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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF DNA COMPOSITION AND DNA MODIFICATION BY CHLOROACETALDEHYDE

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SUMMARY

The separation of common and modified deoxyribonucleosides derived from DNA hydrolyzates was examined under different chromatographic conditions on silica-based octadecyl (C_{18}) columns, involving hydrophobic interactions with the matrix. A novel method for the analysis of the DNA composition is described. It involves the removal of RNA contaminants and enzymatic hydrolysis of DNA, first to deoxyribonucleoside monophosphates and then dephosphorylation of the latter to deoxyribonucleosides. Hydrolysis conditions were sought to avoid deamination of dA and dC residues to dI and dU contaminants, respectively. Elution of these contaminants and the artifacts (ribonucleosides derived from RNA) is described in relation to the elution of deoxyribonucleosides. Chromatographic separation of the hydrolyzate derived from a 15-μg sample of DNA under selected separation conditions and on one high-performance liquid chromatographic column is achieved in 18 min at room temperature. Detection of modified components (and contaminants) present in minute amounts is enhanced with the use of a diode-array detector. The power of this technique lies in its ability to characterize and quantitate accurately the amount of modified species present in the DNA structure (less than 2% of all the other residues). Examples of the composition analysis of DNA derived from a prokaryote (*Escherichia coli* B) and a eukaryote (salmon sperm) are described. Details of quantitation (calibration graphs) of different nucleosides are furnished for peak-area integration by commercially available software, and spectral properties of the nucleoside in the elution buffer are described for quantitation by other means. Application of the composition analysis is shown here for probing the DNA conformation in solution by chemical means, while using chloroacetaldehyde as the modifying agent.

INTRODUCTION*

More than a decade ago, ion-exclusion and ion-exchange chromatographic methods were described for the separation of common and modified nucleosides in order to analyze the base composition of nucleic acids[1–5]. Recently, separations

* Abbreviations: dN, deoxyribonucleoside; rN, ribonucleoside; A, adenosine; C, cytidine; G, guanosine; I, inosine; T, thymidine; U, uridine; CAA, chloroacetaldehyde.

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involving hydrophobic interactions on reversed-phase chromatographic matrices have been explored in order to obtain fast and sensitive separations of the nucleosides[6–9]. The need for novel methods of DNA analysis is realized with renewed interest in the role of the modified components of DNA[10–12]. 5-Methyldeoxycytidine (m^5dC) and 6-methyldeoxyadenosine (m^6dA), the minor components of eukaryotic and prokaryotic DNAs, respectively, are present primarily in less than 2% of all the other residues[12]. In general, DNA samples are often available only in microgram amounts. Several procedures involving hydrophobic interactions have been described recently, which offer fast and sensitive methods for the separation of deoxyribonucleosides[6–9]. We recently described a microprocessor-controlled (hardware and software) high-performance liquid chromatographic (HPLC) system for data acquisition, data processing and analysis of the deoxynucleosides[13–15]. A method of DNA analysis at the sub-nanomolar level is described here. Details of the sample (DNA) purification, hydrolysis to deoxyribonucleosides, and their separation on a single reversed-phase column are reported. The study of nucleoside composition is demonstrated for probing the DNA conformation in solution by chemical means, using chloroacetaldehyde (CAA) as the modifying agent.

EXPERIMENTAL

The nucleoside separations were performed on a 250 mm × 4.6 mm I.D. column packed with 5-μm silica-based C_{18} reversed-phase chromatography material and installed with a guard column (Separation Group, Hesperia, CA, U.S.A., Model Vydac 201HS54 or Supelco, Belfonte, CA, Model Supelcosil LC-18-DB). The guard column was fitted with a 2-cm cartridge, containing 5-μm packing material. The deoxyribonucleosides and *E. coli* DNA (strain B) were purchased from Sigma (St. Louis, MO, U.S.A.). DNA from crude salmon sperm milt, obtained from Reliable Chemical (St. Louis, MO, U.S.A.) was purified in this laboratory by using Marmur's procedure[16]. The DNA sample was purified to remove RNA contaminants. The enzymes nuclease P1, pancreatic DNase I and calf intestinal alkaline phosphatase were obtained from Boehringer-Mannheim Biochemicals (Indianapolis, IN, U.S.A.) and pancreatic RNase was obtained from Worthington Biochemical (Freehold, NJ, U.S.A.).

