J. Pharm. Pharmacol. 1988, 40: 809-811
Communicated May 24, 1988

Equilibrium binding of spermine and histamine to salmon sperm DNA and poly(dGdC)

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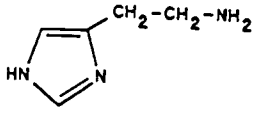
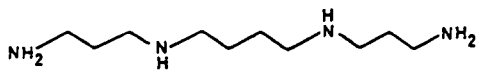
Abstract—Studies using the solute-enhanced phase partition technique demonstrate that the endogenous amines histamine and spermine bind to both salmon sperm DNA and poly(dGdC) in a markedly cooperative fashion. Both compounds exhibited DNA sequence-dependence effects in their mode of binding. The linear aliphatic spermine molecule binds more strongly than histamine to both DNA types.

Polyamines are believed to perform a variety of functions in-vivo (Morris & Marton 1981). Certain of these functions involve their interaction with DNA. For example, spermine (I) induces the formation of condensed forms of DNA in-vitro (Marx & Ruben 1983; Wilson & Bloomfield 1979; Widom & Baldwin 1980; Marx & Reynolds 1982) and is probably involved in the stabilization of condensed forms of DNA in-vivo (Morris & Marton 1981). Recent studies suggest that spermine may alter the structure of DNA (Wang et al 1979; Behe & Felsenfeld 1981; Chen et al 1984; Rich et al 1984; Thomas & Bloomfield 1985; Feuerstein et al 1986; Basu et al 1987). Studies by Scolnik et al (1984, 1985) suggest that the endogenous amine, histamine (I), interacts with DNA in a manner similar to that proposed by Abraham (1981) for the polyamines, probably resulting in the modulation of genomic expression of DNA. Thus, it is of interest to examine the equilibrium binding of both spermine and histamine to DNA. These two molecules represent two different classes of physiological amines: i.e. a linear aliphatic polyamine exemplified by spermine and spermidine, and an aralkylamine containing a heterocyclic amino functionality, such as histamine and tryptamine.



Histamine



Spermine

I

This present study focuses on equilibrium binding at low bound drug to DNA base pair ratios (i.e. low r). Previously, interesting phenomena, such as cooperativity and site selectivity have been observed at low r (Winkle & Krugh 1981). The phase partition method was chosen for determining the binding; this method has been used previously to obtain low r data (Waring et al 1975; Davanloo & Crothers 1976; Hogan et al 1979; Winkle & Krugh 1981; Graves & Krugh 1983). Since both spermine and histamine are predominantly cationic at physiological pH, the current studies employed the solute-enhanced phase partition technique described by Krugh et al (1981). Both histamine and spermine were examined for their ability to bind cooperatively to salmon sperm DNA and to poly(dGdC). The results of these binding studies are presented below.

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Materials and methods

Materials. [^{3}H(G)]Histamine (specific activity 7·8 Ci mmol^{-1}) and [terminal methylenes-^{3}H(N)]spermine hydrochloride (specific activity 30 Ci mmol^{-1}) were purchased from ICN Radiochemicals, Irvine, CA. Histamine hydrochloride, spermine hydrochloride and salmon sperm DNA were obtained from the Sigma Chemical Co., St. Louis, MO. Poly(dGdC) was obtained from PL Biochemicals, Belmont, CA. Nonan-1-ol and sodium tetraphenylboron (gold label) were purchased from the Aldrich Chemical Co., Milwaukee, WI. [^{3}H]Histamine and carrier histamine were combined to produce an appropriate specific activity between 7×10^{-10} and 7×10^{-11} counts min^{-1} mmol^{-1}. [^{3}H]Spermine and carrier spermine were similarly combined producing specific activities between 6×10^{-10} and 5×10^{-11} counts min^{-1} mmol^{-1}. Salmon sperm DNA was prepared for use according to the method described by Muller & Crothers (1975). Concentrations of the DNA solutions were determined using the following ε (per base): salmon sperm DNA, $\varepsilon_{260} = 6{\cdot}6 \times 10^3$ M^{-1}cm^{-1}; poly(dGdC), $\varepsilon_{254} = 7{\cdot}1 \times 10^3$ M^{-1}cm^{-1}.

