Studies of the Binding of Calcium and Lanthanum Ions to D-Lyxose and D-Ribose in Aqueous Solutions Using Proton Magnetic Resonance

Robert E. Lenkinski and Jacques Reuben*[1]

Contribution from the Isotope Department, The Weizmann Institute of Science, Rehovot, Israel. *Received August 25, 1975*

Abstract: Aldoses having a cis arrangement of three consecutive hydroxyl groups in one of their tautomeric species form weak complexes with metal ions in aqueous solution. Procedures are outlined for the analysis of proton NMR data obtained for solutions containing aldoses and metal ions in two particular systems: the case in which the aldose exists primarily in two anomeric forms, one of which binds to metal ions, and the system in which the aldose exists in all four anomers of which only one *does not* interact with metal ions. The first method was used in the analysis of data obtained for the interactions of D-lyxose with $LaCl_3$, $La(ClO_4)_3$, and $CaCl_2$. The second method was applied to data obtained for the interaction of D-ribose with $LaCl_3$ and $CaCl_2$. From these analyses it is found that predominantly 1:1 metal-sugar complexes are formed in aqueous solution. The results presented confirm the suggestion that metal complexation occurs with the pyranose in a conformation which has an ax-eq-ax arrangement of the three consecutive *cis*-hydroxyl groups.

The formation of complexes between simple carbohydrates and metal ions has been an area of long-standing interest in the chemistry and biochemistry of sugars.[2] Recent attention has been focused on the interactions of metal ions with cyclitols and aldoses in aqueous solutions.[3] The study of metal ion interactions with aldoses is complicated by the fact that the solution formed upon the addition of a crystalline aldose to a solvent (e.g., D_2O) can contain as many as four *major* species: the α- and β-pyranoses and the α- and β-furanoses. Interconversions between all species are possible through a series of tautomeric rearrangements. These interconversions, after sufficient time, establish an equilibrium composition of the four forms which is a function of their relative free energies. Angyal has reported that the addition of certain metal ions to the aqueous solutions of aldoses may alter the relative amounts of each of the four species present by the formation of weak metal-sugar complexes with some, but not all, of the above mentioned species.[4] This effect has been observed in aldoses which have a cis arrangement of three hydroxyl groups on consecutive carbons in any of their isomeric forms. For the pyranoside forms, there is evidence that the metal-sugar complex is formed with the sugar in a conformation having an ax-eq-ax arrangement of the three hydroxyl groups.[5]

The composition of aldoses in solution can be studied by proton NMR since the four forms have characteristic resonances in the anomeric region, which are easily resolvable.[6] The NMR spectrum of an equilibrium mixture of an aldose yields three parameters for each species: a chemical shift, δ, a spin-spin coupling constant, J, and a relative intensity, I. Effects of the addition of a metal ion to an aldose, measured through the changes in these three parameters, should provide a means of monitoring the metal-sugar equilibria. The present study was undertaken with the aims of developing a methodology with which to analyze the changes produced by metal ions in all of the three NMR parameters of the sugar species and applying this methodology to several systems in order to obtain formation constants for the metal-sugar complexes. Accordingly, D-lyxose, an aldose which in D_2O exists almost exclusively in the pyranose forms, and D-ribose, which has all of the four forms present, were chosen respectively as a simple and complex system to study. The interactions of both sugars with calcium and lanthanum ions were measured. In the following sections, we present an outline of the methodology used to analyze the NMR data and the results obtained from these analyses.

Methodology

Binding Constants from Integrated Intensity Measurements. In this section treatments for a simple case (a) and a more complex case (b) are presented.

(a) Consider a two-component system (a_1 and a_2) in which only one component (a_1) binds to a metal ion, M, with a formation constant, K_f. Assuming a 1:1 complex is formed, the equilibrium expression can be written as

$$M + a_1 \rightleftarrows Ma_1 \qquad (1)$$

with

$$K_f = [Ma_1]/[M][a_1] \qquad (2)$$

where Ma_1 is the metal-sugar complex and [ ] denotes an equilibrium concentration. From eq 2 a Scatchard equation[7,8] can be derived

$$\frac{[Ma_1]}{a_{1T}[M]} = K_f - K_f\frac{[Ma_1]}{a_{1T}} \qquad (3)$$

where the subscript T denotes the total molar concentration of the species present. In the absence of metal ions, one can measure the equilibrium ratio of the two species present, R_0, defined by

$$R_0 = a_{1T}{}^0/a_{2T}{}^0 \qquad (4)$$

At the ith metal addition, the concentrations of a_1 and a_2 will change to preserve the following relationship

$$R_0 = [a_1]^i/[a_2]^i \qquad (5)$$

By definition $S_T = a_{1T} + a_{2T}$ and since a_2 does not interact with the metal ion, $[a_2]^i = a_{2T}$. After each addition of metal ions the ratio of the two species R_i can be measured, which is given by

$$R_i = a_{1T}{}^i/a_{2T}{}^i \qquad (6)$$

Substituting the above expressions into eq 3 yields the experimentally useful Scatchard expression,

$$\frac{(R_i - R_0)(1 + R_i)}{R_i[M_T(1 + R_i) - S_T(R_i - R_0)]} = K_f - K_f\left(\frac{R_i - R_0}{R_i}\right) \qquad (7)$$

