Quantitative Characterization of the Binding of Histamine by Heparin†

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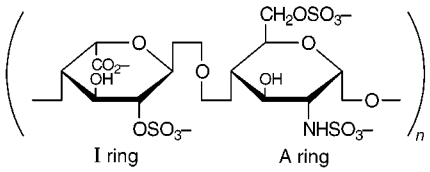
Received March 19, 1998; Revised Manuscript Received August 4, 1998

ABSTRACT: Tissue histamine is stored in mast cell granules, presumably as a histamine−heparin complex. Heparin is a polyelectrolyte, with a fraction of its anionic charge neutralized by condensed counterions. The interaction of heparin with histamine in aqueous solution was quantitatively characterized by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Binding constants were determined from chemical shift−pH titration data for the C2H proton of the imidazolium ring for a wide range of histamine, heparin, and Na^+ concentrations. The results indicate a binding stoichiometry of 1 histamine per heparin disaccharide repeat unit. The binding is electrostatic, as indicated by the strong dependence of the binding constant on Na^+ concentration. From an analysis of the binding constants using the counterion condensation theory of polyelectrolytes, it was determined that the binding of H_2A^{2+} results in displacement of 1.72 Na^+ ions from the counterion condensation volume of heparin and that H_2A^{2+} makes 2 ionic interactions with heparin. The displacement of Na^+ from the counterion condensation volume of heparin by H_2A^{2+} was also studied by ^{23}Na NMR. From ^{23}Na spin−lattice relaxation time data, it was determined directly that 1.78 Na^+ ions are displaced per H_2A^{2+} bound by heparin. The results are discussed in terms of the ion exchange process which takes place when histamine is released by mast cells.

Tissue histamine is localized in the specialized membrane-enclosed secretory granules of mast cells (*1*). The granules, which account for ∼50% of the total mast cell volume, are comprised primarily of heparin and basic protein (*2*). For example, heparin accounts for ca. 30% of the dry weight of isolated granules from rat peritoneal mast cells, and basic protein accounts for ca. 35% (*3*). The nature of the mechanism by which histamine is stored in granules has not been established; however, evidence suggests that heparin provides the histamine binding sites (*2*, *4*, *5*).

Heparin is a highly negatively charged polysaccharide, the structure of which is largely accounted for by repeating sequences of the trisulfated disaccharide {(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glycopyranose):



Because of its high negative charge density, heparin is a polyelectrolyte; i.e., a fraction of its negative charge is neutralized by bound counterions (*6*−*14*). Counterions can bind to polyelectrolytes by site-specific electrostatic interactions and by territorial (or delocalized) electrostatic interactions (*15*, *16*).

At the pH in mast cell granules (5.2−6.0) (*17*, *18*), histamine is a diprotonated dication. In a previous study, we found that diprotonated histamine binds to heparin in aqueous solution and that, at pH 5.2−6.0, the binding is site-specific, with the imidazolium group located in a binding pocket with a high affinity for the imidazolium ring (*5*).

To further characterize the heparin−histamine binding interaction, we have measured binding constants over a range of heparin, histamine, and sodium concentrations. The results are analyzed using counterion condensation theory to determine ionic and nonionic contributions to the binding (*15*, *16*, *19*).

The results are also of interest with respect to the release of histamine from mast cells. Release of histamine is a multistep process, which includes cell membrane−granule membrane fusion followed by exposure of the granule contents to extracellular fluid (*3*). Histamine is then released from the histamine−granule complex by an ion exchange process involving sodium ions of the extracellular fluid (*3*, *20*−*22*). In the present study, the sodium/histamine exchange has been characterized by analyzing the binding constants in terms of counterion condensation theory and more directly by ^{23}Na nuclear magnetic resonance (NMR)[1] spectroscopy.

MATERIALS AND METHODS

Beef lung heparin (sodium salt) (153 USP units/mg, molecular mass of 16−17 kDa) and histamine hydrochloride were obtained from Sigma Chemical Co. The heparin had

† This research was supported in part by National Institutes of Health Grants AI 24216 and HL 56588 and by the University of California, Riverside, Committee on Research.

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[1] Abbreviations: NMR, nuclear magnetic resonance; T_1, spin−lattice relaxation time; CPMG, Carr−Purcell−Meiboom−Gill; H_2A^{2+}, diprotonated histamine.

S0006-2960(98)00625-4 CCC: $15.00
Published on Web 09/17/1998

an average anionic charge of 3.6 per disaccharide repeat unit, as determined by conductometric titration (*14*, *23*).

