HIGH-SPEED CHROMATOGRAPHY OF NUCLEOSIDE MONOPHOSPHATES BY MICROCOLUMN LIQUID CHROMATOGRAPHY

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UDC 547.963.32:544

The aim of the present work was to increase the efficacy of the chromatographic separation of nucleoside monophosphates [1-6] by shortening the time of chromatograpy, improving the resolution, and contracting the peaks, which permits working with smaller amounts of substances and the determination of the amount of pseudouridylic acid together with other nucleotides in a direct alkaline hydrolysate of tissues of biological materials [7].

The direct alkaline hydrolysates and microcolumn (0.5 × 90 mm) containing Dowex 1 × 8 were prepared on KhZh 1305. To shorten the time of chromatographing the nucleotides and for the good resolution of the pseudouridylic acid and 3'-AMP we used a principle that was the opposite of methods described previously [2, 3], i.e., the eluting system was composed of a stepwise-increasing gradient of pH values of the eluting solution from 2.25 to 2.55 and a steeper concentration gradient of salt in it from 0.0 to 0.25 M NaCl (Fig. 1). The use of this gradient for separating nucleoside 5'-monophosphates permitted the time of chromatography to be shortened and the peaks of the nucleotides to be narrowed (Fig. 2). Fig. 3 gives, together with the conditions, the pattern of another chromatogram of nucleoside 5'-monophosphates differing from the preceding ones by the fact that the microcolumn can be used without regeneration for 20 and more analyses.

With the aid of the first MCLC method described we have determined the nucleotide compositions of direct alkaline hydrolysates of tissues of rat liver, cotton seeds, kenaf, and mung beans:

	AMP	GMP	CMP	UMP	ΨMP	Yield, % on the weight of defatted flour
Cotton plant	22,8	29.4	25 6	20,4	1,8	0,55
Mung bean	19,4	32.5	21,5	24.3	2 3	0,32
Kenaf	23 1	33,4	22,7	19.4	1,4	1.00
Rat liver	19,0	31.4	29.2	18,7	1.7	1.83

The separation of the direct alkaline hydrolysates into isolates [8] showed that the hydrolysate contained — in addition to mononucleotides — oligonucelotide that had not undergone hydrolysis under the action of alkali because of the presence in them of 2'-O-methylated nucleotides. The amount of oligonucleotides was specific for each material studied. Their percentage contents in the direct alkaline hydrolysates were as follows: rat liver — 5.81; kenaf — 14.0; mung bean — 0.35; cotton plant — 22.5.

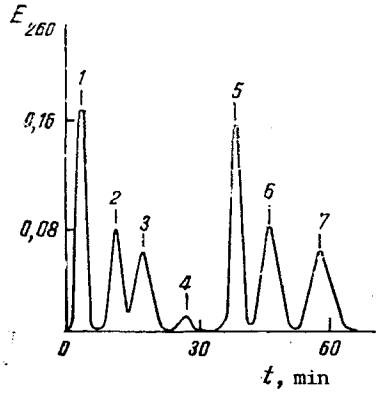

Fig. 1. Microcolumn liquid chromatography of a direct alkaline hydrolysate of rat liver tissues: 1) 3'(2')-CMP; 2) 2'-AMP; 3) 3'-AMP; 4) 3'(2')-ψMP; 5) 3'(2')-UMP; 6) 2'-GMP; 7) 3'-GMP.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 580-581, July-August, 1985. Original article submitted February 12, 1985.

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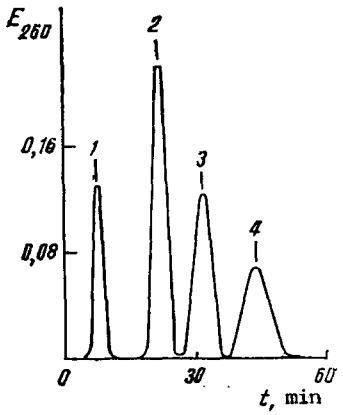

Fig. 2. High-speed MCLC of nucleoside 5'-monophosphates: 1) 5'-CMP; 2) 5'-AMP; 3) 5'-UMP; 4) 5'-GMP.

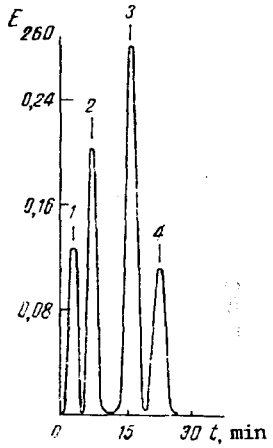

Fig. 3. Chromatographic separation: 1) 5'-CMP; 2) 5'-UMP; 3) 5'-AMP; 4) 5'-GMP. pH gradient from 3.0 to 4.15 and NaCl gradient from 0 to 0.4 M.

Thus an effective method for the MCLC of nuceloside 3'(2')-monophosphates and nucleoside 5'-monophosphates had been developed and the nucleotide compositions of the direct alkaline hydrolysis of a number of plants and or rat liver tissues have been determined

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