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# STRUCTURAL–FUNCTIONAL CHARACTERISTICS OF PEPTIDE ENDOTOXINS

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*The biological activity and structural characteristics of peptide fractions and individual peptides from the blood sera of patients with an endogenous intoxication syndrome have been investigated.*

The accumulation in the blood of biologically active peptides with a medium molecular mass (500-5000 Da, MMM peptides) is one of the factors of the development of intoxication in a number of diseases — acute burn toxemia, malignant tumors, hepatic and renal inufficiencies, sepsis, etc. [1, 2]. The commonest feature of the MMM proteins is a membranotropic action. By changing the structural–functional characteristics of cell membranes, the peptides exert an inhibiting action on a whole series of metabolic processes — processes involving the transport of ions and amino acids and the functioning of the system of hemostasis and of the enzyme and immune systems [3-5]. The MMM peptides are a product of an intensified catabolism and proteolysis of blood proteins. In spite of the fact that the MMM peptides, otherwise known as peptide endotoxins, were first described in 1968, and many workers have studied their biological activity, physiologial action, and structure, many questions of the origin of the pathogenic peptides and the mehanism of their action on the patient's organism remain unclear.

We have investigated the blood sera of patients with acute and chronic renal insufficiency, since it is known that in these pathologies the increase in the level of MMM peptides in the blood is most pronounced — 20 to 10-fold in comparison with healthy donors [6].

The blood serum was initially separated with the aid of gel chromatography on a column (1. 5 × 100 cm) of Sephadex G-25 equilibrated with 0.01 M sodium acetate solution, pH 6.7, which yielded 11 protein-peptide fractions (Fig. 1). Screening in bilayer phospholipid membranes (BPMs) showed that the greatest activity was possessed by fraction IX, absent from the blood of healthy donors [7]. Fraction IX increased the conductivity of the BPMs in the presence of sodium or potassium ions by two orders of magnitude: it amounted to $9.36 \times 10^{-8}$ ohm$^{-1}$/cm$^2$, as compared with a norm of $2.3 \times 10^{-10}$ ohm$^{-1}$/cm$^2$.

After accumulation in preparative amounts, this fraction was concentrated and separated on a column (1. 5 × 90 cm) of TSK gel HW-40 equilibrated with a 0.05 M solution of ammonium bicarbonate, pH 8.0. This gave nine fractions (Fig. 2) of which IX-3, IX-5, and IX-7 possessed membranotropic activity in relation to BPMs. Fraction IX-3 increased the conductivity of the BPMs by two orders of magnitude — $4.7 \times 10^{-8}$ ohm$^{-1}$/cm$^2$ at a norm of $3.0 \times 10^{-10}$ ohm$^{-1}$/cm$^2$, while fractions IX-5 and IX-7 were less active — $6.8 \times 10^{-9}$ ohm$^{-1}$/cm$^2$ and $5.35 \times 10^{-9}$ ohm$^{-1}$/cm, respectively, at a norm of $3 \times 10^{-10}$ ohm$^{-1}$/cm$^2$. It was established by N-terminal analysis that therse fractions were heterogeneous and contained from three to five peptides. We made a further investigation of the mist active fraction IX-3.

A characteristic manifestation of the activity of the MMM peptides, together with the disturbance of the functions of biological membranes, is the immunodeficient state caused by them [5]. We therefore investigated their influence on the cell immunity reaction in model experiments and studied the action of the MMM peptides on the natural killing system and, in particular on the activity of the natural killers lymphocytes (NKs) and on the synthesis of one of the key cytokines of the immune system, interleukin-2 (IL-2). IL-2 is part of the system regulating the level of natural membrano- and cytotoxicity in the organism; its presence is necessary for potentiating and maintaining the activity of the NKs [8, 9].

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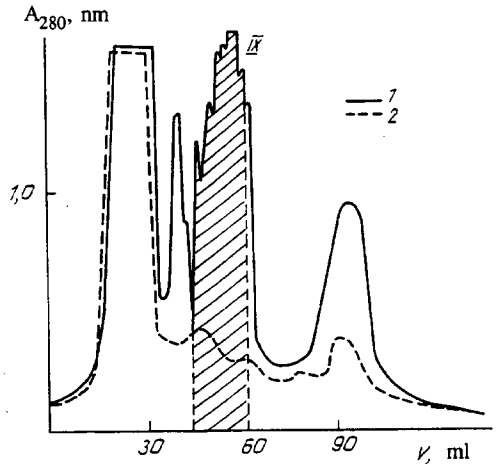


Fig. 1. Gel chromatography of the blood serum of a patient with chronic renal insufficiency (1) and of a healthy donor (2) on a column (1.5 × 100 cm) of Sephadex G-25 in 0.01 M sodium acetate solution, pH 6.7. Rate of elution 20 ml/h.