A plot of $\nu/[M] \equiv (R_i - R_0)(1 + R_i)/R_i[M_T(1 + R_i) - S_T(R_i - R_0)]$ against $\nu \equiv ([R_i - R_0]/R_i)$ should yield a straight line with a slope of $-K_f$ and an intercept on the abscissa of 1.0. If complexes of higher stoichiometry are formed, the shape of the curve will be affected as well as the value of the intercept.[9]

(b) Consider a four-component system (a_1, a_2, a_3, and a_4) in which only one component, a_4, does not interact with metal ions. For this case, one can write the Scatchard equation

$$\frac{[Ma_i]}{a_{iT}[M]} = K_{fi} - K_{fi}\frac{[Ma_i]}{a_{iT}} \qquad (8)$$

for $i = 1, 2, 3$. In the absence of metal ions, four ratios can be measured.

$$R_i^0 = a_i^0/S_T \qquad (9)$$

(for $i = 1, 2, 3, 4$). After the jth metal addition (assuming S_T is kept constant) four ratios can again be measured.

$$R_i^j = a_{iT}^j/S_T \qquad (10)$$

(for $i = 1, 2, 3, 4$), where $S_T = \Sigma_{i=1}^4 a_{iT}$. If a_4 does not interact with the metal, then $[a_4]^j = a_{4T}^j$ and the four ratios will change with each metal addition preserving the relationship

$$R_4^0 = R_4^j S_T/S_F^j \qquad (11)$$

where the total free sugar concentration is given by $S_F^j = \Sigma_{i=1}^4 [a_i]^j$. Equation 11 can be rearranged to give

$$S_F^j = R_4^j S_T/R_4^0 \qquad (12)$$

Noting that

$$[a_i]^j = R_i^0 S_F^j \qquad (13)$$

$$a_{iT}^j = R_i^j S_T \qquad (14)$$

$$[Ma_i]^j = a_{iT}^j - [a_i]^j \qquad (15)$$

$$[M]^j = M_T^j - \sum_{i=1}^{3} [Ma_i]^j \qquad (16)$$

the following expressions for $[Ma_i]^j$ and $[M]^j$ can be derived:

$$[Ma_i]^j = (R_i^j - R_i^0 R_4^j/R_4^0)S_T \qquad (17)$$

$$[M]^j = M_T^j - S_T\left[\sum_{i=1}^{3} (R_i^j - R_i^0 R_4^j/R_4^0)\right] \qquad (18)$$

Substituting the appropriate expressions into eq 8 one obtains the experimentally useful Scatchard expression

$$\frac{\left(1 - \frac{R_i^0 R_4^j}{R_i^j R_4^0}\right)}{\left[M_T^j - S_T \sum_{i=1}^{3} (R_i^j - R_i^0 R_4^j/R_4^0)\right]} = K_{fi} - K_{fi}\left[1 - \frac{R_i^0 R_4^j}{R_i^j R_4^0}\right] \qquad (19)$$

or in Scatchard's notation: $\nu/M_F = K_{fi} - K_{fi}\nu$.

Formation Constants and Structural Parameters from Chemical Shifts and Coupling Constants. If δ is defined as the observed shift, in the presence of metal ions, referenced to the shift with no metal present, then the following equation is obtained[10]

$$\delta/[M] = K_f\delta_M - K_f\delta \qquad (20)$$

where $[M]$ is the equilibrium concentration of metal ion, K_f is the formation constant for the metal-sugar complex, and δ_M is its limiting shift. If the binding is weak, the assumption that $M_T \approx [M]$ is usually made. If the observed spin-spin coupling constant with no metal present is J_0, the observed spin-spin coupling constant upon metal addition is J, and assuming $[M] \approx M_T$, one obtains

$$(J - J_0)/M_T = K_f J_M - K_f J \qquad (21)$$

where J_M is the spin-spin coupling constant in the metal-sugar complex.

The internal consistency of shift and spin-spin coupling data can be checked by plotting δ against J according to eq 22, which is derived from eq 20 and 21.

$$\delta = J\delta_M/(J_M - J_0) - J_0\delta_M/(J_M - J_0) \qquad (22)$$

Such a plot should yield a straight line with a slope of $\delta_M/(J_M - J_0)$ and an intercept on the abscissa of J_0. Note that eq 22 is free of any assumption concerning the values of $[M]$.

