Journal of Chromatography, 277 (1983) 305—307
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1796

Note

Isocratic reversed-phase high-performance liquid chromatographic separation of deoxyribonucleosides and ribonucleosides

SEBASTIAN P. ASSENZA and PHYLLIS R. BROWN*

Department of Chemistry, University of Rhode Island, Kingston, RI 02881 (U.S.A.)

and

ALAN P. GOLDBERG

Analytical Instruments Division, E.I. du Pont de Nemours and Company, Inc. Wilmington, DE 19898 (U.S.A.)

(Received May 6th, 1983)

There is increasing evidence that the pattern of methylation in DNA bases influences gene expression in vertebrate cells. In addition, the methylation of DNA which occurs in animals treated with carcinogens is suspect in tumor formation. Since the methylated components could serve as an indicator of DNA function or damage, much research has focused on the determination of both major and minor fragments [1].

Many different analytical techniques have been used to determine the components of DNA. Reversed-phase high-performance liquid chromatography (RPLC) is found to be ideally suited for separation and quantitation of DNA fragments in its various forms [2—5]. However, the methods developed thus far have several disadvantages: (1) the time required for the separation of major and minor compounds is too long for routine application; (2) isocratic separation of these compounds from RNA fragments have been difficult to achieve; (3) it is difficult to assay DNA components in the presence of RNA components.

Therefore, we developed an isocratic RPLC separation of some important major and minor deoxyribonucleosides and ribonucleosides. The method is sensitive and selective for the determination of 5-methyldeoxycytidine (m_5dC),

0378-4347/83/$03.00

7-methyldeoxyguanosine (m_7dG), and 6-methyldeoxyadenosine (m_6dA) along with their ribonucleosides and other nucleic acid components.

EXPERIMENTAL

Apparatus

The chromatographic system was a Du Pont 8820 (Du Pont Instruments, Wilmington, DE, U.S.A.) which included a controlled-temperature column compartment, 254-nm fixed-wavelength detector, HP 3380A integrator (Hewlett-Packard, Avondale, PA, U.S.A.) and a strip-chart recorder (Houston Instruments, Austin, TX, U.S.A.). Separations were achieved with a Zorbax-TMS (Du Pont) column (250 × 4.6 mm I.D.) packed with 6-μm trimethylsilica.

Chromatographic conditions

Eluents were prepared with 2.0% methanol (glass-distilled, Burdick and Jackson Labs., Muskegon, MI, U.S.A.) in 4.0 m*M* $(NH_4)_2HPO_4$ and 4.0 m*M* $NH_4H_2PO_4$, pH 4.0 (v/v); the phosphate salts were the highest grade available from Fisher Scientific (Fair Lawn, NJ, U.S.A.). The flow-rate was 2.0 ml/min and the column temperature was 35°C.

Standards

The nucleic acid components were obtained from Vega Biochemicals (Tucson, AZ, U.S.A.) and Sigma (St. Louis, MO, U.S.A.); standard solutions were prepared in the mobile phase at concentrations of approximately 100 μM.

RESULTS AND DISCUSSION

Although the separation of deoxyribonucleosides and ribonucleosides has been reported using gradient elution with octadecylsilica reversed-phase columns [4, 5], we were able to use isocratic elution to achieve excellent resolution of the major deoxyribonucleosides and ribonucleosides together with some methylated analogues. The analysis of the majority of the nucleosides can be obtained in less than 14 min (Fig. 1). If, however, it is also important to determine the concentrations of the 6-methyl analogues of adenosine and the dimethyl analogues of guanosine, the analysis then requires approximately 40 min. In comparison to the previously published methods, the relatively short analysis time of our method is attributed to the use of the trimethylsilica reversed-phase packings. In addition, because we used isocratic elution, the total analysis time is even more rapid because the column need not be flushed and equilibrated between injections. In addition, detection limits are lower (ca. 5–15 pmol) with isocratic elution since there is no baseline drift.

