by other workers when studying other neurospecific proteins (S-100, 14-32) [4, 6, 7]. The discovery of NSP in other organs and tissues can evidently be explained by the innervation of these organs.

Previously the writers noted species specificity of NSP 10-40-4: Antibodies obtained against NSP 10-40-4 from human brain give a distinct precipitation zone in agar gel with human and guenon brain extracts, a weaker zone with bovine, dog, and rabbit brain extracts, and a negative reaction with rat and guinea pig brain extracts [1].

The use of solid-phase immunoenzyme analysis enabled quantitative estimation of the degree of crossing of the antigenic determinants of NSP 10-40-4 for different species of animals. The percentage of crossing was determined from dilution curves of human and animal brain extracts during activation of the plate by antibodies against NSP 10-40-4 from human brain [3]. The results of assay are given in Table 2. Reactivity of human brain extract was taken at 100%. It will be clear from Table 2 that the reactivity of brain extracts of different species of mammals relative to antibodies against NSP 10-40-4 from human brain differs considerably.

A method of immunoenzyme assay of neurospecific antigen 10-40-4 has thus been developed, by means of which its concentration in human brain and other organs has been measured and the degree of crossing of the antigenic determinants of NSP 10-40-4 for different species of mammals has been determined.

LITERATURE CITED

1. T. P. Klyushnik and G. Sh. Burbaeva, Biokhimiya, 48, 1203 (1983).
2. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).
3. T. Chard, Radioimmunologic Methods [in Russian], Moscow (1981), p. 174.
4. D. A. Hullin, K. Brown, P. A. M. Kynoch, et al., Biochim. Biophys. Acta, 628, 98 (1980).
5. J. Kohn and M. Wilchek, Biochem. Biophys. Res. Commun., 107, 878 (1982).
6. D. Schmechel, P. J. Marangos, and M. Brightman, Nature, 276, 834 (1978).
7. F. Suzuki, T. Nakajima, and K. Kato, J. Biochem. (Tokyo), 92, 835 (1982).
8. A. Voller, D. E. Bidwell, and A. Bartlet, Bull. World Health Org., 53, 55 (1976).
9. M. B. Wilson and P. K. Nakane, in: Immunoflourescence and Related Staining Techniques, Amsterdam (1978), p. 215.

DETERMINATION OF ANTIBODIES AGAINST STREPTOCOCCUS GROUP A POLYSACCHARIDE IN HUMAN SERUM BY AN IMMUNOENZYME METHOD

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Previous investigations revealed a high level of antibodies against *Streptococcus* group A polysaccharide (A polysaccharide) in acute rheumatic fever and other diseases caused by a streptococcus of this group [1, 6]. Antibodies against polysaccharide of streptococcus of group A-variant, containing a determinant common for different groups of polysaccharides were found in the same sera, but at a lower level, by radioimmunoassay [6]. A determinant common for A polysaccharide and polysaccharide of group L *Streptococcus* (L polysaccharide) also was found [3, 8]. Antibodies against this determinant could not be found in human sera by immunodiffusion in gel [1], but they were not studied by more sensitive method.

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