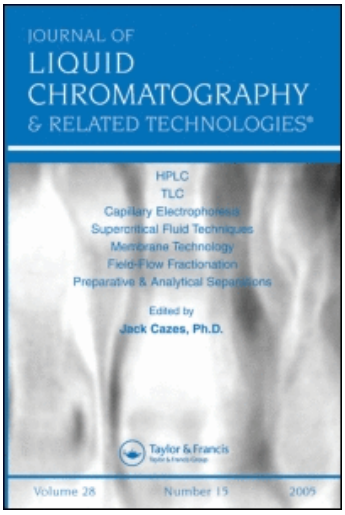

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AN HPLC METHOD FOR THE MEASUREMENT OF POLYAMINES AND LIPIDIC AMINES BINDING TO DNA

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ABSTRACT

An HPLC method is employed to measure the binding of polyamines and lipidic amines to DNA. DNA interaction is expressed as the % DNA peak size exclusion. The natural polyamine, spermine, has clearly demonstrated the highest ability for binding. Among the synthetic lipidic amines tested 1,2-hexadecanediamine has been found to be the most active.

INTRODUCTION

Natural polyamines such as spermine, spermidine, putrescine, are widely distributed in biological systems and have been shown to be critical for cell growth and carcinogenesis.[1,2] It is well known that the association of cationic polyamines with negatively charged DNA induces significant structural changes in DNA in cell-free systems.[3] Spermidine and spermine can cause DNA to condense and aggregate[4] and induce both B-to-Z and B-to-A transitions in certain DNA sequences.[5,6] The hypothesis that structural transitions and condensation in specific DNA sequences caused by polyamines may be related to nucleosome formation and the condensation of DNA into chromatin is gaining experimental support.

Measurements of polyamine-DNA interactions might yield therapeutically active compounds. The ability of organic molecules to bind to nucleic acids by various mechanisms such as intercalation and ionic interaction have previously been studied by a variety of techniques, including equilibrium dialysis,[7,8] absorption spectroscopy,[9,10] and fluorescence spectroscopy.[7,11] Additionally as the DNA or RNA interaction is best described as a dynamic equilibrium, an HPLC method based on DNA or RNA size exclusion was introduced.[12] This method was modified by several investigators and used for testing intercalating agents,[13] other small organic molecules,[14] and crude plant extracts.[15]

Although a variety of methods for HPLC analysis of polyamines have been reported,[16] the interaction of polyamines with DNA has not been studied by HPLC up to now. Natural polyamines (spermine, spermidine, putrescine, cadaverine) together with a number of lipidic diamines and amino alcohols have been studied by DNA size exclusion and the results are presented in this paper. The lipidic diamines and amino alcohols tested, have been previously synthesized[17,18] and preliminary screening has shown interesting biological activities (unpublished data).

MATERIALS AND METHODS

Materials

Calf thymus DNA, activated type XV was purchased from Sigma Chemical Co. Doxorubicin hydrochloride USP (adriamycin HCl) was gifted from Pharmacia. HPLC (MeOH) grade solvents were obtained from Lab Scan. Thrice-deionized water was obtained from a Millipore purification system.

Mobile phase was degassed with ultrasonic prior to use. All solvents and solutions for HPLC analysis were filtered through 0.22 μm Millipore membrane filters before injection. DNA and test samples were dissolved in deionized water.

Apparatus and Chromatography

A Hewlett-Packard (Palo Alto, CA, USA) model 1050 HPLC, isocratic pump and a Rheodyne injector Model 7125 fitted with a 20 μL loop were used. A line source detector at 254 nm was used in the validation experiments. The signals were recorded on a Hewlett-Packard Model HP3395 integrator. The column was a 4 x 250 mm, 5 μm octadecylsilane column (Lichrospher RP-18). The column was equilibrated with a H_2O : MeOH, 80 : 20 mixture. Test samples and DNA solutions were then introduced in a ratio 1 : 1 (v / v) into the sample loop (20 μL) without incubation. Flow rate was maintained at 1 mL/min and the free DNA eluted in approximately 1 min. After the appearance of DNA peak, the column was washed with MeOH (100 %) for 20 min to elute the sample mixture. DNA binding is expressed as a % DNA peak exclusion.