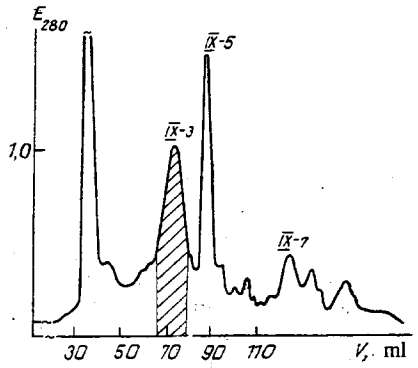


Fig. 2. Separation of fraction IX on a column (1.5 × 90 cm) of TSK gel HW-40 in 0.05 M ammonium bicarbonate solution, pH 8.0. Rate of elution 18 ml/h.

The activity of the NKs of animal spleen cells was tested by using the membranotropic test with [$^3$H]uridine [10] in a ratio of effector to target of 100:1 on the 7th, 14th, and 30th days after the injection of the MMM peptides. It was found that the MMM peptides exerted no direct influence on the activity of the NKs.

We then investigated the intensity of the synthesis of IL-2 in the same period as the determination of the activity of the NKs. We evaluated the total T-growth activity of the production of lymphocytes stimulated by the mitogen concanavalin A (ConA). Since the main effect of these products is due to IL-2, with a definite probability we characterized the T-growth activity as the degree of synthesis of IL-2.

It was established that the MMM peptides exerted an influence on the synthesis of IL-2 and that the effect had a two-phase nature; on the 7th day after the administration of the MMM peptides to the animals a considerable (by 57%) suppression of the synthesis of IL-2 was observed. In the middle of the experiment, on the 14th day, there was a 15% activation of the production of this lymphokine as compared with a control, and on the 30th day 27% suppression of the synthesis of IL-2 was recorded (Fig. 3). The results obtained show an influence of the MMM peptides on the capacity of the lymphocytes for producing IL-2 under the action of a mitogenic stimulus in the direction of a stable inhibition of the production of this lymphokine.

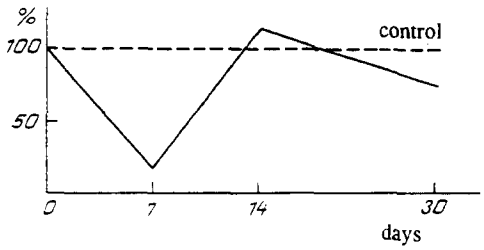


Fig. 3

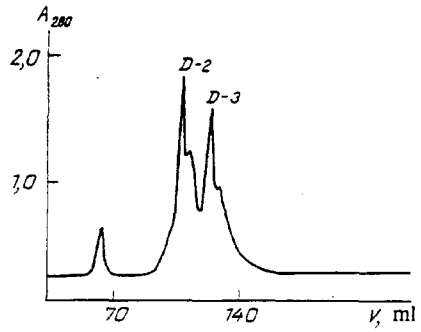


Fig. 4

Fig. 3. Production of IL-2 in mice. The production of IL-2 (in percentages) is plotted along the axis of ordinates, and the time after injection of the MMM peptides along the axis of abscissas.

Fig. 4. Gel chromatography of fraction IX-3 on a column (1.5 × 100 cm) of Sephadex G-25 in 0.01 M sodium acetate solution, pH 6.7. Rate of elution 13 ml/h.