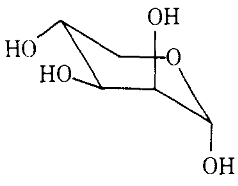
Experimental Section

NMR spectra were recorded on a Bruker HFX-10 spectrometer operating at 90 MHz for protons equipped with an internal deuterium locking system. Coupling constant measurements were performed on a 1 Hz/cm scale. Chemical shift measurements were made on a 2 Hz/cm scale and were referenced to internal *tert*-butyl alcohol (ca. 10 mM) standard. Each spectrum was integrated at least three times and an average value obtained from these measurements. For D-ribose, the integrals were referenced to internal *tert*-butyl alcohol, which in turn was calibrated by comparison to internal tetramethylammonium chloride. The concentration of tetramethylammonium chloride was determined by the Mohr titration for chloride.[11] The temperature for each NMR titration was measured using the peak separation of a methanol sample in conjunction with a temperature calibration curve supplied by the instrument manufacturer.

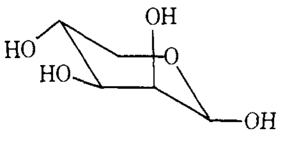
D-Lyxose and D-ribose were obtained commercially from Sigma Chemical Co. These sugars were recrystallized from D_2O at least four times to minimize the HDO peak in their NMR spectra. Calcium was introduced as the anhydrous chloride salt. Lanthanum was introduced as the hydrated chloride or perchlorate salts which were recrystallized from D_2O several times to minimize the amount of H_2O present as water of crystallization. The concentrations of stock solutions of calcium were determined using the Mohr method. The concentrations of lanthanum stock solution were determined by a titration with EDTA using arsenazo as an indicator.[12]

Results and Discussion

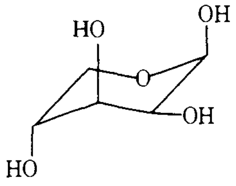
D-Lyxose. The proton NMR spectrum of D-lyxose in D_2O shows two resonances in the anomeric region, corresponding to the α- and β-pyranose forms, **1** and **2**, respectively, the α resonance being at lower field than the β resonance.[13] Upon addition of calcium or lanthanum salts an increase of the β form is observed accompanied by a shift of this resonance to lower field with negligible changes in its spin-spin coupling constant. The integrated intensities obtained on treatment of a 0.32 M solution of D-lyxose with between 0.226 and 4.74 M $CaCl_2$ were analyzed using eq 7. Figure 1 shows a Scatchard plot for the D-lyxose-$CaCl_2$ titration. Similar experiments were performed by treating 0.32 M D-lyxose in D_2O with between 0.11 and 1.76 M $La(ClO_4)_3$ and between 0.13 and 1.16 M $LaCl_3$. A Scatchard plot for the intensities obtained from the $La(ClO_4)_3$ titration is also shown in Figure 1. As seen from



1



2(C1)



3(1C)

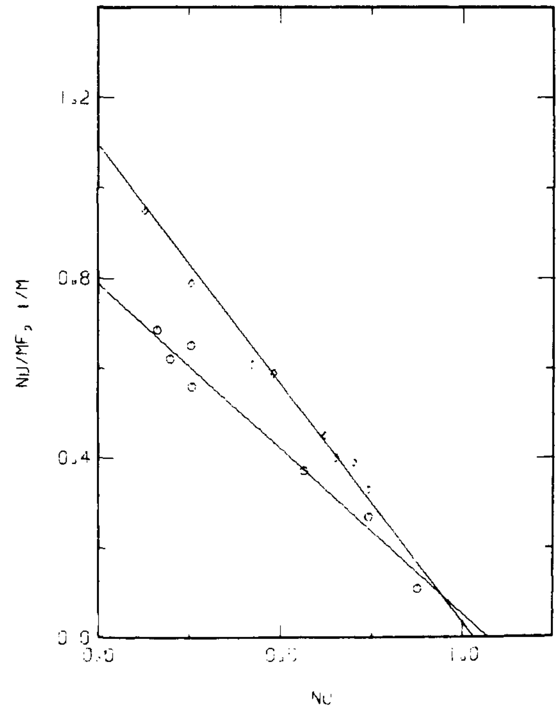


Figure 1. Scatchard plots for D-lyxose binding to calcium ions (circles for $CaCl_2$), and lanthanum ions [diamonds for $La(ClO_4)_3$] constructed according to eq 7.

