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

Kh. R. Nuriddinov, M. R. Nuriddinova, and R. N. Nuriddinov

UDC 547.963.32:543.544

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].

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 722-723, September-October, 1985. Original article submitted February 12, 1985.

0009-3130/85/2105-0685$09.50

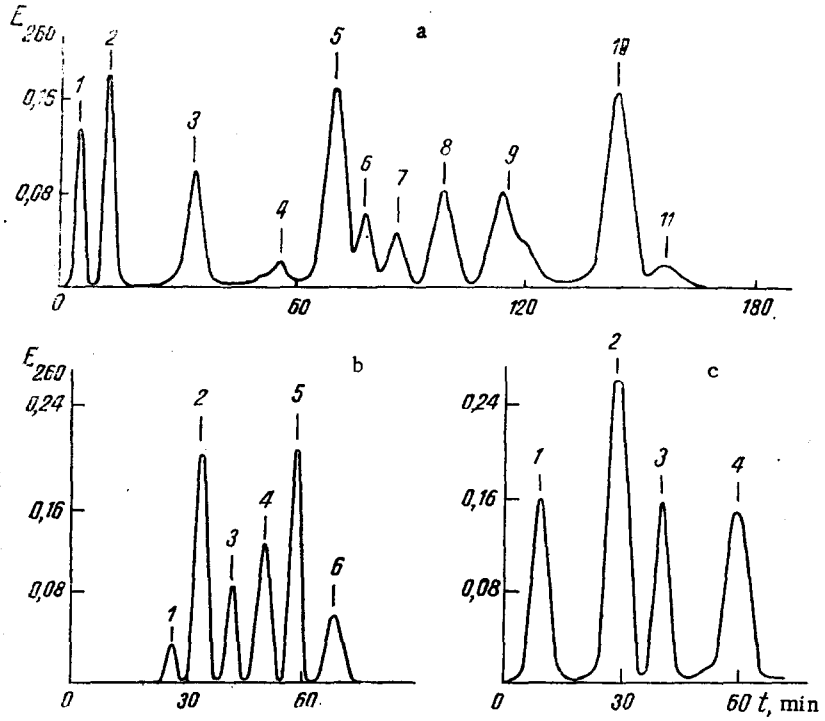

Fig. 1. Microcolumn liquid chromatography: a) nucleotide 5'-mono-, -di-, and -triphosphates: 1) 5'-CMP; 2) 5'-AMP; 3) 5'-UMP; 4) 5'-CDP; 5) 5'-GMP; 6) 5'-UDP; 7) 5'-CTP; 8) 5'-ADP; 9) 5'-GDP + 5'-UTP; 10) 5'-ATP; 11) 5'-GTP; b) 1) 5'-UMP; 2) 5'-GMP; 3) 5'-UDP; 4) 5'-GDP; 5) 5'-UTP; 6) 5'-GTP; c) the dinucleotides of a pyrimidyl-RNase hydrolysate of a pyrimidyl-RNase hydrolysate of cotton-plant km RNA: 1) APCP; 2) GPCP; 3) APUP; 4) GPUP.

LITERATURE CITED

1. G. I. Baram, M. A. Grachev, N. I. Komarova, M. P. Perelroyzen, Yu. A. Bolvanov, S. V. Kuzmin, V. V. Kargaltsev, and E. A. Kuper, J. Chromatogr., 264, 69 (1983).
2. Kh. R. Nuriddinov and R. N. Nuriddinov, Khim. Prir. Soedin., 248 (1983).
3. G. N. Nikulina, Methods of Determining Nucleotides in Plants [in Russian], Leningrad (1980), p. 37.
4. V. V. Vlasov, N. M. Melamed, V. E. Chizhikov, and M. A. Tukalo, Bioorg. Khim., 2, 892 (1976).
5. Kh. R. Nuriddinov, M. R. Nuriddinova, and R. N. Nuriddinov, Khim. Prir. Soedin., 110 (1981).
6. M. A. Grachev, in: The Ultramicroanalysis of Nucleic Acids [in Russian], Moscow (1973), p. 104.
7. Kh. R. Nuriddinov, M. R. Nuriddinova, and R. N. Nuriddinov, Khim. Prir. Soedin., 746 (1982).
8. J. X. Khym, J. Chromatogr., 124, 415 (1976).