Although the deoxy standards were clearly separated, interferences from contaminating RNA in biological samples can hamper the determination of concentrations of hydrolysates of DNA. Using the procedure described by Kuo et al. [4] to prepare DNA samples prior to chromatography, the resulting DNA/RNA enzymic hydrolysates were analyzed under our conditions. The uridine fractions from RNA were found to elute between the cytidine (C) and deoxycytidine (dC) peaks (Fig. 1). Some minor components present in

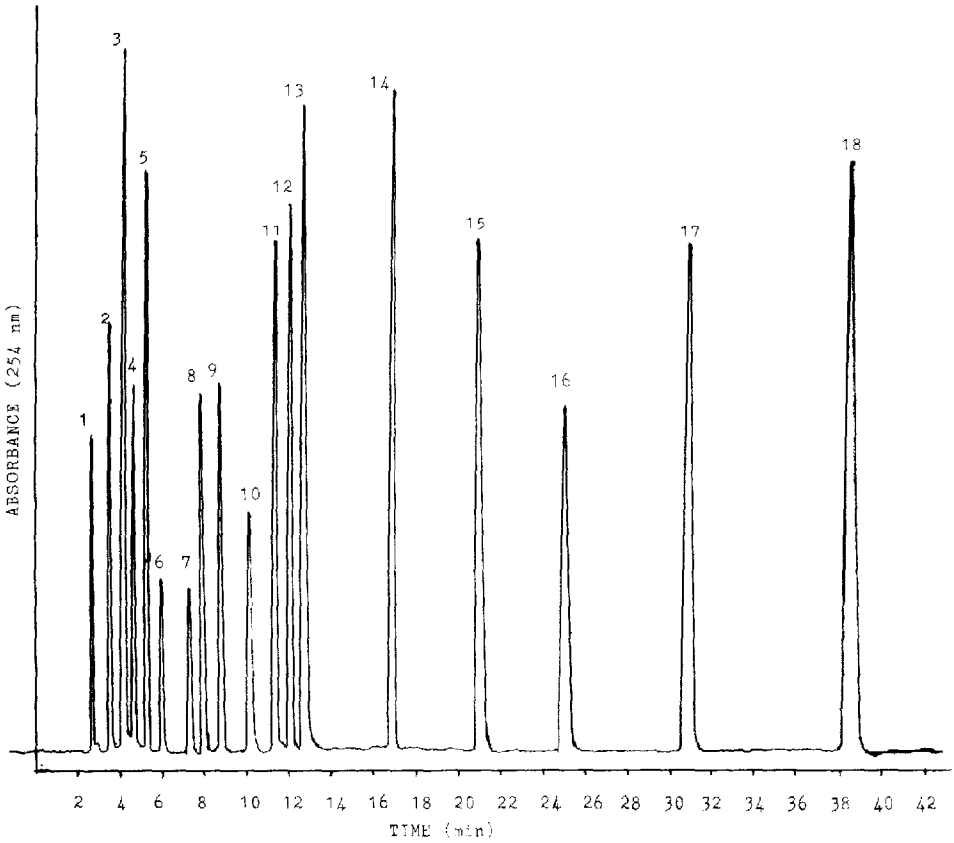

Fig. 1. Isocratic separation of major and minor deoxyribonucleosides and ribonucleosides. Conditions are given in the text. Peaks: 1 = C, cytidine; 2 = dC, deoxycytidine; 3 = I, inosine; 4 = G, guanosine; 5 = m_5dC, 5-methyldeoxycytidine; 6 = dI, deoxyinosine; 7 = dG, deoxyguanosine; 8 = m_7G, 7-methylguanosine; 9 = dT, thymidine; 10 = A, adenosine; 11 = m_1G, 1-methylguanosine; 12 = m_7dG, 7-methyldeoxyguanosine; 13 = dA, deoxyadenosine; 14 = m_1dG, 1-methyldeoxyguanosine; 15 = m_2^2G, 2,2-dimethylguanosine; 16 = m_6A, 6-methyladenosine; 17 = m_2^2dG, 2,2-dimethyldeoxyguanosine; 18 = m_6dA, 6-methyldeoxyadenosine.

RNA digests, such as 1-methylinosine, 2-methylguanosine and 1-methyladenosine, which interfere only with the resolution of ribonucleosides, do not interfere with the resolution of the deoxyribonucleosides. Therefore, in order to use this system for the assay of hydrolysates of RNA, conditions must be modified to obtain optimal resolution of the hydrolysates of interest. However, for routine assays of digests of DNA, the rapid and efficient isocratic separation as illustrated in Fig. 1 can be very useful in studies of DNA methylation and function.

REFERENCES

1 M.E. Ehrlich and R.H. Wang, Science, 212 (1981) 1350.
2 M. Ehrlich and K. Ehrlich, J. Chromatogr. Sci., 17 (1979) 531.
3 J.K. Christman, Anal. Biochem., 119 (1982) 38.
4 K.C. Kuo, R.A. McCune, C.W. Gehrke, R. Midgett and M. Ehrlich, Nucl. Acids Res., 8 (1980) 4763.
5 R.A. Hartwick, S.P. Assenza and P.R. Brown, J. Chromatogr., 186 (1979) 647.