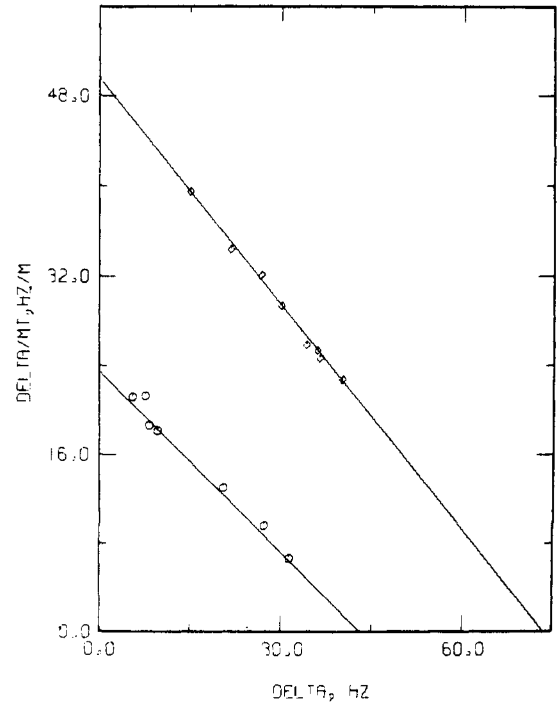


Figure 2. The variation of δ/M_T with δ (as in eq 20) for the shift obtained by treatment of D-lyxose with $CaCl_2$ (circles) and $La(ClO_4)_3$ (diamonds).

Table I. Binding Parameters for D-Lyxose with Calcium and Lanthanum Salts

	Intensity analyses		Shift measurements	
Salt	K_f, M^{-1}	n	K_f, M^{-1}	δ_M, Hz
$CaCl_2$	0.8 ± 0.1	1.07	0.6 ± 0.1	41 ± 2
$LaCl_3$	1.1 ± 0.1	1.03	0.8 ± 0.1	72 ± 3
$La(ClO_4)_3$	0.9 ± 0.1	1.06	0.7 ± 0.1	73 ± 3

Figure 1 these plots are straight lines with intercepts on the abscissa of ca. 1.0, indicating the formation of 1:1 metal-sugar complexes in solution. It is clear that a sufficient range of chemical saturation (ν values of between 0.2 and 0.8) was covered to ensure the accurate determination of formation constants.[8]

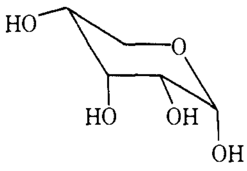
The chemical shifts obtained from the calcium titration were analyzed using eq 20. These results are shown in Figure 2. The assumption that $[M] = M_T$, made in using eq 20, was checked by comparing the [M] values obtained from an analysis of the intensity measurements with the M_T values from the experiment. The error in assuming $[M] = M_T$ ranges from 18 to 4%, respectively, for the lowest to the highest $CaCl_2$ concentration. The shifts obtained from the lanthanum titration were analyzed in a similar manner to that employed for the calcium results. The $LaClO_4$ results are also shown in Figure 2. The assumption that $[M] = M_T$ was checked as for the calcium experiments and the errors ranged between 20 and 6%. The results obtained from these analyses of shift and intensity data for D-lyxose with calcium and lanthanum are summarized in Table I. The discrepancies between the formation constants determined from shift data and intensity data arise from the assumption that $[M] = M_T$ made in using eq 20. It is clear that the condition $[M] = M_T$ does not hold in this system.

An examination of the formation constants, presented in Table I, shows that the complexation of the β-D-lyxopyranose form with calcium and lanthanum ions is relatively weak. The good agreement between the constants obtained for $LaCl_3$ and $La(ClO_4)_3$ provides evidence that the anion plays a minor role in complex formation. We suggest that the similarity in association constants obtained from the lanthanum and calcium experiments arises from the fact that, in solution, the β-D-lyxopyranose exists predominantly as the C1 conformer[14] (**2**) whereas only the higher energy 1C conformer (**3**) which has the appropriate ax-eq-ax arrangement of consecutive hydroxyl groups is expected to bind to metal ions.[5] The net energy required to bring about the conformational transition is probably of the same order of magnitude as the difference in the free energies of complex formation with lanthanum and calcium.

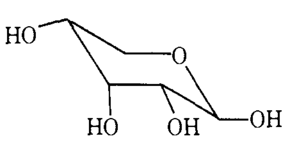
Changes in the relative populations of the two conformers (C1 and 1C) brought about by metal-sugar complexation are also reflected in the downfield shift of the anomeric resonance which is undergoing a change from an ax to an eq environment.[6] In addition, complexation of a metal ion to a ligand in solution may bring about an electric field perturbation near the nuclei within the ligand resulting in a shift which is commonly referred to as the "complex formation shift". Thus the shifts observed upon the addition of metal ions in the spectrum of the sugar may arise through either one or both of the above effects. We suggest that only a combination of these two effects can account for the variations observed in the δ_M values shown in Table I, since the magnitudes of the shifts arising from metal-induced conformational changes are not expected to depend on the metal ion present.

D-Ribose. The proton spectrum of D-ribose has four resonances in the anomeric region. From higher to lower field, these correspond to the α-pyranose (**4**), the β-pyranose (the C1 conformer (**5**) and the 1C conformer (**6**)), the β-furanose (**8**), and the α-furanose (**7**) forms, respectively. Upon the addition of metal ions, the intensities of these four resonances change; the α-pyranoside and α-furanoside peaks increase while the peaks of the two β forms decrease in intensity. Of the four resonances, only the peak of the β-furanose remains unshifted in the presence of metal ions. A concomitant decrease in the splitting of the β-pyranoside resonance is observed.