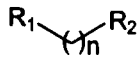
All tested compounds were measured at three different concentrations (0.25, 0.10, 0.05 mg/mL) and tested in triplicate.

RESULTS AND DISCUSSION

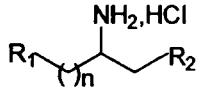
The structures of the compounds tested are shown in Figure 1. % DNA peak size exclusion are summarised in Table 1. An example of complete (100 %) peak exclusion is demonstrated in Figure 2.

All the compounds containing two or more amino groups (**1-6**, **9-11**), except compound **14**, exhibited a complete DNA binding (100 % DNA peak exclusion) at a concentration of 0.25 mg/mL (Table 1). Monovalent molecules (one aminogroup) such as hexadecanamine (**7**), the classical intercalating agent doxorubicin (**15**) and the aminoalcohols **8**, **12**, **13** demonstrated a weaker effect on DNA, ranging from 13 ± 2 % up to 75 ± 4 %.

At the concentration of 0.1 mg/mL the effect caused by the monovalent molecules has substantially decreased (0 - 19 ± 2 %), while divalent or polyvalent molecules retained their potency (85 ± 7 - 100 %). At even lower concentration (0.1 mg/mL) spermine (a tetravalent molecule) exhibited a



	n	R_1	R_2
1	2	NH_2,HCl	NH_2,HCl
2	3	NH_2,HCl	NH_2,HCl
3	4	NH_2,HCl	NH_2,HCl
4	5	NH_2,HCl	NH_2,HCl
5	3	NH_2,HCl	$NH(CH_2)_4NH_2$,2HCl
6	3	NH_2,HCl	$NH(CH_2)_4NH(CH_2)_3NH_2$,3HCl
7	16	H	NH_2,HCl
8	2	NH_2,HCl	OH



	n	R_1	R_2
9	14	H	NH_2,HCl
10	12	H	NH_2,HCl
11	14	H	CH_2NH_2,HCl
12	14	H	OH
13	16	H	OH
14	1	C_6H_5	NH_2,HCl

Figure 1. Structures of compounds tested.

complete interaction with DNA (100 %). The trivalent molecule spermidine (**5**) and the divalent molecules **1**, **2**, **3**, **4**, **9**, **10**, **11** showed weaker ability for binding (75 ± 9, 28 ± 2, 65 ± 7, 60 ± 4, 58 ± 5, 23 ± 3, 8 ± 3, 0 % respectively). Spermine showed a measurable peak exclusion (35 ± 3 %) even at the concentration of 0.01 mg/mL.

Table 1

Effect of Compounds Tested on the DNA Peak Size

	Compound	DNA Peak Exclusion[a,b] (%) [c]	[d]	[e]
1	1,2-ethanediamine dihydrochloride	28 ± 2	100	100
2	1,3-propanediamine dihydrochloride	65 ± 7	100	100
3	putrescine dihydrochloride	60 ± 4	100	100
4	cadaverine dihydrochloride	58 ± 5	100	100
5	spermidine trihydrochloride	75 ± 9	100	100
6	spermine tetrahydrochloride	100	100	100
7	hexadecanamine hydrochloride	0	0	13 ± 2
8	ethanolamine hydrochloride	0	19 ± 2	34 ±2
9	1,2-hexadecanediamine dihydrochloride	23 ± 3	100	100
10	1,2-tetradecanediamine dihydrochloride	8 ± 3	100	100
11	1,3-heptadecanediamine dihydrochloride	0	85 ± 7	100
12	2-amino-hexadecanol hydrochloride	0	10 ± 2	65 ± 7
13	2-amino-octadecanol hydrochloride	0	10 ± 3	75 ± 4
14	2-amino-3-phenyl-propanamine dihydrochloride	10 ± 2	15 ± 1	34 ± 2
15	doxorubicin hydrochloride	0	16 ± 4	47 ± 3

[a] Concentration of DNA 0.1 mg/mL.
[b] Mean ± standard deviation based on n=3.
[c] Concentration of compound tested 0.05 mg/mL.
[d] Concentration of compound tested 0.10 mg/mL.
[e] Concentration of compound tested 0.25 mg/mL.