Enzymatic digestion conditions

To remove RNA contaminants from the DNA sample, the sample was first dissolved in the buffer used for RNase digestion [50 m*M* N-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid buffer (pH 7.7) with 0.15 *M* sodium chloride and 15 m*M* sodium citrate] and then pancreatic ribonuclease[17] (RNase) was added to the solution (0.1 enzyme unit per 1.0 A_{260} unit of DNA), followed by incubation at 45°C for 4 h. The reaction mixture was then freed from ribonucleosides by dialyzing the mixture against 20 m*M* sodium succinate buffer (pH 5.5) in microdialysis chambers (Hoefer Scientific Instruments, San Francisco, CA, U.S.A., Model EMD-101).

DNA hydrolysis was carried out with 2–4 A_{260} units (100–200 μg) of DNA in a total volume of less than 150 μl. First, the DNA was degraded to deoxyribonucleoside monophosphates by the addition of 10 μl of buffer [30 m*M* sodium acetate with 10 m*M* zinc chloride (pH 5.3)] for each A_{260} unit of DNA and an enzyme mixture

containing nuclease P_1 from *Penicillium citrinum*[18] (1 unit/μl) and pancreatic deoxyribonuclease[19] (DNase I, 0.1 unit/μl). A 1-μl volume of the enzyme mixture was added for each A_{260} unit of DNA, and the reaction mixture was incubated at 37°C for 2 h. Dephosphorylation of the deoxynucleotides was accomplished by the reaction of alkaline phosphatase. The pH of the reaction mixture was raised by the addition of 10 μl of 1 *M* sodium glycinate buffer (pH 8.0 or 8.8), then one unit of alkaline phosphatase was added for each A_{260} unit of the DNA digest, and the mixture was incubated at 37°C for 3 h.

HPLC and data processing equipment

The DNA analysis was performed with a chromatographic system consisting of (a) a manual injection valve (Rheodyne, Cotati, CA, U.S.A., Model 7125), (b) a programmable mobile phase gradient pump (Perkin-Elmer, Norfolk, CT, U.S.A., Series LC4) and (c) a diode-array effluent monitor (LKB, Bromma, Sweden, Model 2140 Rapid Spectral Detector). A minicomputer (Zenith Data Systems, St. Joseph, MI, U.S.A., Model Z-248, IBM AT-compatible) was used to operate the detection unit and also to collect and store the absorption spectrum of the effluent every 0.3 s (for example, from 220 to 300 nm) with the aid of a software program (LKB, Model Wavescan-EG).

A silica based reversed-phase C_{18} HPLC matrix of 5-μm spherical beads was used for chromatography. Separations were carried out at different temperatures and with different gradients of the mobile phase (methanol) in order to optimize the chromatographic conditions. The different elution gradient systems used for this work are described in Table I. Peak areas of the chromatogram were digitized at a wavelength of interest (*e.g.*, at 260 nm) using a commercially available software program (Nelson Analytical, Cupertino, CA, U.S.A., Model 2000, Version 3.6).

TABLE I

ELUTION GRADIENT PROGRAMS FOR THE SEPARATION OF DEOXYRIBONUCLEOSIDES

System	*Stage*	*Time (min)*	*Mode*	*Elution solvent (%)*		
				*Buffer**	*Methanol***	*THF****
A	Equil.	10	Step	100	0	0
	Start	5	Step	100	0	0
	End	15	Linear	82	18	0
B	Equil.	10	Step	95	5	0
	Start	8	Linear	78	22	0
	End	10	Linear	15	85	0
C	Equil.	10	Step	95	5	0
	Start	8	Linear	53	47	0
	End	7	Linear	15	60	25

* Buffer for system A contains 10 m*M* ammonium phosphate with 2.5% methanol (pH 5.3); buffer for system B contains 20 m*M* sodium succinate with 0.5 m*M* sodium azide (pH 5.3 or 5.5); sodium azide is routinely added to the buffer to prevent bacterial growth.

** 100% methanol.

*** 100% tetrahydrofuran.

Quantitation of each deoxyribonucleoside peak was based on the calibration graph for the reference compound which was pre-calibrated and stored in the Nelson software. From the nanomoles of the deoxyribonucleodes, the percentage nucleoside composition of DNA was derived.

Modification of DNA with chloroacetaldehyde

A DNA sample from *E. coli* (two A_{260} units) was allowed to react with 0.1 *M* chloroacetaldehyde[20] (CAA) in 0.2 *M* sodium succinate buffer (pH 5.5) at 37°C for 1 h. After the modification, the reaction mixture was extracted twice with two volumes each of diethyl ether to remove the unreacted CAA. Then the product was hydrolyzed and analyzed as described above.