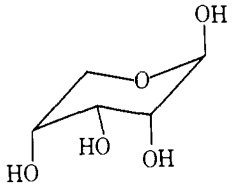
The integrated intensities obtained upon treatment of a 0.634 M solution of D-ribose with between 0.08 and 2.76 M of $CaCl_2$ at 27 °C were analyzed using the appropriate Scatchard equation (eq 19). The plots thus constructed are shown



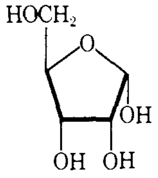
4



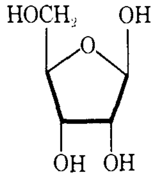
5(C1)



6(1C)



7



8

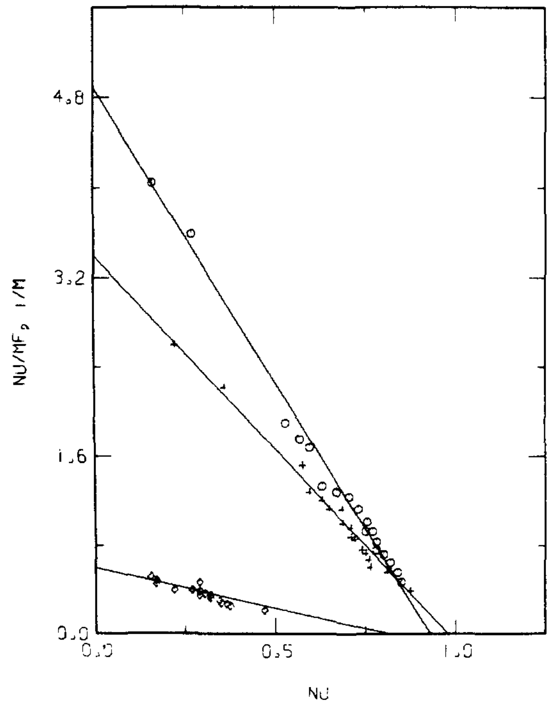


Figure 3. Scatchard plots for the interaction of D-ribose with $CaCl_2$ at 27 °C constructed according to eq 19. The α-D-ribofuranose, the α-D-ribopyranose, and the β-D-ribopyranose are represented by circles, crosses, and diamonds, respectively.

in Figure 3. The procedure was repeated for the data obtained on treatment of a 0.317 M solution of D-ribose with between 0.12 and 1.87 M of $CaCl_2$ at 52 °C. The plots for this experiment are shown in Figure 4. The intensities obtained upon treatment of a 0.317 M solution of D-ribose with between 0.14 and 1.49 M $LaCl_3$ at 52 °C were analyzed in a similar manner. The Scatchard plots for this system are presented in Figure 5. The formation constants obtained from these plots are summarized in Table II.

The Scatchard plots shown in Figures 3, 4, and 5 are in general straight lines with intercepts on the abscissa of between 0.8 and 1.1, again confirming the formation of 1:1 metal–sugar complexes. The deviations from linearity seen in Figures 4 and 5 for the β-D-ribopyranose form indicate the presence of some sort of systematic error in the experiments.[8] Such systematic errors will not appreciably affect the slope ($-K_f$) of these plots, if there is a sufficient number of points for ν values between 0.2 and 0.8. The plots constructed for the α-D-ribopyranose and α-D-ribofuranose satisfy this condition. For the β-D-ribopyranose, since only part of the appropriate range has been covered, the formation constants obtained by this method are

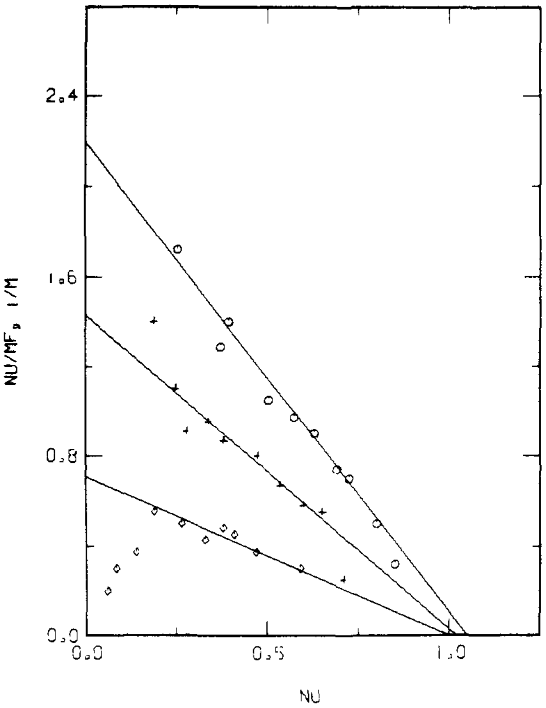


Figure 4. Scatchard plots for the interactions of D-ribose with $CaCl_2$ at 52 °C constructed according to eq 19. The symbols have the same meaning as in Figure 3.