^{1}H NMR measurements were made on 99% H_2O/1% D_2O solutions containing histamine or heparin plus histamine. Heparin concentrations are expressed in terms of the concentration of the disaccharide repeat unit unless indicated otherwise. ^{1}H NMR spectra were measured at 400 and 500 MHz and 25 °C with Varian XL-400 and VXR-500S NMR spectrometers. The H_2O resonance was suppressed by selective presaturation for 1−2 s before application of the nonselective observation pulse. ^{1}H chemical shifts were measured relative to the resonance for the methyl protons of internal *tert*-butyl alcohol (δ = 1.2365 ppm) or 2,2-dimethyl-2-silapentane-5-sulfonate (DSS, δ = 0.0 ppm).

^{23}Na NMR spectra were measured at 52.9 MHz and 25 °C with a Varian XL-200 spectrometer. Samples were contained in 10 mm tubes. ^{23}Na spin−lattice (T_1) and spin−spin (T_2) relaxation times were measured with inversion−recovery and Carr−Purcell−Meiboom−Gill (CPMG) pulse sequences, respectively. Relaxation delays of at least $20T_1$ were used in all relaxation time measurements, and spectra were measured at 10−20 different longitudinal or transverse relaxation periods in the T_1 and T_2 determinations, respectively. T_1 values were determined by a three-parameter (I_o, A, and T_1) fit of inversion−recovery data to the equation:

$$I_t = I_o(1 - Ae^{-t/T_1})$$

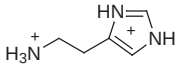
T_2 values were determined by a two-parameter (I_o and T_2) fit to the equation:

$$I_t = I_o e^{-t/T_2}$$

T_1 and T_2 were found to be equal, indicating $\omega\tau_c \ll 1$ (the extreme narrowing condition), where ω and τ_c are the ^{23}Na NMR frequency and correlation time, respectively (*24*).

RESULTS

Determination of Binding Constants from Histamine Chemical Shift Data. The chemical shifts of the carbon-bonded protons of histamine:



change over the pH regions where the imidazolium and ammonium groups are titrated, as illustrated by the chemical shift−pH titration curves in Figure 1. The binding of histamine by heparin causes a displacement of the chemical shift−pH titration curves for histamine to higher pH (Figure 2) (*5*). Binding constants for the interaction of histamine with heparin were determined from chemical shift−pH titration curves for histamine in solution with heparin. Because acid dissociation constants for histamine were needed as a function of NaCl concentration for determination of the binding constants, acid dissociation constants were first determined from chemical shift−pH titration data for free histamine.

The relative magnitudes of the chemical shift changes in Figure 1 for the C2H and C4H protons of the imidazolium ring and for the CH_2 protons adjacent to the imidazolium ring (CH_2a) and the ammonium group (CH_2b) indicate that

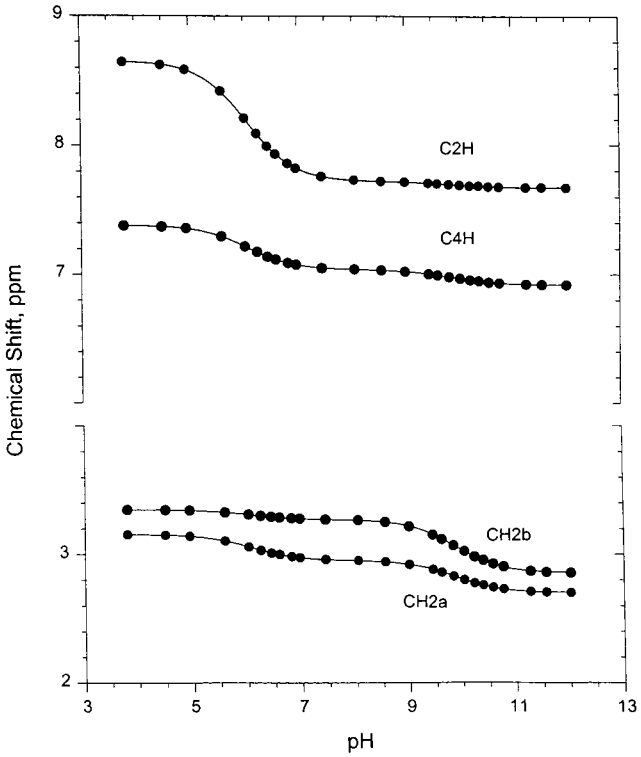


FIGURE 1: Chemical shift−pH titration curves for the carbon-bonded protons of histamine. Resonance assignments are given in the text. [Histamine] = 0.001 M; $[Na^+]_{total}$ = 0.050 M. The lines through the points are predicted by the simultaneous fit of all the data to a diprotic acid model.