It seems that the ability of binding is determined by the number of amino groups. The potency of the phenomenon, which has to be attributed to ionic interactions, is decreased when the number of amino groups decreases. These observations are in full agreement with recent NMR studies which showed that spermine binded to t-RNA more strongly than spermidine.[19]

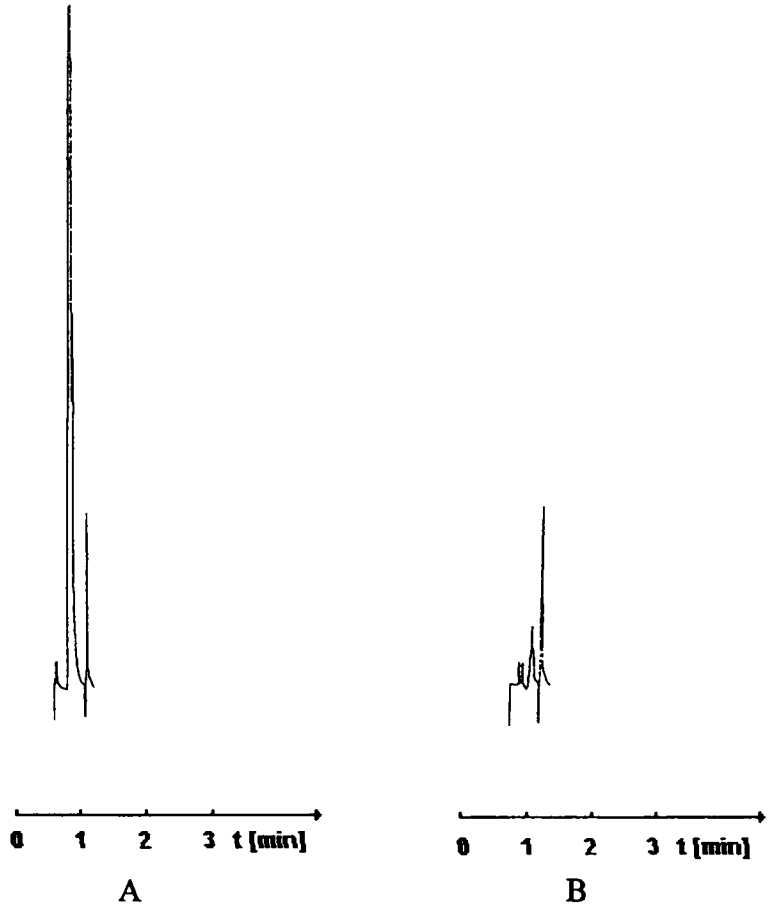


Figure 2. Chromatograms of DNA (0.1 mg/mL) (A) and DNA (0.1 mg/mL) + 1,3-heptadecanediamine dihydrochloride (0.25 mg/mL) (B). Chromatographic conditions: column, octadecylsilane Lichrospher RP 18 (4 x 250 mm, id 5 μm); eluent, water-methanol 80:20 (v/v); flow rate, 1.0 mL/min; injection volume, 20 μL; detector UV set at 254 nm; temperature, 25 °C

Among the divalent molecules, the presence of a long chain resulted in weaker effects, as concluded by comparison of compounds **9**, **10** to **1** and **11** to **2**. Comparing compounds **1-4**, the system of 1,3-diamino groups showed the best result indicating that this is the optimum distance between the two amino groups.

CONCLUSIONS

The binding of natural polyamines and other related synthetic cationic compounds to DNA was studied for the first time by an HPLC method. Compounds containing two or more amino groups exhibited stronger effect than the classical intercalator doxorubicin. The ratio DNA : ligand determines the potency of the observed peak exclusion. The described chromatographic method could serve as a valuable pre-screen antitumor *in vitro* assay, since it possesses simplicity, reproducibility, considerable reliability and it is of low cost.

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