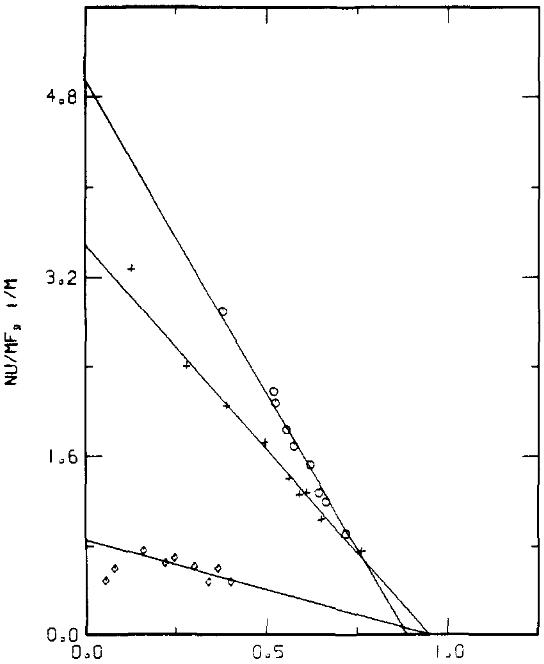


Figure 5. Scatchard plots for the interactions of D-ribose with $LaCl_3$ at 52 °C constructed using eq 19. The symbols have the same meaning as in Figure 3.

probably less reliable than those reported for the other anomers in Table II. It should be noted that since each of the three anomers binds to metal ions with a different formation constant, it is difficult in this particular system to cover the appropriate range of ν values for each anomer simultaneously.

The values obtained for the formation constants of the calcium complexes of the three anomers of D-ribose at 27 °C are in good agreement with those reported by Angyal.[5] From a comparison of the lanthanum and calcium formation constants presented in Table II, it is clear that for the two α anomers that

Table II. The Binding Parameters Obtained from Scatchard Plots of Signal Intensity Data for D-Ribose with the Chloride Salts of Calcium and Lanthanum

			Anomer		
Salt	Temp, °C		α_F	α_p	β_p
$CaCl_2$	27	K_f, M^{-1}	5.3 ± 0.1	3.4 ± 0.1	0.8 ± 0.1
		n	0.93	0.98	0.82
$CaCl_2$	52	K_f, M^{-1}	2.2 ± 0.2	1.4 ± 0.1	0.7 ± 0.1
		n	1.05	1.03	1.01
$LaCl_3$	52	K_f, M^{-1}	5.7 ± 0.2	3.6 ± 0.1	0.9 ± 0.1
		n	0.89	0.95	0.86

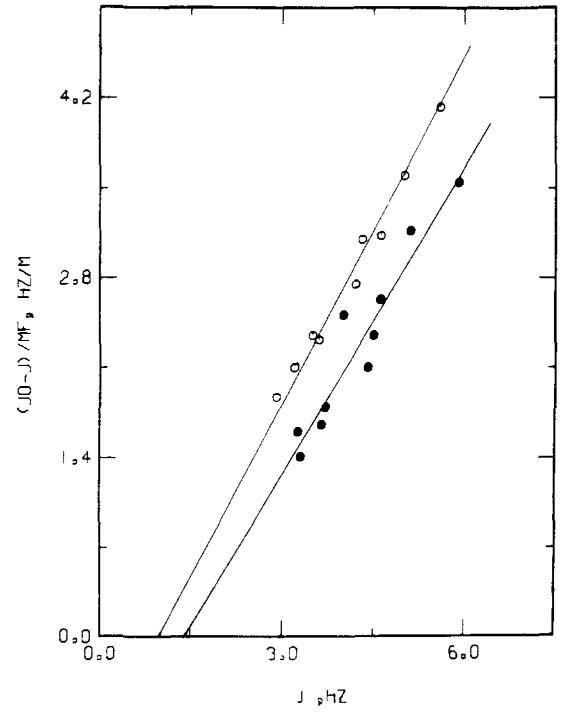


Figure 6. The variation of $(J_0 - J)/[M]$ with J for the β-D-ribopyranoside form of D-ribose upon treatment with $CaCl_2$ (closed circles) and $LaCl_3$ (open circles) at 52 °C.

bind, complexation with lanthanum ions is by at least a factor of 2 as strong as that with calcium ions. It is difficult to comment on any variation in the constants for the other anomer, since the difference between the calcium and lanthanum values borders on the range of experimental uncertainties. We suggest that, as in the case of D-lyxose, the similarity observed in the formation constants for the β-D-ribopyranose can be rationalized in terms of the energetics involved in metal-induced changes of the populations in the C1 and 1C conformers, **5** and **6**, respectively.