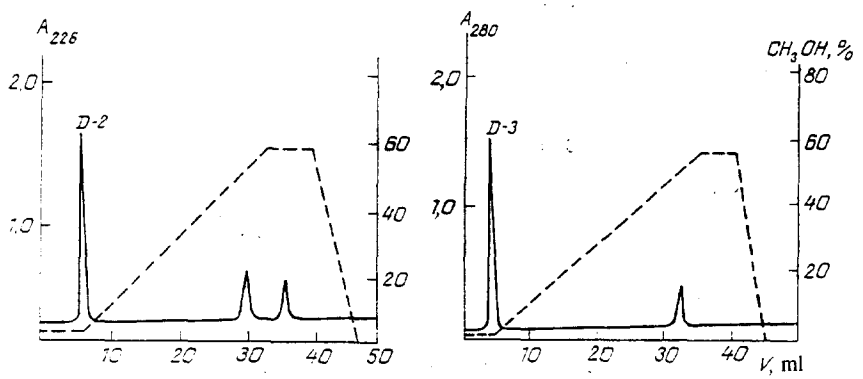


Fig. 5. HPLC of fractions D-2 and D-3 on a column (4.6 × 50 mm) of Zorbax ODS in a concentration gradient of methanol in 0.1% trifluoroacetic acid. Rate of flow 1 ml/min.

In view of its membranotropic action and participation in the development of an immunodeficient state, fraction IX-3 was used to obtain MMM peptides in the homogeneous state. For this purpose, the peptide fraction was subjected to chromatography on a column (1.5 × 100 cm) of Sephadex G-25 equilibrated with a 0.01 M solution of sodium acetate, pH 6.7 (Fig. 4). Three fractions were obtained, two of which — D-2 and D-3, exhibiting a membranotropic action — were further purified by high-performance liquid chromatography (HPLC) (Fig. 5). Peptides D-2 and D-3 retained membranotropic activity: D-3 increased the conductivity of a BPM for sodium and potassium ions by two orders of magnitude, and D-2 increased the mean life of a membrane.

The homogeneity of the peptides isolated was shown by determining terminal amino acid residues, by isoelectric focusing, and by partition TLC. The molecular masses (M) of the peptides were determind with the aid of HPLC in the presence of markers: for peptide D-2, M was 5660 Da, and, for peptide D-3, 2500 Da. The amino acid compositions of peptides D-2 and D-3 are given in Table 1. Analysis of the N-terminal amino acid residues of the peptides permitted the detection of the dansyl derivatives of ε-lysine and *o*-tyrosine, but only *o*-tyrosine in the case of D-3.

Thus, From the blood serum of patients with a disturbed renal function we have isolated fractions of MMM peptides possessing a membranotropic action and an immunomodulating effect, and also homogeneous peptides with a membranotropic activity.

TABLE 1. Amino Acid Compositions of the Peptides from the Blood Serum of Patients with Acute and Chronic Renal Insufficiency

| Amino acid | Peptide D-2 | Peptide D-3 | Amino acid | Peptide D-2 | Peptide D-3 |
|---|---|---|---|---|---|
| Asx | 4.50 | 3.20 | Met | 0.78 | – |
| Thr | 2.67 | 0.92 | Ile | 1.11 | 2.30 |
| Ser | 4,54 | 0.17 | Ley | 3.33 | – |
| Glx | 5.71 | 4.87 | Tir | 1.50 | 2.80 |
| Pro | 3.33 | 1.81 | Phe | 1.55 | 1.24 |
| Gly | 3.52 | 2.60 | His | 1.02 | – |
| Ala | 2.80 | 0.19 | Lys | 2.67 | – |
| Cys | – | 1.10 | Arg | 1.70 | – |
| Val | 2.64 | 1.41 | | | |

## EXPERIMENTAL

The blood serum was fractionated by gel chromatography on Sephadex G-25 (Pharmacia, Sweden) and TSK gel HW-40 (Toyo Soda, Japan), and by HPLC on a Zorbax ODS column (4. 6 × 50 mm) (Du Pont, USA).

N-Terminal amino acid residues were determined by Gray's method. The amino acid compositions of the peptides were established with the aid of a Biotronic C-7000 amino acid analyzer (FRG), and for this the peptides were hydrolyzed with 5.7 N HCl in evacuated sealed tubes at 110°C for 24 and 72 h.

The biological activities of the peptide fractions and peptides were determined on BPMs obtained by Kalikulov's method [11]. The formation of the membranes and the measurement of their electrical characteristics were performed as in [12]. As the target cells we used tumor cells of human erythromyeloleukemia K-562. The activity of the synthesis of IL-2 was tested by the use of 96-hour T-lymphoblasts activated by ConA. The NK activities and the production of IL-2 were studied on mice — hybrids of the first generation (CBSA × C57B1/$6_j$)$F_1$.

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