titration of the H_2A^{2+} form of histamine takes place in a stepwise fashion and that the imidazolium ring is titrated first. The procedure used to determine acid dissociation constants involved fitting chemical shift−pH titration data of the type shown in Figure 1 to a diprotic acid model:

$$H_2A^{2+} + H_2O \rightleftharpoons H_3O^+ + HA^+; K_{A1} = \frac{[H_3O^+][HA^+]}{[H_2A^{2+}]} \quad (1)$$

$$HA^+ + H_2O \rightleftharpoons H_3O^+ + A; K_{A2} = \frac{[H_3O^+][A]}{[HA^+]} \quad (2)$$

Thus, K_{A1} and K_{A2} are acid dissociation constants for the imidazolium and ammonium groups, respectively. The chemical shift−pH titration data were fit to eq 3:

$$\delta_o = f_{H_2A}\delta_{H_2A} + f_{HA}\delta_{HA} + f_A\delta_A \quad (3)$$

where f represents the fractional concentration and δ the chemical shift of the form indicated. The fractional concentrations are given by eqs 4−6:

$$f_{H_2A} = [H^+]/D \quad (4)$$

$$f_{HA} = K_{A1}[H+]/D \quad (5)$$

$$f_A = K_{A1}K_{A2}/D \quad (6)$$

where $D = [H^+]^2 + K_{A1}[H^+] + K_{A1}K_{A2}$. The values determined for K_{A1} and K_{A2} are reported in Table 1. The lines through the points in Figure 1 are theoretical curves

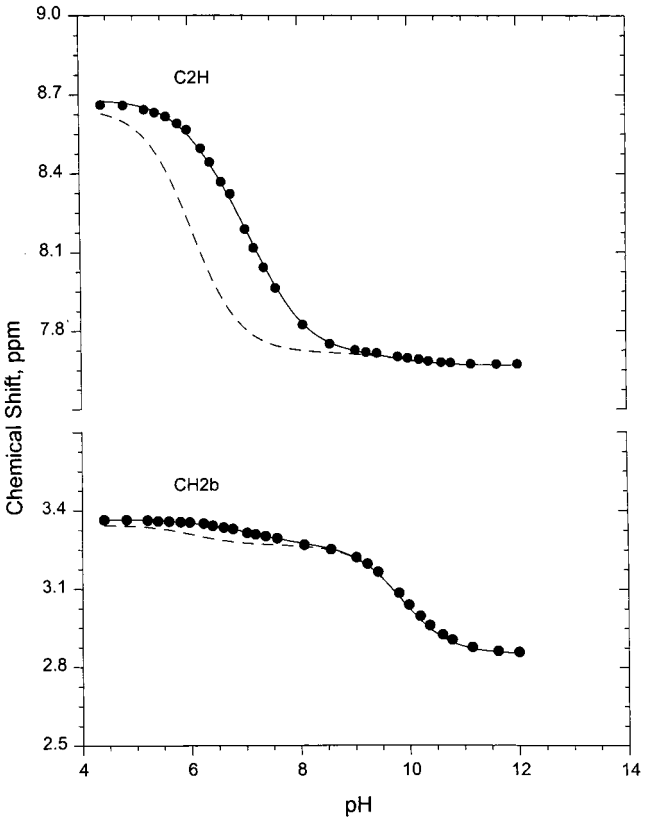


FIGURE 2: Chemical shift−pH titration data for the C2H and CH_2b protons of histamine (0.001 M) in solution with 0.001 M heparin disaccharide repeat unit. The total sodium concentration was 0.013 M. The solid lines through the experimental points are the chemical shift−pH titration curves predicted by the nonlinear least-squares fit of the data to the histamine−disaccharide binding model. The dashed lines are the chemical shift−pH titration curves predicted for the C2H and CH_2b protons of free histamine.

Table 1: Acid Dissociation Constants for Histamine as a Function of Sodium Chloride Concentration[a]

[NaCl], M	pK_{A1}	pK_{A2}
0.05	6.07 ± 0.02	9.91 ± 0.03
0.10	6.11 ± 0.03	9.93 ± 0.02
0.15	6.17 ± 0.02	9.93 ± 0.04
0.20	6.18 ± 0.04	9.96 ± 0.03
0.25	6.21 ± 0.03	9.95 ± 0.05
0.30	6.24 ± 0.02	9.95 ± 0.02
0.35	6.24 ± 0.05	9.99 ± 0.04
0.40	6.23 ± 0.01	9.97 ± 0.01
0.45	6.25 ± 0.04	10.00 ± 0.03

[a] 25 °C.

predicted by the acid dissociation constants obtained from the simultaneous fit of all the chemical shift−pH titration data in Figure 1 to the diprotic acid model using nonlinear least-squares methods.

