Chemistry of Natural Compounds, Vol. 38, No. 6, 2002

QUANTITATIVE DETERMINATION OF SERUM ALBUMIN BY IMMUNOENZYME ANALYSIS

Sh. S. Tashmukhamedova and Sh. R. Usmanov UDC 547.962.3

Immunoenzyme analysis (IEA) is a highly sensitive method for diagnosing many diseases. IEA differs from other radioimmunological methods in sensitivity, stability of the ingredients, and high resolution [1]. IEA is based on an antigen—antibody reaction and the detection of the resulting complex using immunoglobulins labeled with enzymes [1].

IEA is used to diagnose infectious and somatic diseases, to monitor the effectiveness of treatment, to determine quantitatively specific reagents for producing vaccines and sera, to standardize biopreparations, and to detect allergens and autoantibodies [2]. We used IEA for quantitative determination of human serum albumin (HSA) in blood serum of liver cirrhosis patients.

We observed 27 patients with verified diagnoses. The degree of liver-cell disfunction from liver cirrhosis was estimated using the Child—Pugh criteria [3]. The patients were examined under stress and divided into three groups: A, mild; B, moderate; C, serious degree of liver-cell disfunction. There were nine patients in each group.

The analytical results revealed a correlation between the degree of seriousness of the liver-cell disfunction and the amount of albumin in blood serum. Thus, the correlation coefficient for group A was 0.99; B, 0.74; C, 0.99. This correlation characterizes the degree of hepatodepressive syndrome, which fundamentally changes the theoretical premises that a lowered HSA content is axiomatic in liver-cirrhosis patients. Opinions that the volume of preserved hepatocytes and the HSA concentration are not absolutely correlated apparently confused many authors who combined groups of patients with liver cirrhosis without dividing them according to the seriousness of the pathological process (according to Child—Pugh), where a compensatory synthetic effort occurs in the early stages and HSA catabolism decelerates. This is followed by an interruption of adaptation and progression of liver cirrhosis.

The IEA was developed in two steps. These were immobilization of the HSA antibodies and preparation of antibody—enzyme conjugates. The carriers for the immobilization of HSA antibodies were microporous capron membranes of pore size 0.2 μm and mass 15 mg [2]. Antibodies were prepared by immunization of rabbits and further purified over affinity sorbent based on HSA. The acute dose of antigen (HSA) per rabbit was prepared by emulsification of complete Freund's adjuvant (PAF Cabbiochem, USA) (0.5 mL) and lyophilized albumin (10 μg) in physiological saline (0.5 mL). The emulsion was injected (0.1 mg) into the knee every 10 days. Blood was collected at the peak of the immune response, in our instance, on the 50th day. The titer of specific antibodies to albumin was determined by the Ouchterlony precipitation reaction. The antibody titer was 1:32. The resulting antiserum was precipitated by ammonium sulfate. For further purification, serum containing HSA antibodies was passed over a column containing affinity sorbent with immobilized HSA. Then, the column was eluted with buffer (0.9% NaCl, pH 7.2) until the absorptions of the buffer added to the column and the effluent were zero. The flow rate was 0.5 mL/min. Then, a desorbing buffer (0.05 M tris-NaOH with 1 M KCl, pH 9.8) was added to the column. The effluent was collected in 3-mL portions. The amount of antibodies bound to the polyamide was determined from the difference in the protein content in solution before and after incubation by spectrophotometry [3].

For immobilization, polyamide membranes were placed in weighing bottles with HCl (2.5 N) and incubated for 2 h. The excess of acid was removed by washing the membranes with distilled water and borate buffer (0.05 M, pH 8.2). This activated the polyamide membranes. Then, glutaraldehyde solution (2.5%) was added to the bottles, which were incubated for 2 h at room temperature in the same buffer. After incubation the membranes were again washed with buffer. Antibodies were added (100 μg/mL) and incubated for 48 h at 4°C with constant stirring. Unbound antibodies were removed by washing with buffer until traces of protein disappeared. The amount of antibodies bound to the polyamide was determined from the difference

Second Tashkent State Medical Institute, Beruni Tashkent State Technical University. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 487-488, November-December, 2002. Original article submitted October 21, 2002.

 0009-3130/02/3806-0614$27.00

in the protein content in solution before and after incubation by spectrophotometry. The amount of antibodies bound to the polyamide was 50 μg/mL (in one bottle).

In the second step, enzyme marker was added to the antibodies. This was α-amylase purified from a commercial preparation of amylorisin P10x (GOST 20264.1975), which differs from the enzymes usually used (alkaline phosphatase, peroxidase, etc.) in its availability and high catalytic activity. The enzyme we selected satisfies the requirements set for enzyme markers in IEA. It has a high specificity and specific catalytic activity that enables it to be observed as an enzyme marker at very low concentrations. It retains its high enzymatic activity after chemical modification to prepare conjugates with antibodies. The method for determining the enzyme and substrate concentrations is also highly sensitive [2].

Conjugates of antibodies to α-amylase were prepared as follows. α-Amylase (1 mg) in borate buffer (0.1 M, pH 8.2) was treated with antibodies (1 mg/mL), stirred vigorously, and treated dropwise with glutaric dialdehyde (0.1 mL, 0.01%) in the same buffer. After incubation for 16 h, it was dialyzed against NaCl (0.1 M) and centrifuged. The protein content was determined. The amylase activity was found by iodometry [3]. The activity of the conjugate was 33762.32 units/h. Bottles with immobilized antibodies were washed three times with tris-HCl buffer (0.05 M, pH 7.5) and equilibrated in the same solution containing $CaCl_2$ (30 mM) and ethyleneglycol (5%). The added solution is blood serum from liver-cirrhosis patients diluted 100 times by the same buffer. We now present the conditions for performing the immune reactions.

The first immune reaction: After adding serum, solutions are incubated for 15-20 min at 37°C. Unreacted components are washed away three times by tris-HCl buffer (0.05 M). Then, a solution of antibody conjugates with enzyme (100 μg in each, 250 μg/mL) is added to the bottles. The bottles are incubated for 20 min and again washed with the same buffer.

The second immune reaction: Membranes are treated with substrate (0.3 mL, 0.5% starch solution). Hydrolysis is carried out for 25 min at 37°C. The hydrolysis products (0.1 mL) are treated with iodine (10 mL). HSA was determined on photoelectric colorimeter. The amount of HSA is determined from a calibration curve constructed using HSA isolated by affinity chromatography [4] at concentrations from 0.5 to 500 μg/mL.

Thus, quantitative determination of HSA used an immunoenzyme analysis method that differs from known methods in its high sensitivity, shorter analysis time, high resolution, and unambiguous interpretation. A correlation between the degree of seriousness of liver-cell disfunction and the amount of albumin in blood serum was found during the analysis of the results.

REFERENCES

1. S. E. Severin and G. A. Solov′eva, *Practicum in Biochemistry* [in Russian], Meditsina, Moscow (1989), pp. 307-309.
2. Sh. S. Tashmukhamedova, M. M. Rakhimov, and Kh. K. Sultanov, *Vopr. Med. Khim.*, No. 3, 39 (1995).
3. S. E. Severin and G. A. Solov′ev, *Practicum in Biochemistry* [in Russian], Meditsina, Moscow (1989), pp. 83-112.
4. M. M. Rakhimov and Sh. R. Usmanov, *Kime va Farmatsiya*, No. 4, 30 (1998).