Solvent partition equilibrium binding. The equilibrium binding of spermine and histamine to salmon sperm DNA or to poly(dGdC) was investigated using the solute-enhanced partition analysis method described by Krugh et al (1981). The organic phase was nonan-1-ol containing 5 mM sodium tetraphenylboron, and the aqueous phase was sodium phosphate buffer (10 mM sodium phosphate, 1 mM sodium EDTA, pH = 7·0) containing 0·1 M sodium chloride. Solutions of spermine, histamine, salmon sperm DNA and poly(dGdC) were prepared in this buffer. DNA concentrations in the samples ranged from 5×10^{-6} to 1×10^{-4} M. Histamine and spermine total concentrations in the samples ranged from 1×10^{-6} M to 5×10^{-5} M. Either 100 or 200 μL of aqueous solutions of histamine or spermine were combined with the appropriate DNA preparation, and the resulting mixtures were mixed with 100 or 200 μL of 5 mM sodium tetraphenylboron in nonan-1-ol. Samples were shaken for two days at 23°C on a New Brunswick wrist-action shaker and then centrifuged at 5000 rev min^{-1} to separate the phases. After physically separating the aqueous and organic layers of the samples, 20 or 30 μL aliquots were removed, dissolved in a mixture of 1 mL of phosphate buffer and 4 mL of Aqualite counting fluid (Baker) and the [^{3}H]content in the samples was determined by scintillation spectrometry on a Beckman Model L5 1800 Scintillation Spectrometer. To determine the partition coefficients for histamine and spermine, 100 or 200 μL aqueous samples of the appropriate tritiated amines containing no DNA were shaken for two days with the organic solution. The [^{3}H]-content in the two phases was determined as previously described. The partition coefficients were: spermine, partition coefficient (aqueous/organic) = 9·20; histamine, partition coefficient (aqueous/organic) = 0·26. Using the counts min^{-1} values

from the aqueous and organic phases and the appropriate partition coefficient, the concentrations of DNA-bound and free ligand were calculated for the two amines. Data were then presented as Scatchard plots (Scatchard 1949).

Results and discussion

The solute-enhanced phase partition method described by Krugh et al (1981) was employed to collect equilibrium binding data for the binding of spermine and histamine to salmon sperm DNA and to poly(dGdC). Phase partition methods have been used previously to study the binding of a variety of molecules to DNA, e.g. distamycin (Davanloo & Crothers 1976; Hogan et al 1979), actinomycin D and *N*-hydroxy-*N*-acetyl-2-aminofluorene (Winkle & Krugh 1981), daunorubicin (Graves & Krugh 1983), ethidium (Winkle et al 1982) and several other drugs (e.g. Waring et al 1975). The solute-enhanced phase partition method incorporates an organic-soluble anion to enhance the partitioning of positively charged drugs between non-polar organic solvents and an aqueous buffer solution. Krugh et al (1981) demonstrated the effectiveness of this method in their study of the binding of daunorubicin to calf thymus DNA. However, when this method is used, there is the possibility of the formation of drug-ion complexes. Such complex formation can occur to a significant degree when the drug and the partitioning ion are both "planar" polycyclic molecules, e.g. ethidium ion and tetraphenyl boron. Significant drug-ion complexation will complicate drug-DNA binding analysis. To check whether the presence of tetraphenylboron would affect the binding of either spermine or histamine to the DNAs used in this study, preliminary binding studies were conducted with 1, 3 and 5 mM sodium tetraphenylboron in the nonan-1-ol. The data from the analysis of all three organic phases were identical. Further, the partition coefficients for both spermine and histamine remained constant over the drug concentration ranges used, suggesting the absence of any strong drug-drug or drug-tetraphenylboron interactions.

