AN APPROACH TO THE DEVELOPMENT OF IMMUNOCHEMICAL METHODS FOR PEPTIDE ELIMINATION FROM THE BLOOD OF PATIENTS WITH RENAL FAILURE

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UDC 616.61-008.64-092:616.153.96-008.6-089.816:621.928

KEY WORDS: renal failure, peptides, conjugation, immunosorbent, antibodies.

Treatment of patients with kidney diseases is a very complex problem. Advanced and severe forms of kidney disease are distinguished by particularly marked and persistent toxic manifestations, leading to apathy, polyneuritis, anemia, encephalopathy, convulsions, and mental disorders [4].

This symptom-complex is the result of a disturbance of the excretory function of the kidneys and accumulation of toxic metabolites of protein and nonprotein nature in the body. To remove them in medical practice methods such as hemodialysis, hemodiafiltration, and hemoperfusion are used. However, only end products of nitrogen metabolism (urea, creatinine, etc.) pass through the semipermeable membrane of the hemodialysis apparatus, and metabolites with a higher molecular weight are retained and accumulate. These substances include peptides with a molecular weight of 500 to 10,000 daltons, which are uremic toxins [7]. To remove these substances efficiently membranes with increased permeability have been produced [1], but as a result of their use, besides metabolites, substances essential for physiological activity, such as hormones, vitamins, and amino acids, also are removed, so that the patients' state is worsened.

The method of hemoperfusion, utilizing the universal adsorption properties of charcoal adsorbents and ion-exchange resins, is nowadays used for detoxication. Although this method gives better results, it is nonspecific [3].

It is therefore essential to devise more specific methods of detoxication. The most promising method is the immunochemical, which is distinguished by its extremely high sensitivity and specificity. For such a method functionally important peptides must be isolated in a homogeneous form and antibodies obtained against them, which will be used to produce immunosorbents.

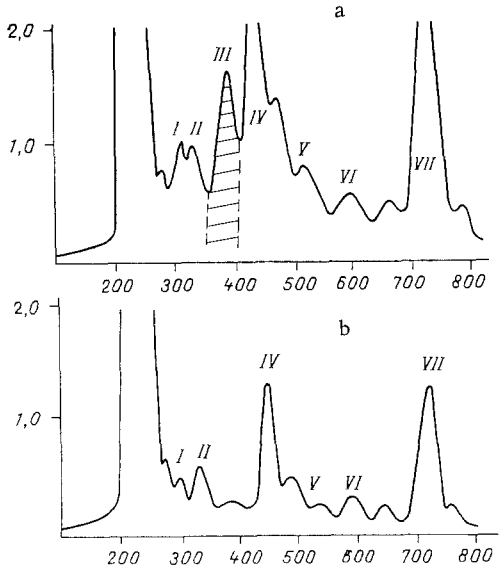

Fig. 1. Gel-chromatography of blood serum on column with Sephadex G-25: a) patients with CRF; b) healthy donors. Abscissa, volume of eluate (in ml); ordinate, optical density at 280 nm.

Laboratory of Protein and Peptide Chemistry, Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 8, pp. 245-247, August, 1984. Original article submitted April 4, 1983.

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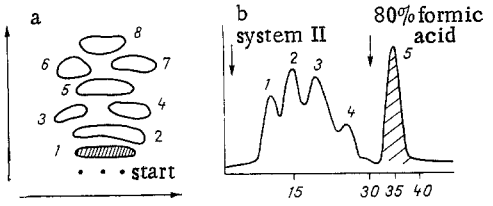

Fig. 2

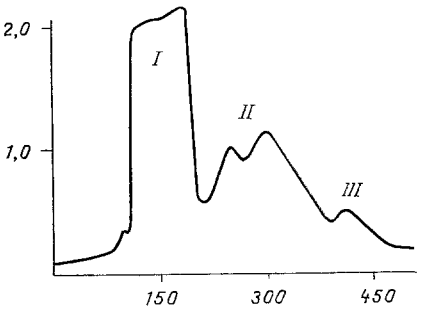

Fig. 3

Fig. 2. Separation of fraction III by peptide mapping method (a) and by partition chromatography on column with cellulose MN-300 (b).

Fig. 3. Gel-chromatography of conjugated peptides on column with Sephadex G-250. Abscissa, volume of eluate (in ml); ordinate, optical density at 280 nm.

This paper is devoted to the isolation and conjugation of a blood serum protein from uremic patients, whose concentration correlates with the degree of uremic intoxication.