The chemical shift−pH titration curves for histamine in solution with heparin are shifted to higher pH, as illustrated by the chemical shift data in Figure 2 for the C2H and CH_2b protons of histamine. The dashed curves in Figure 2 represent the chemical shift−pH behavior calculated for the C2H and CH_2b protons of free histamine using pK_A values determined above for histamine; the data points are for 1.00 mM histamine in solution with 1.00 mM heparin disaccharide repeat unit and a total Na^+ concentration of 0.013 M. Due to the binding of protonated histamine by heparin, the curves

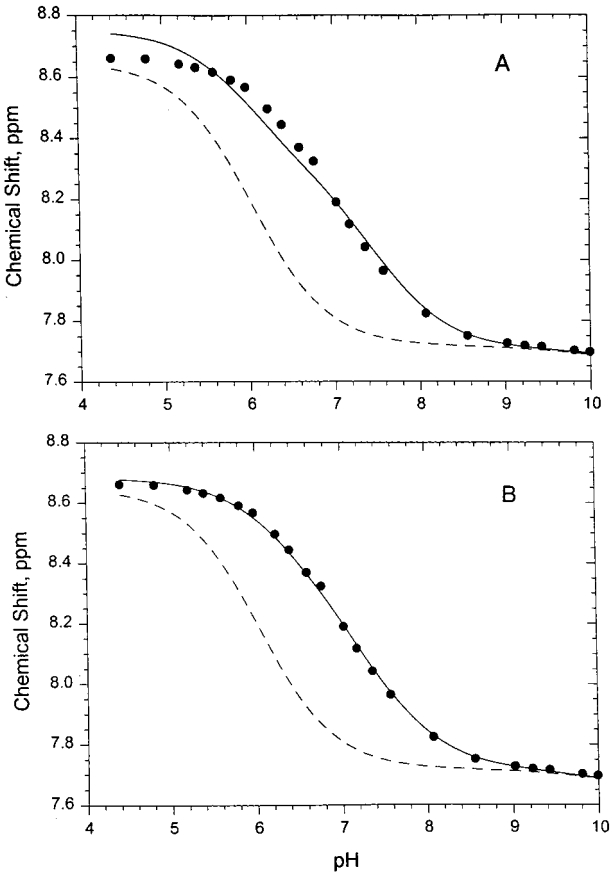


FIGURE 3: Chemical shift−pH titration data for the C2H proton of histamine (0.001 M) in solution with 0.001 M heparin disaccharide repeat unit. The total sodium concentration was 0.013 M. The solid lines in (A) and (B) are the chemical shift−pH titration curves predicted by the nonlinear least-squares fit of the data to (A) the histamine−tetrasaccharide binding model and (B) the histamine−disaccharide binding model, respectively. The dashed lines are the predicted chemical shift−pH titration curves of free histamine.

for both the C2H and the CH_2b protons are shifted over the pH region where the imidazolium group is titrated. Binding constants for the interaction of histamine with heparin were determined using chemical shift−pH titration data of the type shown in Figure 2. Initial attempts to determine binding constants by fitting the chemical shift−pH titration data to a model in which a tetrasaccharide unit was the combining unit were unsuccessful; a typical fit for a tetrasaccharide combining unit is illustrated by the results in Figure 3A. The data could be successfully fit to a model in which the heparin disaccharide repeat unit is the combining unit, as illustrated by the results in Figure 3B. A 1:1 histamine−heparin disaccharide stoichiometry has also been determined by equilibrium dialysis for histamine−heparin binding (25).

The model is defined by eqs 1, 2, and 7:

$$Hep^{x-} + H_2A^{2+} \rightleftharpoons Hep{-}H_2A;\ K_{Bd} = \frac{[Hep - H_2A]}{[Hep^{x-}][H_2A^{2+}]} \quad (7)$$

where $[Hep^{x-}]$ represents the concentration of the heparin disaccharide repeat unit and K_{Bd} is the binding constant for binding of histamine to the disaccharide combining unit. Binding constants were determined by a nonlinear least-squares fit of the chemical shift−pH titration data to the

deprotonation/binding model. The procedure involved fitting the data to the model equation:

$$\delta_o = f_{H_2A}\delta_{H_2A} + f_{HA}\delta_{HA} + f_A\delta_A + f_{HepH_2A}\delta_{HepH_2A} \quad (8)$$

where f and δ are defined as for eq 3. The fractional concentrations were calculated for each experimental point using the mass balance equations for histamine and heparin, the values determined above for the acid dissociation constants, and the binding constants, which were optimized by the nonlinear least-squares fitting procedure. The values determined above for δ_{H2A}, δ_{HA}, and δ_A were used in the fitting procedure.