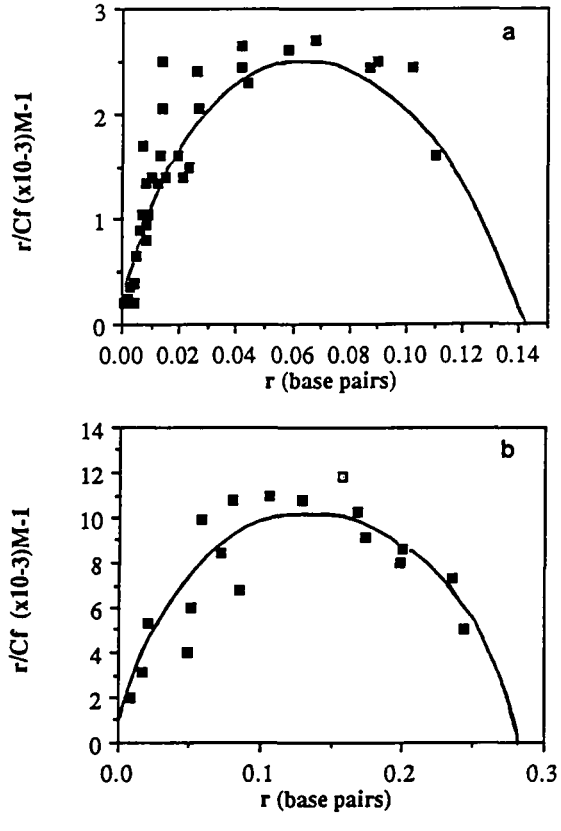

FIG. 1. Scatchard plots of equilibrium binding data for histamine with (a) salmon sperm DNA, and (b) poly(dGdC). Both [DNA] and [poly(dGdC)] ranged between 5×10^{-6} and 1×10^{-4}M. [Histamine] ranged between 1×10^{-6} and 5×10^{-5}M. T = 23°C, [NaCl] = 0·1 M. The curves represent the McGhee-von Hippel modelling.

Scatchard plots of the data obtained from the equilibrium binding studies of spermine and histamine to salmon sperm DNA and poly(dGdC) are presented in Figs 1, 2. The data show

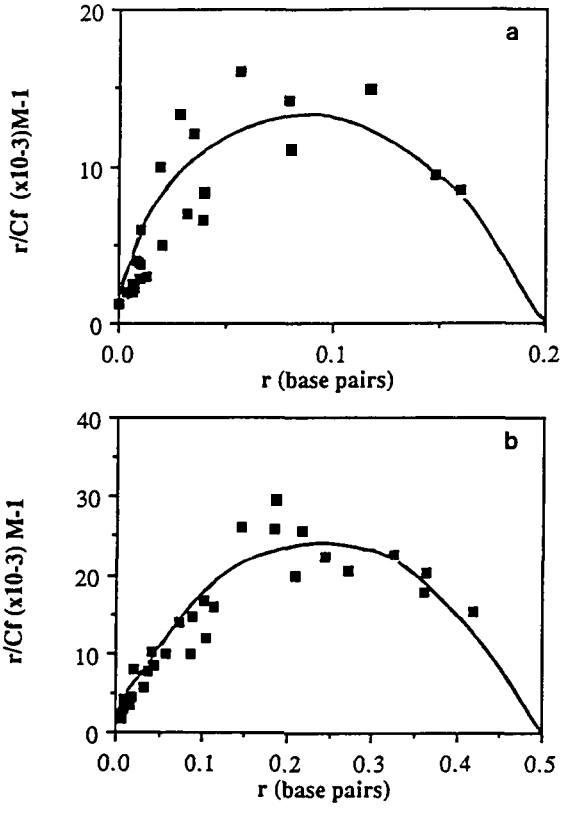

FIG. 2. Scatchard plots of equilibrium binding data for spermine with (a) salmon sperm DNA, and (b) poly(dGdC). Both [DNA] and [poly(dGdC)] ranged between 5×10^{-6} and 1×10^{-4}M. [Spermine] ranged between 1×10^{-6} and 5×10^{-5}M. T = 23°C, [NaCl] = 0·1 M. The curves represent the McGhee-von Hippel modelling.