RESULTS AND DISCUSSION

Effect of column temperature on the separation of deoxyribonucleosides

The results in Fig. 1 indicate that the *k'* values of the nucleosides are significantly temperature dependent from 24 to 30°C. However, no appreciable decrease in these values occurs for most species at temperatures above 30°C, that is, increasing the column temperature over 30°C does not reduce the analysis time. However, at 30°C the separation can be concluded in less time and the dG and dT pair can be more readily resolved, m^5dC —a modified component present is less than 2% of the other DNA components— is eluted close to the rG peak (deoxyinosine, dI, derived from the deamination of dA, is eluted after rG, but before dG). In analogy with the separation of nucleosides on ion-exchange and reversed-phase columns, increasing the column temperature affects the elution of purine nucleosides more than that of pyrimidine nucleosides[4,7]. As the column temperature is raised, purines exhibit less hydrophobicity; therefore, they are eluted earlier on these columns. If the objective is to measure m^5dC, separation at room temperature can yield satisfactory results. However, a chromatogram with sharper peaks results if the analysis is carried out at a temperature higher than 30°C[1,2].

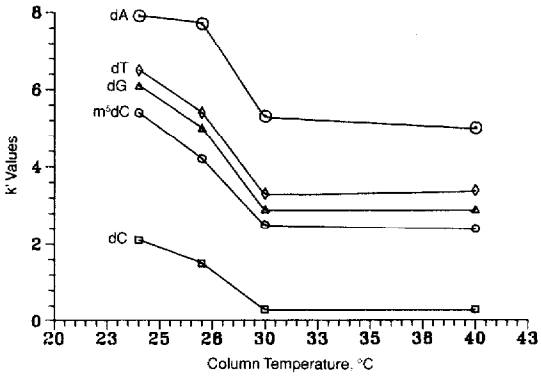

Fig. 1. Effect of temperature on the elution of deoxyribonucleosides. An authentic mixture of five nucelosides was injected into a Vydac column and elutions were carried out at different temperatures, using the same elution system (system A in Table I).

Effects of pH and mobile phase on the separation of nucleosides

Deamination of dA and dC must be avoided during DNA hydrolysis and HPLC of nucleosides. Under significantly acidic chromatographic conditions, *e.g.*, at pH 4, an acid-catalyzed deamination is observed. Similarly, under the alkaline conditions generally used for the phosphatase reaction, *e.g.*, at pH > 8, a base-catalyzed deamination of the two nucleosides occurs to a great extent. Under both conditions, deamination is further increased if the column temperature or the temperature of the phosphatase reaction is raised above 37°C (results not shown here). The separation of nucleosides (k' values) under three sets of conditions involving slightly different pH conditions (pH 5.3 *vs.* 5.5) and gradients of methanol are shown in Table II (*cf.*, Table I) for the composition of elution systems A and B). The results in columns 1 and 2 in Table II were obtained by using different methanol gradients and the same pH, whereas those in columns 2 and 3 were obtained by using the same gradient but different pH. Perhaps dC ionizes differently under two separation conditions, since dC exhibits a pK_a of 4.3. At pH 5.3, dC may exhibit some protonation, but perhaps none at pH 5.5. However, dC was eluted similarly under the three elution conditions. dA exhibits a pK_a of 3.8, far away from the two elution pH values; therefore, differences in the elution of dC and dA cannot be explained on the basis of differences in their ionizations in the elution systems used here. As noted for elution system B at pH 5.5 (Table II, column 3), ribonucleosides were eluted earlier than their deoxy derivatives. dG, a purine nucleoside, should exhibit more hydrophobicity than dT and therefore should be eluted later than the pyrimidine nucleoside. However, the results indicate that methanol abolished the hydrophobic character of dG, causing it to be eluted earlier than dT, whereas methanol failed to reduce the hydrophobic character of the other purine (dA) nucleosides to any degree.