A value of 15 020 ± 300 was obtained for K_{Bd} from the simultaneous fit of both sets of chemical shift data in Figure 2 to the model equations. The solid curves through the experimental points are theoretical chemical shift−pH curves predicted by the parameters determined from the nonlinear least-squares fit. Binding constants were also determined by fitting just the C2H data to eq 8. The fit is shown in Figure 3B. The value obtained for the binding constant (K_{Bd} = 15 050 ± 460) is essentially identical to that obtained by fitting simultaneously both sets of chemical shift data. Thus, the remainder of the calculations were done using only C2H chemical shift data.

Binding constants were determined over a range of histamine, heparin, and sodium concentrations. To illustrate, representative chemical shift−pH titration data are presented in Figure 4. In Figure 4A, the open circles are data for the titration of 0.001 M histamine in solution with a total Na^+ concentration of 0.35 M; the solid squares, open squares, and solid circles are data for 0.001 M histamine plus 0.010, 0.050, and 0.100 M heparin, respectively, in 0.35−0.40 M Na^+ solution. As the heparin:histamine ratio is increased, the chemical shift−pH titration curves are displaced to higher pH, indicating increased binding of histamine by heparin. In Figure 4B, the open circles are for 0.001 M histamine in solution with a total Na^+ concentration of 0.20 M; the solid squares, open squares, and solid circles are for 0.001 M histamine in solution with 0.050 M heparin and total Na^+ concentrations of 0.575, 0.375, and 0.175 M, respectively. As the concentration of Na^+ decreases, the displacement of the chemical shift−pH titration curves increases, indicating more binding of histamine by heparin as the concentration of Na^+ decreases. Binding constants determined by fitting these and other chemical shift−pH titration data are reported in Table 2. The smooth curves through the data points in Figure 4 are predicted by the nonlinear least-squares fits.

Determination of the Number of Na^+ Displaced by Histamine by ^{23}Na NMR. Displacement of Na^+ from the counterion condensation volume which surrounds heparin by histamine was quantitatively characterized by measuring ^{23}Na spin−lattice (T_1) relaxation times as a function of solution conditions. ^{23}Na spin−lattice relaxation times, which depend on the magnitudes and rates of fluctuations of local electric field gradients at the ^{23}Na nucleus, decrease upon binding to heparin (*14*, *24*, *26*). ^{23}Na T_1 data could be fit by single-exponential functions, which indicates that exchange of Na^+ between its free and bound forms is fast (*24*), in which case the observed T_1 value can be treated in terms of a two-site (free and bound) exchange-averaged model (*24*, *26*, *27*).

