differentiated as fractions of coagulant and anticoagulant components. The coagulant fractions 1-8 were found to contain caseinolytic and thrombin-like proteases, probably causing a stimulation of clotting, while in the anticoagulant fractions 10-17 and claiming the role of active principle were found "direct" and "indirect" fibrinolysins, phospholipases A_2, and components blocking the effects of thrombin and thrombin-like proteases on fibrinogen (fibrogenin treated with them lost its capacity for forming clots under the influence of thrombin or thrombin-like proteases from the mamushi venom).

The results of electrophoresis and of isoelectric focussing revealed a predominant content of basic components in the fraction of the coagulant group, while the anticoagulant fractions were represented mainly by acidic proteins.

The same results were obtained when the mamushi venom was chromatographed on DEAE-cellulose, but in this case it was possible to detect and collect preparatively a smaller number (13) of protein fractions likewise differentiated with respect to their influence on the clotting of blood and the pI values of the proteins that they contained.

LITERATURE CITED

1. É. S. Sadykov, S. I. Ikramova, N. A. Barabanshchikova, and L. Ya. Yukel'son, Khim. Prir. Soedin., 133 (1980).
2. N. A. Barabanshchikova, S. I. Ikramova, É. S. Sadykov, and Ya. Yukel'son, Khim. Prir. Soedin., 265 (1980).
3. N. A. Barabanshchikova, É. S. Sadykov, V. M. Sorokin, and L. Ya. Yukel'son, Dokl. Akad. Nauk UzSSR, No. 1, 75 (1980).

PURIFICATION AND CHARACTERIZATION OF THE PROTEIN HEMOPOIESIS STIMULATOR FROM THE SPLEEN OF HORSFIELD'S TERRAPIN *Testudo horsfieldi*

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The literature contains information indicating the participation of humoral factors from the spleens of many animals in the process of postradiation recovery [1, 2]. However, there is no single opinion in the nature, properties, and mechanism of the action of this substance, known by its effective influence on hemopoiesis.

In the present work we consider the isolation and physicochemical characterization of the component (or components) of terrapin spleen affecting hemopoiesis and promoting postradiation recovery.

In the first stage of purification, an extract of terrapin spleen was fractionated by gel filtration on Ultragel AcA-34. The gel chromatograms of the spleen extract showed up to seven protein peaks, the best separation of the fraction being achieved by the use as eluent of 0.01 M Tris hydrochloride buffer with pH 7.5. However, for preparative purposes only three fractions were collected. It has been shown previously [3] that up to 26% of the whole weight of the terrapin spleen extract consists of proteins, 1.46% of lipids, and 11.5% of sugars. On gel filtration, the bulk of the proteins (47.3%) in the extract issued in the second fraction which, in accordance with the theory of gel filtration [4], contained components with molecular weights of 60-80 daltons. The same fraction was characterized by the most powerful stimulating activity. The protein hemopoiesis stimulator (PHS) contained in it was subjected to further purification with the aid of ion-exchange chromatography on CM-cellulose. As a result, five protein fractions were obtained of which a powerful biological efficacy was possessed by the last two, 4 and 5, containing the most basic components of the material

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chromatographed. This conclusion was confirmed by the results of electrophoresis and isoelectric focussing, also indicating the predominant presence of basic components in these fractions.

In view of the heterogeneity of the fractions that contained the PHS, investigations on its purification will be continued.

LITERATURE CITED

1. I. N. Kendysh, Usp. Sov. Biol., 73, No. 3, 342 (1972).
2. O. I. Olontseva and M. V. Tikhomirova, Med. Radiol., 26, No. 3, 56 (1981).
3. A. A. Turdyev, D. Kh. Nigmatov, D. Kh. Madzhidova, and R. B. Usmanov, in: 1st All-Union Biophysical Congress [in Russian], Vol. 2, Moscow (1982), p. 234.
4. L. M. Ginodman, Modern Methods in Biochemistry [in Russian], Vol. 1, Moscow (1964), p. 37.

MICROCOLUMN LIQUID CHROMATOGRAPHY OF NUCLEOSIDE 5'-MONO-, -DI-, and -TRIPHOSPHATES ON KhZh 1305

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In the present paper we describe cheap, effective, and rapid methods for the microcolumn liquid chromatography (MCLC) of nucleoside 5'-mono-, -di-, and -triphosphates on KhZh 1305 [1-3]. Microcolumns (0.5 × 90 mm) containing Dowex 1 × 8 ion-exchange resin were prepared by a method described previously [2, 4, 5]. The optimum stepwise gradient of pH values from 2.25 to 4.15 of the eluting solution and the concentration of salt in it from 0 to 0.6 M KCl that permitted the separation of a mixture of nucleoside 5'-mono-, -di-, and -triphosphates in 2.7 h with good resolution (Fig. 1, a) was selected experimentally. 5'-GMP and 5'-UDP, and also 5'-GDP and 5'-UTP, were separated poorly. The nucleotides were identified from the UV spectra of the peaks recorded by the KhZh 1305 microspectrophotometer simultaneously with the chromatogram on the profile of the peaks [1, 2, 6]. For a reliable identification of the nucleotides we also determined the retention times of the individual substances.

For the complete separation of the poorly resolved peaks of 5'-GMP and 5'-UDP and also of 5'-GDP and 5'-ITP we selected experimentally the optimum eluting system composed of a stepwise gradient of pH values of the eluting solution from 2.3 to 2.5 and of concentrations of salt in it from 0 to 0.5 M NaCl. In this chromatographic system, 5'-UMP, 5'-UDP, 5'-UTP, 5'-GMP, 5'-GDP, and 5'-GTP were well separated (Fig. 1b). Such chromatography enables the quantity of the nucleotides and their labeled analogs in production to be monitored with minimum expense.

Similar eluting systems were also made up for the separation of the oligonucleotides of enzymatic hydrolysis of plant rRNAs. Figure 1c, gives one of such chromatograms — the elution profile of the dinucleotides of a pyrimidyl-RNase hydrolysate of cotton-plant RNA [7]. The conditions of chromatography were approximately the same as in the preceding case with a stepwise gradient of pH values from 2.3 to 2.5 and of salt concentrations of 0 to 0.9 M NaCl.

Thus, in KhZh 1305 microcolumns with Dowex 1 × 8 anion-exchange resin it is possible to carry out the high-performance chromatography of nucleoside 5'-polyphosphates and oligonucleotides superior in many parameters to high-performance chromatography performed in high-pressure chromatographs and in small columns at high temperatures [2, 8].

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