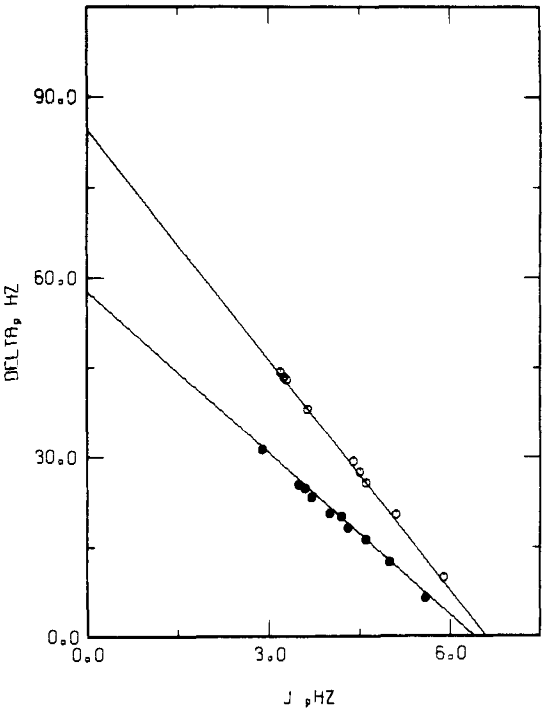


Figure 7. The variation of observed shift, δ, with the observed coupling constant, J, obtained for β-D-ribopyranose with $CaCl_2$ (closed circles) and $LaCl_3$ (open circles) at 52 °C.

A considerable variation with temperature can be seen in the constants obtained for the α anomers. For the calcium binding, these variations correspond to an enthalpy change of ca. −7 kcal/mol, accompanied by an entropy change of ca. −20 eu. These values should be considered as being estimates only of the thermodynamic parameters for the complex formation reaction, since measurements were done only at two temperatures. In addition, the inaccuracies in the constants obtained for the β-D-riboxypyranose prevent us from commenting on any temperature variations observed with this anomer.

Since the assumption that $[M] = M_T$ was found to be in error for the analysis of the shift data for D-lyxose, the shift data for D-ribose were analyzed using eq 20 with [M] values obtained from an analysis of the intensity data. In addition, the coupling constant changes observed on metal addition in the β-D-ribopyranoside were analyzed using eq 21. Plots of $(J_0 - J)/[M]$ against J for the lanthanum and calcium titrations at 52 °C are shown in Figure 6. Plots of δ vs. J for the same anomer were constructed according to eq 22. Examples of this type of plot are presented in Figure 7. The slopes and intercepts of these plots, in conjunction with J_M values obtained from the intercepts of these plots, in Figure 6, give values of J_0 and δ_M, which can be used as a check for internal consistency of the results obtained by the other methods of analysis, since eq 22 contains no assumptions concerning the calculation of [M].

Table III. Limiting Shifts and Coupling Constants for the Calcium and Lanthanum Complexes of D-Ribose

		δ_M, Hz				β_p	
Salt	Temp, °C	α_F[a]	α_p[a]	β_p[a]	β_p[b]	J_M, Hz	J_0,[b] Hz
$CaCl_2$	27	25 ± 2	24 ± 2	44 ± 2	42 ± 2	1.2 ± 0.2	6.5 ± 0.1
$CaCl_2$	52	30 ± 2	28 ± 2	42 ± 2	46 ± 2	1.3 ± 0.2	6.4 ± 0.1
$LaCl_3$	52	38 ± 2	27 ± 2	71 ± 2	68 ± 3	1.3 ± 0.2	6.6 ± 0.1

[a] Determined from the intercept on the abscissa in plots constructed using eq 20. [b] Determined from plots constructed using eq 22 (cf. Figure 7).

The results obtained from the above methods of analysis of shift and coupling constant data are compiled in Table III.

The limiting shift values given in Table III for the β-D-ribopyranose are similar to those reported for the β-D-lyxopyranose in Table I. This similarity supports the interpretation given for the agreement between the formation constants obtained for calcium and lanthanum binding to these anomers. Additional support for this interpretation can be found in the J_M values for the β-D-ribopyranoside which correspond to the coupling constant expected for the 1C conformation.[6] As in the case of the β-D-lyxopyranose, the δ_M values for the β-D-ribopyranose arise from a combination of complex formation shift and a shift caused by metal-induced changes in the populations of conformers. For the other anomers of D-ribose, the shifts observed upon complexation with metal ions must arise solely from complex formation shifts, since no metal-induced changes in conformation are expected.