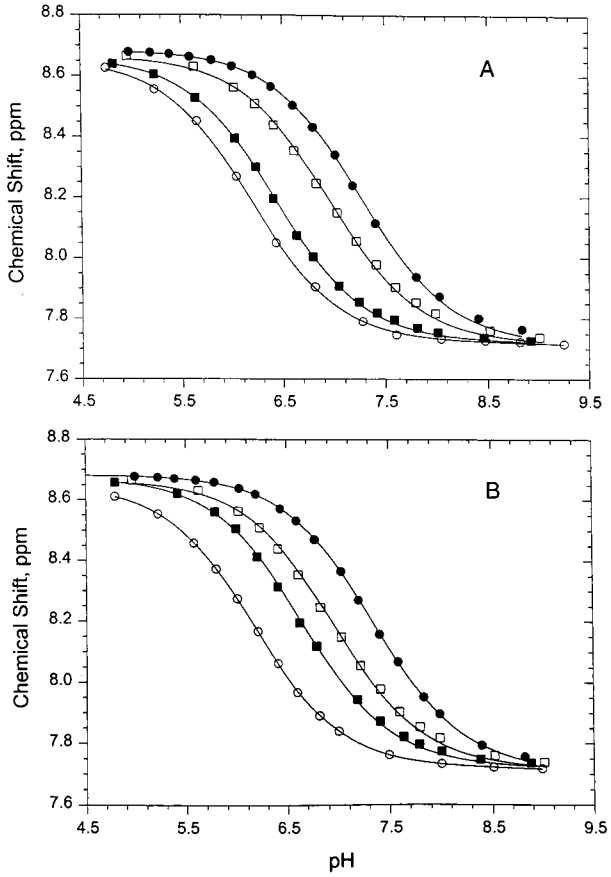


FIGURE 4: Chemical shift−pH titration data for the C2H protons of histamine in solution with heparin. (A) 0.001 M histamine, $[Na^+]_{total}$ = 0.35 M (○); 0.001 M histamine plus 0.010 M heparin, $[Na^+]_{total}$ = 0.395 M (■); 0.001 M histamine plus 0.050 M heparin, $[Na^+]_{total}$ = 0.375 M (□); 0.001 M histamine plus 0.100 M heparin, $[Na^+]_{total}$ = 0.35 M (●). (B) 0.001 M histamine, $[Na^+]_{total}$ = 0.20 M (○); 0.001 M histamine plus 0.050 M heparin, $[Na^+]_{total}$ = 0.575 M (■); 0.001 M histamine plus 0.050 M heparin, $[Na^+]_{total}$ = 0.375 M (□); and 0.001 M histamine plus 0.050 M heparin, $[Na^+]_{total}$ = 0.175 M (●). The solid lines through the experimental data are theoretical curves predicted by the nonlinear least-squares fits.

The exchange-averaged T_1s are population-weighted averages of the T_1s for the free and bound forms:

$$\frac{1}{T_{1o}} = \frac{P_f}{T_{1f}} + \frac{P_b}{T_{1b}} \quad (9)$$

where T_{1o} is the observed T_1 and T_{1f} and T_{1b} the T_1s and P_f and P_b the molar fractions of Na^+ in the free and bound forms. Substitution of $P_f = 1 - P_b$ into eq 9 leads to

$$P_b = \frac{T_{1b}(T_{1f} - T_{1o})}{T_{1o}(T_{1f} - T_{1b})} \quad (10)$$

P_b is equal to $[Na^+]_b/[Na^+]_t$, where $[Na^+]_b$ and $[Na^+]_t$ are the concentrations of bound and total Na^+.

In Figure 5 are plotted ^{23}Na T_1 values as a function of pH for solutions which contained (A) 0.050 M NaCl, (B) 0.002 M heparin plus 0.050 M total Na^+, and (C) 0.002 M heparin, 0.004 M histamine, and 0.050 M total Na^+. The T_1 of 0.050 M total Na^+ in solution with 0.002 M histamine is independent of pH and equal to that of 0.050 M NaCl (data not shown). In solution with heparin alone (data set B), Na^+ is present in three forms: free, bound to the CO_2H form of

Table 2: Formation Constant of Histamine−Heparin Complex[a,b]

[histamine], M	[heparin][c]/[histamine]	$[Na^+]_{total}$, M	K_{Bd}[d]
0.001	50	0.58	24
0.001	10	0.40	54
0.001	50	0.38	82
0.001	100	0.35	75
0.001	100	0.35	97
0.001	10	0.20	228
0.001	50	0.18	282
0.001	1	0.10	773
0.001	1	0.050	2.68×10^3
0.001	10	0.035	3.19×10^3
0.001	1	0.020	1.02×10^4
0.001	1	0.015	1.85×10^4
0.001	1	0.014	1.51×10^4
0.001	1	0.010	3.90×10^4
0.001	1	0.0070	7.24×10^4
0.001	1	0.0035	2.39×10^5
0.0008	1	0.0029	3.46×10^5
0.0006	1	0.0022	4.15×10^5
0.0004	1	0.0014	9.90×10^5
0.0003	1	0.0011	1.47×10^6

[a] As determined using chemical shift−pH titration data for histamine C2H protons. [b] 25 °C. [c] In terms of the concentration of the heparin disaccharide repeat unit. [d] The average relative standard deviation of individual K_{bd} values was ±10%.

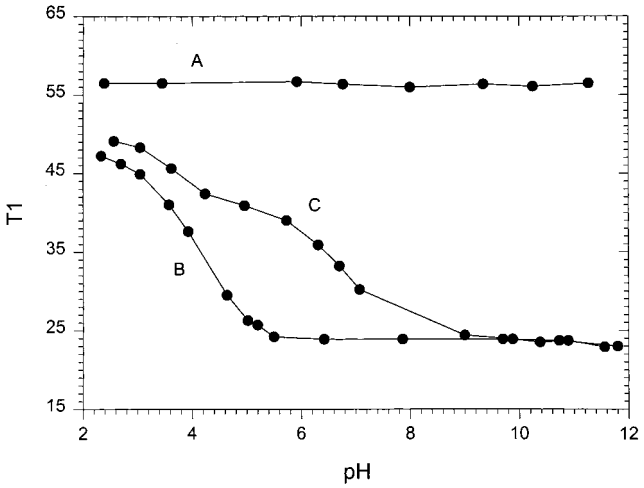


FIGURE 5: ^{23}Na spin−lattice relaxation times as a function of pH for (A) 0.050 M Na^+; (B) 0.050 M total Na^+, 0.002 M heparin; and (C) 0.050 M total Na^+, 0.002 M heparin, 0.004 M histamine. The lines through the experimental points were drawn to connect the points.

