USE OF SODIUM PYROPHOSPHATE IN THE ION-EXCHANGE CHROMATOGRAPHY OF OLIGONUCLEOTIDES

S. I. Yastrebov, A. I. Lomakin,
and V. V. Gorn

UDC 577.113.4.083:543.544.6

In this paper an example is given of the use of a mixture of potassium phosphate and sodium pyrophosphate, solutions of which possess a high ionic strength, in the eluting system during the anion-exchange HPLC of synthetic oligodeoxyribonucleotides. It has been found that an additional concentration gradient of 0.02-0.1 M sodium pyrophosphate (pH 6.5) in the 0-0.2 M KH_2PO_4 (pH 6.5) in 30% acetonitrile system considerably accelerates the chromatographic process (Fig. 1). Short (up to 10 bases long) oligonucleotides issue in the first 5-7 min, while the resolution of longer ones decreases only slightly (Fig. 2). The time of separation is shortened by a factor of 1.5. The system with sodium pyrophosphate has been tested in the separation of a number of synthetic oligonucleotides with lengths of from 20 to 70 bases (Fig. 3). The purity of the products isolated was practically the same as on chromatography in the usual system with potassium phosphate.

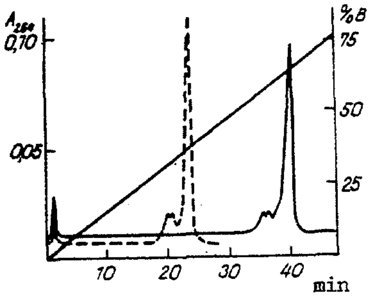

Fig. 1

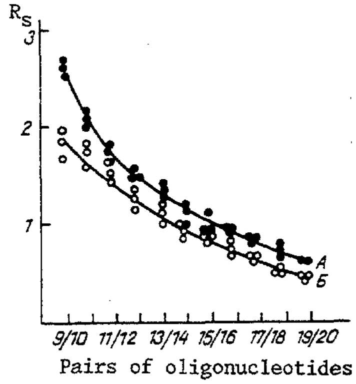

Fig. 2

Fig. 1. Profile of the anion-exchange chromatography of an oligodeoxyribonucleotide ($T_{10}C_2AT_{16}$) on a 3.2 × 250 mm column of Polisil CA (13 μm) in the following eluting systems: full line — buffer A (0.02 M KH_2PO_4 (pH 6.5) in 30% acetonitrile)—buffer B (0.3 M KH_2PO_4 (pH 6.5) in 30% acetonitrile); dashed line — buffer A (0.02 M $Na_4P_2O_7$ (pH 6.5) in 30% acetonitrile)—buffer B (0.2 M KH_2PO_4 (pH 6.5), 0.1 M $Na_4P_2O_7$ (pH 6.5) in 30% acetonitrile).

Fig. 2. Dependence of resolution on the length of homologous pairs of oligoadenylates with lengths of from 9 to 21 nucleotides: A) in the system with phosphate; B) in the system with pyrophosphate. Chromatic conditions as for Fig. 1. The resolution was calculated according to [1].

All-Union Scientific-Research Institute of Molecular Biology. Vektor Scientific-Production Association, Ministry of the Medical Industry, Koltsova, Novosibirsk. Novosibirsk Institute of Bioorganic Chemistry, Siberian Branch, Russian Academy of Sciences. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 148-149, January-February, 1992. Original article submitted February 19, 1990; revision submitted September 20, 1991.

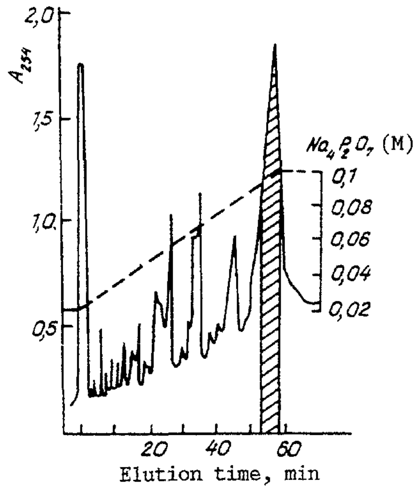

Fig. 3. Elution profile of the isolation by anion-exchange chromatography of a 70-membered oligodeoxyribonucleotide from a reaction mixture. Eluent: 0.02-0.1 M concentration gradient of $Na_4P_2O_7$ in 0.02 M KH_2PO_4, pH 6.5, in 30% acetonitrile, t = 55°C, rate of flow 1.5 ml/min.

The authors thank V. A. Ryabinin and N. N. Karpyshev for providing the synthetic oligonucleotides.

LITERATURE CITED

1. B. Karger, in: Modern Practice of Liquid Chromatography, J. J. Kirkland (ed.), Wiley-Interscience (1974).

SYNTHESIS OF VERONGIAQUINOL AND RELATED COMPOUNDS AND STUDY OF THEIR INHIBITING ACTION ON RAT BRAIN Na^+,K^+-ATPase

B. A. Gorshkov, O. P. Shestak, I. A. Gorshkova,
T. N. Makar'eva, V. L. Novikov, and V. A. Stonik

UDC 577.352.45

The 4-acetamido-2,6-dibromo-4-hydroxycyclohexa-2,5-dienone (VII) (verongiaquinol) isolated from sponges of the family Aplysinidae is a powerful inhibitor of Na^+,K^+-ATPase, interacting specifically with the sulfhydryl groups of the enzyme [1]. In order to establish a structure-activity relationship, we have effected a total synthesis of verongiaquinol (VII) and a number of compounds related to it, (I-VI) and (VII-X), starting from commercial p-hydroxyphenylacetic acid, and, by the method described in [2], have studied their action in relation to Na^+,K^+-ATPase isolated from rat brain by the method of [3]. The bromination and chlorination (Br_2/HOAc and $SOCl_2$, respectively) of this acid led to the 3,5-dibromo- and 3,5-dichloro derivatives the esterification of which (CH_3OH and C_2H_5OH) gave the corresponding Me and Et esters. The corresponding amides were obtained by the aminolysis of the esters (NH_3, MeOH, NaCN). Oxidation of the 3,5-dibromo- and 3,5-dichlorophenylacetic acids and their esters and amides with concentrated HNO_3 gave the known compounds (I), (V), (VII) [4],

---

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 149-151, January-February, 1992. Original article submitted February 27, 1991.