As already indicated, the results of the plots of observed shifts against observed coupling constants (cf. Figure 7) can be used to check the results obtained from the plots of $\delta/[M]$ against δ. Comparisons of the δ_M values obtained from these two methods are in good agreement, showing that all the methods employed are at least internally consistent. In addition, the values for J_0 reported here are in good agreement with those measured from the spectra of D-ribose in the absence of any metal ions. These two observations serve to justify the use of eq 20 and 21 to analyze the shift and coupling constant data obtained from treatment of D-ribose with metal ions.

Conclusions

The methodology outlined here can be applied to analyze the interactions of simple carbohydrates with metal ions. From the results of these procedures it is clear that 1:1 metal-sugar complexes are formed in aqueous solution. The present results bear out previous suggestions that the binding of metal ions to pyranoses occurs with the pyranose ring in a conformation which contains an ax-eq-ax arrangement of three consecutive hydroxyl groups.

Acknowledgment. This work was supported by a grant from the United States-Israel Binational Science Foundation.

References and Notes

(1) Address correspondence to this author.

(2) For a review see: J. A. Rendleman, Jr., *Adv. Carbohydr. Res.*, **21**, 209 (1966).

(3) (a) S. J. Angyal and K. D. Davies, *Chem. Commun.*, 500 (1971); (b) S. J. Angyal, D. Greeves, and V. A. Pickles, *Carbohydr. Res.*, **35**, 165 (1974); (c) S. J. Angyal, *Tetrahedron*, **30**, 1695 (1974).

(4) S. J. Angyal, *Aust. J. Chem.*, **25**, 1957 (1972).

(5) S. J. Angyal, *Pure Appl. Chem.*, **35**, 131 (1973).

(6) For a recent review of NMR in carbohydrate see: G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, **73**, 669 (1973).

(7) G. Scatchard, *Ann. N.Y. Acad. Sci.*, **51**, 660 (1949).

(8) D. A. Deranleau, *J. Am. Chem. Soc.*, **91**, 4044 (1969).

(9) D. A. Deranleau, *J. Am. Chem. Soc.*, **91**, 4050 (1969).

(10) For a review of the methods of measurement of binding constants using NMR measurements see: R. C. Foster and C. A. Fyfe, *Prog. Nucl. Magn. Reson. Spectrosc.*, **4**, 1 (1969).

(11) A. I. Vogel, "A Textbook of Quantitative Inorganic Analysis", Wiley, New York, N.Y., 1961, pp 259–260.

(12) M. M. Woyski and R. E. Harris, *Treatise Anal. Chem.*, **8**, 57 (1963).

(13) (a) R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, **44**, 249 (1966). (b) Conformers 1C and C1 correspond respectively to $^1C_4$ and $^4C_1$ according to the new rules: *J. Chem. Soc., Chem. Commun.*, 505 (1973).

(14) S. J. Angyal, *Angew. Chem., Int. Ed. Engl.*, **8**, 157 (1969).

Poly(γ-benzyl L-glutamate) Helix-Coil Transition. Pretransition Phenomena in the Liquid Crystal Phase[1]

Russell W. Duke,[2a] Donald B. Du Pré,[2a] William A. Hines,[2b] and Edward T. Samulski*[2b]

Contribution from the Department of Chemistry, University of Louisville, Louisville, Kentucky 40208, and the Departments of Physics and Chemistry and Institute of Materials Science, University of Connecticut, Storrs, Connecticut 06268.

Received September 4, 1975

Abstract: Measurements of the macroscopic properties of synthetic polypeptide liquid crystals indicate that the helix-coil pretransition behavior parallels that reported in dilute solution studies. As varying amounts ($\leq$10% by volume) of TFA were added to PBLG in dioxane (24 mg/cm^3 dioxane), observations were made on the pitch in zero magnetic field; in orientated nematic structures of the same liquid crystals, measurements of the diamagnetic susceptibility anisotropy, $\Delta\chi$, and the reorientation rate in a magnetic field were made. The results obtained indicate that the asymmetric part of the intermolecular potential increases across the pretransition range while the twist elastic constant K_{22} remains unchanged. Even small amounts of TFA ($\sim$1% by volume) are sufficient to induce a sharp increase in the fluidity, reminiscent of the behavior of dilute, isotropic solutions of PBLG (i.e., breakdown of an aggregated network). In addition, this work demonstrates that extracting microscopic polypeptide side chain structural information from $\Delta\chi$ measurements is feasible. Specifically, the average orientation of the PBLG side chain as well as its mobility can be monitored as TFA is added to the liquid crystal. The sum of evidence supports the idea that, in the pretransition region, the helix remains rodlike while there is an increased mobility of the side chain as TFA is added.

I. Introduction

The helix-coil transition continues to stimulate considerable interest among both theorists and experimentalists. Over the last two decades, the former have produced numerous new or refined mathematical models which exhibit order-disorder phase transitions with provisions for fitting the abruptness of the transition "cooperativity" to experimental observations of the helix-coil transition.[3a] And, as new physical parameters become experimentally accessible, the measurement and usefulness of such parameters are frequently demonstrated via