Quantitation of chromatographic peaks

Software programs were recently described for the direct, computer-assisted acquisition and display of chromatographic data from conventional HPLC equip-

TABLE II

SEPARATION OF DEOXYNUCLEOSIDES UNDER THREE SEPARATION CONDITIONS

Nucleoside	pK_a*	k'		
		*System A*** *(pH 5.3)*	*System B*** *(pH 5.3)*	*System B**** *(pH 5.5)*
dCyd	4.3	1.7	1.6	1.7
m^5dCyd	4.4	4.6	2.8	3.0
dGuo	2.5	5.2	3.1	3.4
dThd	9.8	5.5	3.5	3.8
dAdo	3.8	7.9	4.6	4.8
Br^8Guo	–	9.6	5.2	5.4
m^6dAdo	4.2	10.2	5.7	5.9

* pK_a values are from G. W. Fasman (Editor), *Handbook of Biochemistry and Molecular Biology*, CRC Press, Boca Raton, FL, 3rd ed., 1975.

** See Table I for composition of the methanol gradient.

*** Br^8Guo, 8-bromoguanosine, used as a marker for retention time in HPLC.

ment[13–15]. Programs were written for a dual-microprocessor HPLC controller and described necessary utility routines to calibrate the instruments, and to acquire, display and store chromatographic data in real time. Here, we describe the use of commercially available software programs in order to calibrate the instrument and integrate peak areas of the chromatogram. In addition, spectral properties of the nucleosides are described here for quantitation by other means.

Relationship between the amount of nucleosides injected and the peak area originated by a software program. The standard solution of each nucleoside, prepared in the elution buffer (pH 5.5), was first assayed for its concentration by diluting a known volume of each sample, in triplicate, to a solution in which the molar absorptivity is known (*e.g.*, 0.10 *M* hydrochloric acid) and the pK_a of the nucleoside lies at least one pH unit away from the pH of the solution. Various amounts of the standard nucleoside solution were then injected and the peak areas were derived by the software and expressed in microvolts-seconds. The linear relationship between the amount of nucleoside injected and the peak area derived for each nucleoside is shown in Table III. Eqn. 1 gives the least-squares fit for the calibration graph, and eqn. 2 solves for the unknown amount of deoxynucleosides in nanomoles from the peak-area input.

Quantitation and characterization of peaks by absorption properties of the nucleosides. Spectral data and molar absorptivities (pH 5.5) at three different wavelengths for each nucleoside are listed in Table IV. This information is necessary for the characterization and quantitation of the peaks by other software programs and also manually. In the latter instance the peak area is derived[4] from the product of peak height × peak width at half maximum peak height × 1.06. Peak heights and peak widths of each nucleoside depend on several chromatographic conditions, including the flow-rate[12]. A comparison of the peak-height method with a microprocessor-contr olled, data-handling system, carried out recently, indicates a greater degree of confidence with the latter method[15]. The results of quantitation with a commercially available software program are described here (see below).

TABLE III

RELATIONSHIP BETWEEN THE AMOUNT OF NUCLEOSIDE (NANOMOLES) INJECTED AND PEAK AREA DERIVED BY SOFTWARE (NELSON ANALYTICAL, CUPERTINO, CA, U.S.A., MODEL 2000, VERSION 3.6)

Deoxyribo-nucleoside	*Eqn. 1**	*Eqn. 2**	*Correlation coefficient*
dCyd	$Y = 2.5775 \cdot 10^5 X - 2.7870 \cdot 10^5$	$X = 3.8798 \cdot 10^{-6} Y + 1.0813$	0.9996
5MedCyd	$Y = 1.7426 \cdot 10^5 X + 3.2180 \cdot 10^4$	$X = 5.7259 \cdot 10^{-6} Y - 1.842 \cdot 10^{-1}$	0.9927
dGuo	$Y = 3.5423 \cdot 10^5 X - 1.5232 \cdot 10^5$	$X = 2.8231 \cdot 10^{-6} Y + 4.3000 \cdot 10^{-1}$	0.9968
dThd	$Y = 2.8929 \cdot 10^5 X - 2.8548 \cdot 10^5$	$X = 3.4568 \cdot 10^{-6} Y + 9.8686 \cdot 10^{-1}$	0.9999
dAdo	$Y = 3.7727 \cdot 10^5 X + 5.0478 \cdot 10^5$	$X = 2.6506 \cdot 10^{-6} Y - 1.3380$	0.9999
6MedAdo	$Y = 3.8937 \cdot 10^5 X - 1.3288 \cdot 10^5$	$X = 2.5683 \cdot 10^{-6} Y + 3.4128 \cdot 10^{-1}$	0.9999

* X = nanomoles of nucleoside; Y = peak area at 260 nm.