clearly that histamine and spermine bind cooperatively to both DNAs, and that histamine binds more weakly than spermine. The data suggest that, for both histamine and spermine, there is a greater binding affinity for poly(dGdC) relative to salmon sperm DNA if the curve maxima (maximum r/c_F) are compared. However, with both drugs, intrinsic binding constants for the binding to poly(dGdC) and to salmon sperm DNA, as extrapolated from the Scatchard plots, appear to be approximately the same. Binding saturation values extrapolated from the plots suggest that, at saturation, there are more base pairs per bound drug for binding to the heterogeneous salmon sperm DNA than there are for binding to the homogeneous poly(dGdC). With both types of DNA, the Scatchard plots for both spermine and histamine display initially increasing slopes, which is indicative of positive cooperativity in the binding.

McGhee-von Hippel binding isotherms were constructed to visual fits for the four sets of data (McGhee & von Hippel 1974). The McGhee-von Hippel parameters for the isotherms are given in Table 1. Comparisons of the parameters for the four systems afforded the same conclusions as obtained by the previous examination of the data.

The McGhee-von Hippel fitted K's and the intrinsic K's

Table 1. McGhee-von Hippel modelling parameters for the equilibrium binding of histamine and spermine to salmon sperm DNA and Poly(dGdC).

	Histamine		
	K[1]	N[2]	w[3]
Salmon sperm DNA	300	7	2000
Poly(dGdC)	500	3·5	6000
	Spermine		
Salmon sperm DNA	1500	5	1500
Poly(dGdC)	2200	2	1000

[1] K is the intrinsic binding constant.
[2] N is the number of base pairs per bound ligand at saturation of binding.
[3] w is the cooperativity factor.

extrapolated from the Scatchard plots, are in good agreement, and suggest that with both DNAs examined, histamine binds more weakly than spermine in the two sets of experiments. This might be due to the decreased cationic nature of histamine at pH 7·0 relative to spermine (Paiva et al 1970; Behe & Felsenfeld 1981). Such an interaction may play a significant role in the DNA binding mechanism, i.e. an electrostatic interaction between the cationic species of the drug and the ionized phosphate residues of the polynucleotide may be operating.

The intrinsic K's, either from extrapolation or from McGhee-von Hippel modelling, for the binding of histamines to salmon sperm DNA and to poly(dGdC) are nearly equal. Likewise, the K's obtained in the same way for the binding of spermine to these two DNAs are nearly equal. However, the curve shapes conveyed by the data sets for both drugs are different for the two DNAs. For both drugs, the parameters w and N are different for the two DNAs. This suggests that, with both spermine and histamine, there is some sequence-dependence in the binding, and might indicate that the binding of both of these amines is not solely dependent upon an electrostatic interaction with the phosphate residues. In this respect, theoretical studies have suggested that spermine may bind preferentially into either the major or minor grove of DNA—depending upon the DNA sequence in the region of binding (Feuerstein et al 1986; Zakrzewska & Pullman 1986). Recent experimental work also suggests the possibility of sequence specificity in spermine binding (Basu et al 1987).

Interestingly, the present study shows that both spermine *and* histamine bind cooperatively to salmon sperm DNA. Cooperativity in the binding could arise from a drug molecule inducing a structural change in the DNA to afford a second DNA structure which is more favourable for subsequent drug binding. In this respect, spermine has previously been shown to alter DNA structure in-vivo by converting it to condensed froms (Marx & Ruben 1983; Wilson & Bloomfield 1979; Widom & Baldwin 1980; Marx & Reynolds 1982). Recent experimental work (Basu et al 1987) and theoretical studies (Feuerstein et al 1986) suggest that spermine may alter the conformation of specific sequences of DNA.

In conclusion, we have presented data on the equilibrium binding of spermine and histamine to salmon sperm DNA and to poly(dGdC). Each molecule binds in a markedly cooperative manner to salmon sperm DNA and to poly(dGdC). Both exhibit DNA sequence effects in their binding characteristics. The linear aliphatic polyamine, spermine, binds more strongly than does the aralkylamine, histamine.

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