heparin, and bound to the CO_2^- form of heparin. The decrease in T_1 as the pH is increased from 2 to 6 reflects both an increased binding of Na^+ as the carboxylic acid group is titrated and a shorter T_1 for bound Na^+; T_1 values for Na^+ bound to the CO_2H and CO_2^- forms of heparin are 0.01134 ± 0.0003 s and 0.00340 ± 0.00004 s, respectively (*14*). The T_1 of Na^+ in solution with heparin and histamine (data set C) is intermediate between those of free Na^+ and Na^+ in solution with heparin over the pH range 2−9, indicating displacement of Na^+ from heparin by histamine over this pH range. The displacement at pH 2 is evidence for the binding of histamine by the CO_2H form of heparin. Chemical shift data for the carbon-bonded protons of heparin indicate the binding of histamine by heparin at pH 2 is delocalized rather than site-specific (5). As the pH is increased and the carboxylic acid group of heparin is titrated, the difference between data sets B and C is increased, indicating increased binding of histamine to the CO_2^- form of heparin. The difference reaches a maximum at pH ∼5.5. As the pH is increased further, the difference decreases, which correlates with titration of the imidazolium ring of histamine and less binding.

The fractional population of bound Na^+ in the heparin/histamine solution was calculated using eq 10 and values of $T_{1b} = 0.00340$ s and $T_{1f} = 0.0564$ s to be 0.0234 at pH 4.96, 0.0277 at pH 5.73, 0.0358 at pH 6.31, 0.0440 at pH 6.70, and 0.0548 at pH 7.07. Using concentrations calculated for the $HepH_2A$ complex and for free heparin (in terms of the disaccharide repeat unit) at the same pH values with the acid dissociation constants in Table 1 and the binding constants in Table 2, it was determined that 0.34 ± 0.03 Na^+ ion remain bound to $HepH_2A$. As discussed in the following section, the fraction of a Na^+ ion condensed on the CO_2^- form of free heparin per heparin anionic charge is estimated by counterion condensation theory to be 0.59 (*11*, *14*). With 3.6 anionic charges per heparin disaccharide on average, this corresponds to 2.12 Na^+ bound per heparin disaccharide repeat unit. Thus, the ^{23}Na spin−lattice relaxation time results indicate that 1.78 Na^+ ions are displaced upon binding of the H_2A^{2+} form of histamine to the CO_2^- form of heparin.

DISCUSSION

Because of its high negative charge density, heparin is a polyelectrolyte with a fraction of its negative charge neutralized by bound counterions. Counterions can bind to polyelectrolytes by site-specific and by territorial (or delocalized) electrostatic interactions (*15*, *16*). The H_2A^{2+} form of histamine binds to heparin by site-specific electrostatic interactions, with the imidazolium group located in a pocket with a specific affinity for the imidazolium ring (5). An exchange-averaged resonance was observed for the C2H proton for all of the conditions studied, indicating fast exchange of histamine between the free and bound forms.

Binding of histamine by sodium heparinate involves an exchange of protonated histamine for Na^+ counterions, as indicated by the strong dependence of the binding constant on Na^+ concentration. The counterion condensation model of polyelectrolyte binding developed by Manning (*15*, *16*) predicts that the fraction of Na^+ ion condensed per anionic charge on the CO_2^- form of heparin, $\theta_{Na^+}^{CO_2^-}$, is 0.59 (*14*). Experimental values of 0.58 and 0.63 have been reported (*28*, *29*). For the bovine lung heparin used in this research, $\theta_{Na^+}^{CO_2^-} = 0.59$ corresponds, on average, to 2.12 sodium ions per heparin disaccharide repeat unit.

In the counterion condensation model, bound counterions are contained in a volume V_p surrounding the polyelectrolyte (*16*). V_p depends on the axial charge spacing as given by eq 11:

$$V_p = 41.1(\xi - 1)b^3 \qquad (11)$$

where $\xi = 7.1/b$ for water at 25 °C and b is the average axial charge spacing in angstroms. Taking the length of a heparin tetrasaccharide unit to be 20.4 Å, b is estimated to be 2.9 for the carboxylate form of heparin having, on average, seven anionic groups per tetrasaccharide segment. V_p has units of cubic centimeter per mole of polyelectrolyte charge. The concentration of delocalized monovalent counterion in