TABLE IV

SPECTRAL PROPERTIES OF DEOXYRIBONUCLEOSIDES UNDER THE CHROMATOGRAPHY CONDITIONS

Values determined in 20 m*M* sodium succinate buffer (pH 5.5), containing 0.5 m*M* sodium azide.

Nucleoside	λ_{max} *at pH 5.5*	*Molar absorptivities at*				*pK*		
		λ_{max}	*254 nm*	*260 nm*	*280 nm*	*Basic*	*Acidic*	*dRib*
dCyd	271	9000	7030	8160	5350	4.3	none	>13
5MedCyd	277	8500	5460	6200	7680	4.4	none	>13
dGuo	254	13270	13270	11380	6680	2.5	9.2	12.4
dThd	267	9650	7890	9420	4630	none	9.8	>13
dAdo	259	15000	14670	14400	1480	3.8	none	>13
Br^8Guo*	261	15600	15040	15530	9220	–	–	–
6MedAdo	265	15400	12170	14850	7220	none	none	>13

* Br^8Guo, 8-bromoguanosine.

Characterization of artifacts in the DNA sample

Detection of RNA contamination and deamination products of dA and dC in DNA hydrolyzates. HPLC analyses of a salmon sperm DNA hydrolyzate prior to and following RNase treatment are shown in Figs. 2 and 3. Elution systems B, also used in Table III, was used for these analyses (see Table I). Satisfactory separations of three of the four ribonucleosides (rC, rG and rA) from the deoxyribonucleosides were achieved under these chromatographic conditions. Although rU was not separated from dC, dU —a product of deamination of dC— was eluted between dC and m^5dC; it was not

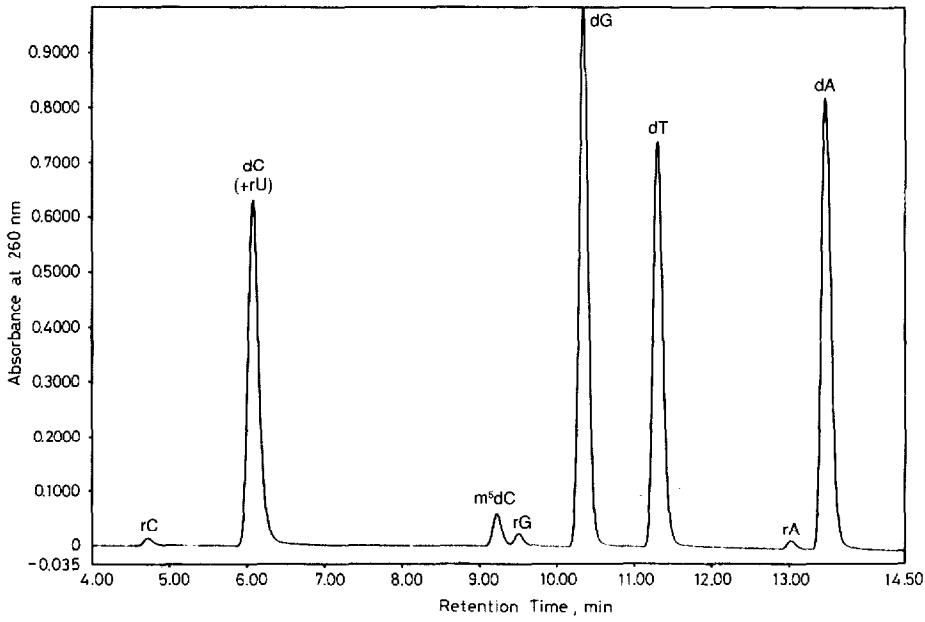

Fig. 2. Analysis of the DNA hydrolyzate, derived from salmon sperm *before* removal of the RNA contaminant. A 15-μg sample of the DNA was separated with elution system B (pH 5.5) (see Table I) at 24°C.