EXPERIMENTAL METHODS

Blood serum from patients with chronic renal failure (CRF) was fractionated by gel filtration on a column with Sephadex G-25 (from Pharmacia, Sweden). A column measuring 2.5 × 100 cm was used and the buffer was 0.01 M sodium acetate, pH 6.7. The rate of elution was 30 ml/h. The fractions were identified on a "Uvicord-2" apparatus (LKB, Sweden), at a wavelength of 280 nm. Blood serum from healthy blood donors was fractionated for comparison. To estimate the molecular weight of the components the ratio V_3/V_0 was calculated from the fractionation curves and compared with its value for marker substances. As markers vitamin B_{12} and angiotensin II, with molecular weights of 1357 and 1368 daltons respectively, were used.

The mixture of peptides was separated by the peptide map method, successively by electrophoresis and chromatography on a plate measuring 20 × 20 cm with a thin layer of cellulose MN-300. Electrophoresis was carried out for 1 h at 500 V. System I (pyridine—acetic acid—water, in the ratio of 25:1:225, pH 6.5) was used as the buffer. System II (pyridine—butanol—acetic acid—water in the ratio of 10:15:13:12 was used for chromatography. The peptides were centrifuged with fluorescamine and eluted with formic acid [2].

For preparative separation of the mixture of peptides we used a modified method of partition chromatography on a column with cellulose MN-300 measuring 1.0 × 10 cm, equilibrated with system II. The mixture of peptides was eluted with system II, and then with formic acid. Components of the fractions were identified by determination of N-terminal amino acids by the method in [8], and also with Ehrlich's reagent.

The peptide was conjugated with glutaraldehyde. Different concentrations of glutaraldehyde were added to an aqueous solution of the peptide in a concentration of 200 μg/ml. The optimal concentration was 1% of the quantity of peptide to be conjugated; conjugates with mol. wt. from 25,000 to 60,000 daltons were formed.

The molecular weight of the conjugated peptides was determined on a column with Sephadex G-50 and by polyacrylamide gel electrophoresis [9]. Bovine serum albumin with mol. wt. of 67,000 daltons was used as the marker. Dialysis against distilled water for 24 h was used for desalination.

EXPERIMENTAL RESULTS

Comparison of the fractionation curves of serum for patients with CRF and healthy blood donors showed that fraction III either was not present in healthy human blood or was present only in small amounts [5, 6]. Investigations to study correlation with the degree of uremic intoxication and the concentration of components of fraction III and their biological activity were published previously [5, 6] (Fig. 1).

Biologically active fraction III was subjected to further fractionation. As a result of separation by the peptide mapping method eight or nine ninhydrin-positive components were found (Fig. 2a).

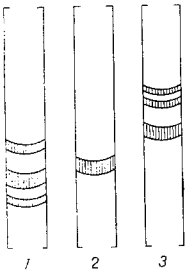

Fig. 4. Polyacrylamide gel disc electrophoresis of fractions obtained by gel-chromatography on column with Sephadex G-50. 1) Fraction I; 2) albumin; 3) fraction II.

On preparative separation of fraction III by partition chromatography on a column with cellulose MN-300, five fractions were obtained (Fig. 2b). Fractions eluted by system II corresponded to the contents of spots 2-8. They were found to contain free amino acids, peptides in low concentrations, and also "Ehrlich-positive" substances, which were tryptophan derivatives.

Fraction 5, eluted with 80% formic acid, was a homogeneous peptide. On identification of DNS-amino acids by Gray's method, ε-DNS-lysine and OH-DNS-tyrosine were found; no N-terminal amino acid was found, indicating that it may have been blocked. The amino-acid composition of the peptide was determined from the results of 24-, 48-, and 72-h hydrolyses, and the following amino acids were present: arginine, lysine, phenylalanine, tyrosine, leucine, valine, 4 alanines, 3 glycines, 2 glutamines, 3 asparagines, proline, threonine, and serine. The molecular weight (2100 daltons) was calculated from the amino-acid composition.

Peptides with mol. wt. of under 10,000 daltons are poor antigens, and it is difficult to obtain antibodies for them. Accordingly, the peptide was conjugated with a bifunctional reagent, namely glutaraldehyde, i.e., the peptides were cross-linked with one another. During gel-filtration of the conjugated peptides on Sephadex G-50, three fractions were obtained (Fig. 3). Determination of the molecular weights of the fractions by disc electrophoresis in the presence of sodium dodecylsulfate showed that fraction I contained components with mol. wt. of about 60,000 daltons, compared with about 40,000 daltons in fraction II (Fig. 4). The writers suggest that components of fraction I, containing alternating peptide components, will be good antigens for obtaining specific antibodies.

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