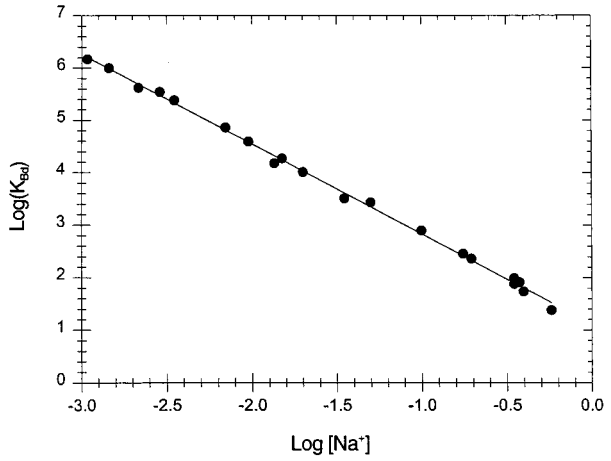


FIGURE 6: Plot of log K_{Bd} vs log $[Na^+]_{total}$. The line through the points is the theoretical line predicted by a linear least-squares fit of the data.

the condensation volume surrounding the polyelectrolyte, C_1^{loc}, is given by eq 12:

$$C_1^{loc} = 10^3 \theta_{Na}^{CO_2^-} V_p^{-1} = 24.3(\xi b^3)^{-1} \qquad (12)$$

C_1^{loc} is estimated to be 0.41 M for the CO_2^- form of heparin. Thus, if the number of Na^+ ions displaced by H_2A^{2+} is greater than 1, release of Na^+ provides an entropic driving force for the binding reaction when the Na^+ concentration in free solution is less than 0.41 M.

According to the theory of polyelectrolyte interactions (*19*), the exchange reaction by which histamine binds to heparin can be quantitatively described by eq 13:

$$H_2A^{2+} + Hep \rightleftharpoons HepH_2A + Z\Psi Na^+ \qquad (13)$$

where Z represents the number of ionic interactions that H_2A^{2+} makes with heparin, Ψ is the apparent fraction of a Na^+ counterion condensed per heparin anionic charge, and $Z\psi$ is the number of Na^+ ions displaced from heparin by H_2A^{2+}. ψ includes both the fraction of a Na^+ ion condensed per heparin anionic charge (θ) and the screening effect of condensed Na^+ ions on the interactions of residual heparin anionic charges. Thus, ψ is larger than θ; $\psi = 0.8$ has been reported for heparin (*11*).

The dependence of the binding constant on Na^+ concentration is given by eq 14:

$$\log K_{Bd} = \log K(\text{nonionic}) - Z\Psi \log [Na^+] \qquad (14)$$

where K_{Bd} is the measured binding constant and K(nonionic) is the contribution to the binding constant from nonionic interactions of histamine with heparin (*19*). If the interaction of the H_2A^{2+} form of histamine with heparin is solely due to electrostatic interactions, K(nonionic) for the binding equilibrium represented by eq 13 is expected to be approximately 1. A sequence-specific nonionic contribution to the binding would result in a K(nonionic) larger than 1 (*11*). log K_{Bd} is plotted vs log $[Na^+]$ in Figure 6. The plot is linear over a wide range of Na^+ concentrations, as predicted by eq 14, with a slope of -1.72 ± 0.02 and an intercept of 1.11 ± 0.03. The value of 13 calculated for K(nonionic) from the intercept indicates a small nonionic contribution to the interaction of histamine with heparin. Because of the increase in the ionic contribution to the binding constant as the Na^+ concentration decreases, the relative importance of the nonionic contribution decreases as the Na^+ concentration decreases.

The value for the slope indicates that, on average, 1.72 Na^+ ions are displaced from heparin by H_2A^{2+}. This value is in excellent agreement with the value of 1.78 determined from the ^{23}Na spin−lattice relaxation time data. Using the value of 0.8 for ψ (*11*), Z is calculated to be 2.2; i.e., H_2A^{2+} makes 2 ionic interactions with heparin which result in the displacement of 1.72 bound Na^+ ions. Research is in progress on the binding of histamine by heparin-derived di-, tetra-, and hexasaccharides to identify the heparin sites involved in the ionic interactions.

Finally, in view of the ion exchange process which is thought to take place when the granule matrix is exposed to both the higher pH and the higher Na^+ concentration of the extracellular fluid during exocytosis (*3*, *20*−*22*), it is of interest to consider more quantitatively the effect of Na^+ concentration on the extent of binding of H_2A^{2+} by heparin. Using the binding constants in Table 2, the fractional concentration of bound histamine in a solution which contains 0.01 M Na^+, 0.001 M histamine, and 0.001 M heparin disaccharide repeat unit is calculated to decrease from 83% to 45% when the pH is increased from 5.5 to 7.4 and then to decrease further to 1.9% when the Na^+ concentration is increased to the blood plasma concentration of 0.14 M.

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BI980625Y