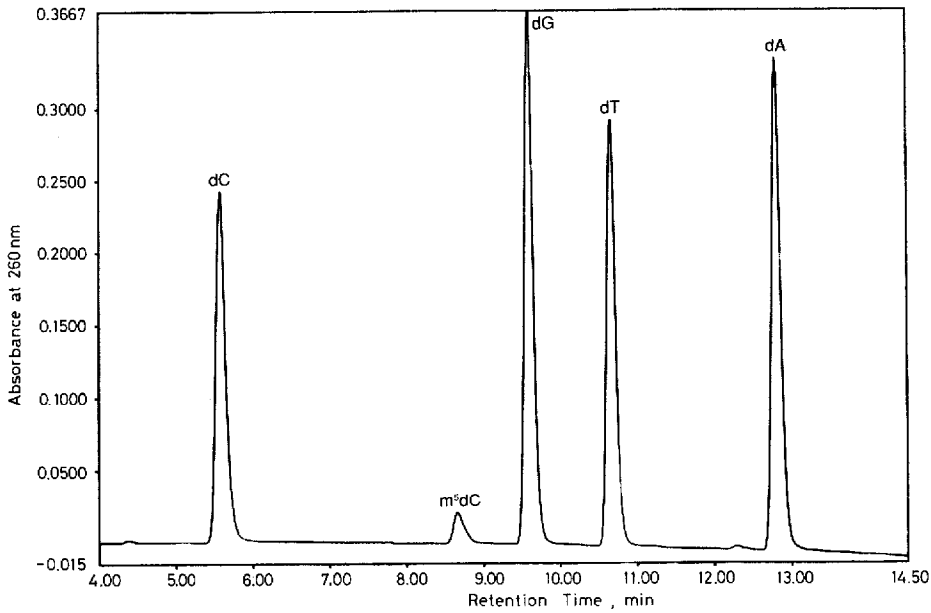

Fig. 3. Analysis of the DNA hydrolyzate, derived from salmon sperm *after* removal of the RNA contaminant by treatment with pancreatic ribonuclease, followed by dialysis. A 15-μg sample of the DNA was separated with elution system B (pH 5.5) (see Table I) at 24°C.

observed under our hydrolysis and analysis conditions. It is noted that rU and dU, which differ only by one hydroxy group, were resolved. This hydroxy group makes rU more polar and causes it to be eluted earlier than dU, but without distinction from dC, which lacks that hydroxyl function but has an additional amino group. Although rU is not separated from dC, its presence in the dC peak can be detected from the shape of the mixed peak, provided that no methanol is used in the starting eluent (results not shown). As the spectra of the two compounds differ significantly, the use of the diode-array detector aids in their identification. The DNA hydrolysis method used here showed no detectable deamination of dA resulting in dI, which was eluted between the rG and dG peaks. Deamination of dA is enhanced under any one of the following conditions: (a) presence of deaminase activity in the alkaline phosphatase, (b) phosphatase digestion carried out at pH > 8, (c) DNA concentration in amounts less than one A_{260} unit per 50 μl of the digest volume and (d) use of column temperatures above 40°C, especially with eluents of very low or very high pH, *e.g.*, in ion-exclusion chromatography[1,2]. Treatment of the DNA sample with RNase, followed by dialysis, effectively removes most RNA contaminants, as shown in Fig. 4.

Compositional analysis of DNA samples derived from two sources

The separation of the *E. coli* (strain B) DNA hydrolyzate is shown in Fig. 4. The minor component, m^6dA, was eluted after dA under our chromatographic conditions. The presence of the methyl group enhanced the hydrophobic interaction of m^6dA with the column matrix. [Although *E. coli* strain C is reported[21] to contain 1% m^5dC, no detectable amount of this residue in strain B was found in our studies.] Similarly, m^5dC

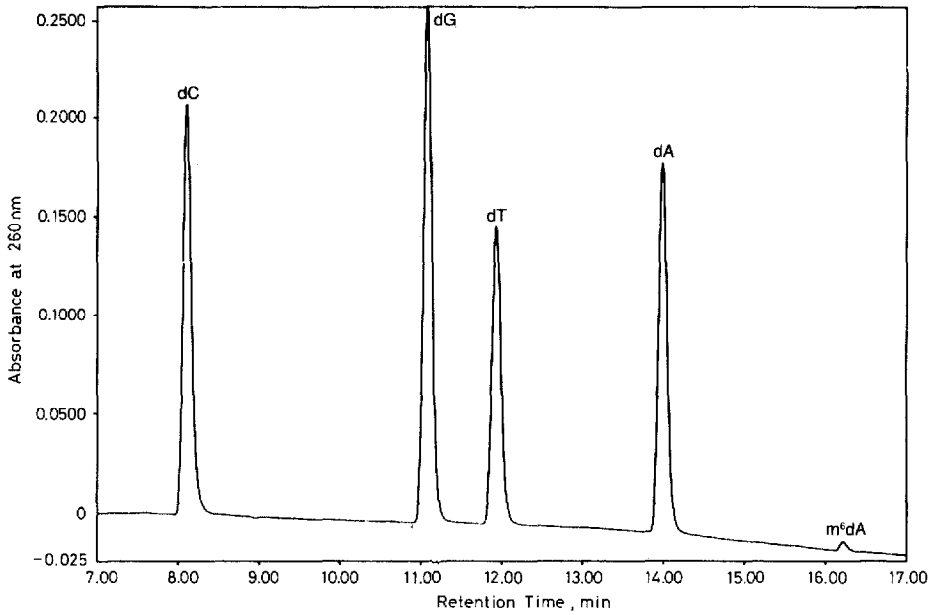

Fig. 4. Nucleoside composition analysis of a DNA sample derived from *E. coli* B. A 15-μg sample after digestion to deoxyribonucleosides was resolved with elution system C (pH 5.5) (see Table I) at 24°C.

was eluted after dC, owing to the extra methyl residue in its structure (see Fig. 3). The m^5dC peak can easily be contaminated with rG and dI contaminants, if present in appreciable amounts (see Fig. 2). Similarly, dC contents can decrease and dT contents increase at the same time by deamination of dC to dU, as dU is eluted with dT in ion-exclusion chromatography[1], although it was not observed in this study. We have recently observed, in crude DNA samples, cytosine deaminase activity responsible for the conversion of dC to dU.

Nucleoside compositions of DNA samples from two different sources, one from a prokaryote (*E. coli* B) and one from a eukaryote (salmon sperm), are shown in Table V. First, Vanyushin *et al.*[21] reported that this particular strain of *E. coli* contains 0.5

TABLE V

MOLAR NUCLEOSIDE COMPOSITION OF DNA SAMPLES

Values are derived from at least three analyses on less than one A_{260} unit (15 μg) of the DNA material.

DNA source	*Concentration (mole-%)**					
	dCyd	*m^5dCyd*	*dGuo*	*dThd*	*dAdo*	*m^6dAdo*
E. coli B	25.9	0.0	26.1	24.1	22.2	1.2
	(0.4)		(0.4)	(0.1)	(0.3)	(0.3)
Salmon sperm	20.3	1.61	22.4	27.9	27.8	0.0
	(0.1)	(0.03)	(0.3)	(0.2)	(0.2)	

* Standard deviation in parentheses.

mole-% of m^6dA. More recently, Kuo *et al.*[6] found that this strain contains 2.5 mole-% while our results indicate that this DNA contains only 1.2 ($\pm$ 0.3) mole-% of m^6dA. Their results[6] for this DNA also differ from the data in Table V for other nucleoside contents. They report (in mole-%) dC, 26.4; m^5dC, not detected; dG, 26.7; DT, 23.4; dA, 23.6; and m^6dA, 2.5. The total content of all five nucleosides from these data equals 102.6 mole-%, whereas our values total 100%. This discrepancy can partly explain the differences between the two results. Our results differ significantly only in dA and m^6dA contents, which we find *ca.* 1.4 mole-% less for each residue than those reported by Kuo *et al*[6]. In Table V, the sum of dA and m^6dA (23.4%) narrowly fails to match the dT contents (24.1%) in *E. coli* DNA. However, considering the standard deviation associated with each residue, the difference is considered to be within the experimental error. A comparison of each residue in the Watson–Crick base pairs (*i.e.*, A = T and G = C) indicates a good correlation, well within the standard deviation.

DNA analysis of salmon milt, shown in Table V, matches very closely such analyses reported by others. For example, *Kuo et al.*[6] reported (in 100 mole-%) this DNA to contain dC, 20.4; m^5dC, 1.57; dG, 22.7; dT, 27.4; dA, 27.8; and m^6dA, not detected. In general, our results show a very good correlation with the published data for the same DNA, thus indicating the reliability of the chromatography system.

Analysis of a chloroacetaldehyde-modified DNA sample, chemical probe of the DNA structure in solution

Chloroacetaldehyde reaction with DNA. The analysis of nucleoside composition is used here to probe chemically the DNA conformation in solution, using CAA as the modifying agent. The results in Fig. 5 indicate a typical analysis of DNA, modified under the conditions described. Reaction parameters, such as CAA concentration, time, and salt concentration, were systematically studied to optimize the reaction with methylated residues in DNA (results to be reported elsewhere). The CAA reaction with *E. coli* B DNA was followed by dialysis, enzymatic hydrolysis, separation and quantitation. The extent of the etheno modification for each residue was derived from the loss in the amount of nucleoside (unreacted DNA, control experiment), while using dT as a reference residue. dT is unable to react with CAA, as it lacks an exocyclic amine function. For example, we find that in Fig. 6, the percentages of dC, dG, dA, and m^6dA residues are modified to their intermediates (e'dN) plus products (edN): 38% (dC), 6.2% (dG), 46% (dA) and 64% (m^6dA).

CAA is known to react with ribo- and deoxyribonucleosides of A and C residues. The reaction product contains an ethene bridge between the exocyclic amine nitrogen and the endocyclic nitrogen[22,23]. We have studied the reaction of CAA with methyl A, C and G derivatives. The methylated counterparts, m^6dAdo and m^5dCyd, exhibit significantly increased reactivity, owing to enhanced nucleophilicity of the exocyclic amine, as opposed to the A and C residues[24]. In normal Watson–Crick base pairing the exocyclic and endocyclic amino groups are hydrogen-bonded and therefore inaccessible. However, the regions containing methyl groups perturb the conformation of the double helix. While methylation of dA in position N-6 aborts hydrogen bonding and possibility yields a "bubble", methylation of dC at the C-5 position can induce a conformational transition from B-DNA to Z-DNA under physiological conditions[25]. The concentration of CAA determines the reactivity of C and A residues in forming an exocyclic product, and the amount of cations (Na^+ or Mg^{2+}) in the

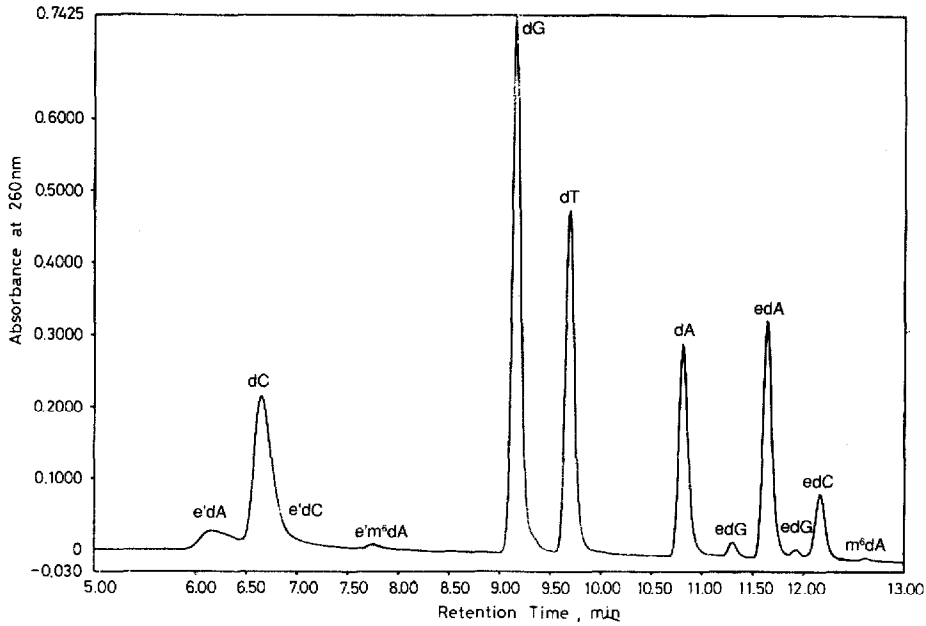

Fig. 5. Analysis of a DNA sample obtained after reaction with 0.1 *M* chloroacetaldehyde at pH 5.5 and 37°C for 1 h, followed by digestion to the nucleosides. Chromatography was carried out with elution system C (pH 5.5) (see Table I) at 24°C.

reaction mixture determines the conformation of the DNA (B-DNA *vs.* Z-DNA)[26], thus controlling the sites available for the reaction. An excessive concentration of CAA was used in the experiment described here (Fig. 6) in order to obtain a modification of the reactive residues. However, moderate reaction conditions were employed to achieve selective modification of the exposed residues in the DNA molecule.

The chromatographic system described here for the separation and analysis of DNA hydrolyzates compares favorably with the currently used methods described by others[6,9]. For example, our analyses are carried out on a single 25-cm C_{18} column as opposed to the 60-cm columns used by others. In addition, we can achieve satisfactory resolution of all five DNA components and of the artifacts at room temperature (*i.e.*, 24°C instead of 45°C) in a short analysis time (18 min instead of 40–60 min) while using only one low-ionic-strength gradient elution buffer which does not precipitate in methanol.

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